2011

On-trap derivatization of short-chain fatty acids

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On-Trap Derivatization of Short-Chain Fatty Acids

by

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Thesis

Submitted to the Department of Chemistry

Eastern Michigan University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

Chemistry

Gas Chromatography

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Acknowledgements

I would like to thank the following people for their support:

Heather Holmes, PhD

Timothy Brewer, PhD

Gavin Edwards, PhD

Ross Nord, PhD

Ruth Ann Armitage, PhD

Harriet Lindsay, PhD

Cory Emal, PhD

Jacyndtha Lisi

Michelle Altenburg

ABSTRACT

It has recently been hypothesized that gastrointestinal microbiota modulate immune response by the production of short-chain fatty acids (SCFAs). There is a need for rapid, selective analytical methodology capable of trace analysis of SCFAs in complex biological matrices. Previous studies by others using multibed-sorbent traps, solid phase microextraction (SPME), and on-fiber derivatization with SPME showed that it is possible to achieve low limits of detection (LODs) for the gas chromatographic (GC) headspace analysis of SCFAs. The goal of this project was to achieve on-trap derivatization of SCFAs by incorporating a derivatizing agent onto a sorbent trap. Preconcentration and derivatization of acetic and propionic acids by the trap prior to entering a GC column produced FID signals for low ppb level concentrations of SCFAs that were competitive with mass spectrometry detection and followed a linear trend over two orders of magnitude. PDAM (1-pyrenyldiazomethane) impregnated poly(acrylate) was monitored on a daily basis and found to be stable up to ten days at room temperature when stored in an amber vial. The most significant challenge resulted from the manual construction of sorbent traps, which can negatively impact chromatographic reproducibility due to interfence of the flow of analytes and carrier gas both into and out of the trap. Alternative trap designs are under consideration to address this issue.

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CHAPTER I: INTRODUCTION

Volatile organic compounds (VOCs) have recently proven to be effective biomarkers for disease. Investigation of human breath[1] using modern analytical instrumentation has shown a correlation between different diseases and their characteristic VOCs. For example, methylated hydrocarbons[2] have been identified in the breath of patients with lung cancer. Moreover, Buszewski *et al.*[2] have suggested other VOCs that can be used as biomarkers such as ethane and pentane for oxidative stress, isoprene for cholesterol metabolism, acetone for diabetes mellitus and ketonemia; sulfur-containing compounds such as dimethylsulfide, methyl mercaptane, and ethyl mercaptane for liver impairment; and nitrogen-containing compounds such as ammonia, dimethylamine, and trimethylamine for uremia and kidney disease. This is important because breath samples from patients can be analyzed for these compounds, thus offering a non-invasive way to monitor the disease state as an alternative to a tumor biopsy. Recently, it has been hypothesized that gastrointestinal microbiota[3,4] play a major role in the immune response for gastrointestinal diseases in humans and mice. Gastrointestinal microbiota have also been found to play a major symbiotic[2] role in providing nutrition to the human body. Huffnagle[4] and Noverr mention that other authors[5,6] state that the colonizing of microbiota in the gastrointestinal tract involves a succession of bacterial populations "waxing and waning" as the host develops and the diet changes. Berg[7] states that the human gut harbors approximately 10^{14} bacteria, and that there are at least 400-500 different species of bacteria that can be further divided into different species. It has also been mentioned that the efficacy of antibiotic[8] treatment can be monitored by determining what microbiota as well as how many are present. Different microbiota

produce specific and characteristic short-chain fatty acids (SCFAs), which are VOCs and have potential for use as biomarkers for evaluating the disease state of the gastrointestinal tract. Therefore, there is a need for rapid and selective analytical methodology capable of such trace analyses of SCFAs in complex, biological matrices.

Pre-column concentration and derivatization techniques such as solid-phase microextraction (SPME), on-fiber derivatization with SPME, purge and trap, on-sorbent derivatization with thermal desorption, and multibed-sorbent traps have been effective in the gas chromatographic (GC) analysis of SCFAs at the low ppb (parts per billion) level. SPME[9] involves using a thin fiber, approximately 100 μm thick, coated with a stationary phase. The fiber is exposed to the headspace or liquid sample to absorb the analytes and is inserted into the injection port of a gas chromatograph where analytes are desorbed thermally. A carrier gas then transfers the analytes to a GC column where they are separated according to differential interactions with the stationary phase. Recently, derivatizing agents[9] have been incorporated onto the SPME fiber. The analytes are derivatized upon entering the stationary phase coating the fiber. This addition of functional groups in the derivatization step allows a greater detector response at the same concentration as the non-derivatized acid, resulting in a lower limit of detection (LODs).

The purge and trap[10] technique involves bubbling an inert gas through a sample to transfer analytes onto a trap consisting of a tube containing a sorbent material that adsorbs the analytes. A carrier gas passes through the trap so that when the analytes are thermally desorbed they are transferred to a GC column for separation.

On-sorbent derivatization[11] with thermal desorption is a technique that involves impregnating the sorbent material with a derivatizing agent and then loading the sorbent

material into a glass capillary tube. The tube is loaded with sample and allowed to react for a specific period of time. After the reaction time has passed, the tube is placed into the injection port of a gas chromatograph that is maintained at a specific temperature where the newly formed derivatives are thermally desorbed from the trap and then carried by way of a carrier gas to a GC column.

A multibed-sorbent trap[12] is a tube that contains discrete beds of sorbent materials of varying adsorption or absorption strengths separated by quartz wool. The analytes are drawn onto the trap by way of a vacuum pump. The trap is resistively heated to desorb the analytes, and upon desorption a carrier gas is passed through the trap to transfer the analytes to a GC column.

On-fiber derivatization has improved sensitivity and LODs of SCFAs, but it is not often done in an automated fashion. Purge and trap has also been effective in achieving lower detection limits of SCFAs due to the preconcentration of analytes prior to injection. Multibed-sorbent traps can be used to perform on-line automated analysis because the different sorbent beds of varying strength can preconcentrate different analytes. So far, on-trap derivatization has not been attempted, but if achieved it has the potential to obtain lower LODs for VOCs such as SCFAs.

CHAPTER II: LITERATURE REVIEW

a.) Preconcentration Methods: SPME

Quantification of various VOCs from agricultural composting operations that contain swine remains that could act as potential biomarkers for a disease was the aim of Akdeniz *et al.*[13] in their recent study. Testing was done under both dry and humid conditions

using SPME with GC-MS. The method outlined involved using GC-MS to produce a standard curve of volatilized dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), pyrimidine, and fatty acids that ranged from acetic through hexanoic, with concentrations ranging from 0.09 to 13.2 ppmv (parts per million by volume) using syringe pump injection. The standard curve for the dry conditions was obtained at 0% relative humidity (RH), while the curve for the humid conditions at 97% RH. Air samples from compost were collected into glass sample bulbs, then sampled by placing a carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber into the bulb, allowing the fiber to remain in the bulb for one hour and then placing the fiber into the GC-MS injection port at 260 °C. The method LODs for dry conditions ranged from 1 to 580 ppbv, and 0.011 to 572 ppbv for humid conditions. Limits of quantitation (LOQs) and limits of linearity were not determined or provided. The % RSDs (% relative standard deviation) ranged from 0.40 to 14.8%. These results suggest that this method has the potential to monitor specific VOCs as biomarkers from agricultural composting operations under both dry and humid conditions.

Clark and Bunch[14] have developed a method using SPME-GC-MS to analyze the flavoring additives in cigarette tobacco. Extractions were performed on Kentucky Reference 1R1 tobacco, and then used to make exactly twenty-five 20.00 mL solutions that were 1 μg/μL in 2,6-dichlorotoluene, 3 M in KCl(aq), and 1 μg/μL in the following 4 compounds: benzaldehyde, tetramethylpyrazine, menthol, and anethole. The solvent was ethanol. The solutions were allowed to equilibrate for two hours. The headspace of each solution was sampled at a variety of temperatures up to 145 °C using three SPME fibers. The fibers (65 μm poly(dimethylsiloxane)/divinylbenzene, 65 μm

Carbowax/divinylbenzene, polyacrylate, and 100 μm methyl silicone) were placed in the headspace for varying periods of time. It was determined that a temperature of 95 °C for 15 minutes with a Carbowax/divinylbenzene SPME fiber achieved optimum extraction. The recoveries of the spike ranged from 1.79 to 3.19 μg, and the % RSDs ranged from 5.3 to 11.7 %. Using the same solution preparation method, standard solutions of 31 different flavoring additives were prepared by spiking the solutions with 1, 5, and 10 μg of the additives. From the calibration curves, LODs for all 31 additives were determined by extrapolation. The LODs ranged from 15 to 5,985 ng/g. LOQs and LOLs were not determined or provided. This method shows that SPME is effective in extracting VOC analytes for GC headspace analysis.

b.) Preconcentration Methods: Purge and Trap

Cormier *et al.*[15] have attempted to use the purge and trap method with GC sniffing analysis and GC-MS to determine the odor-active components in milk, which are SCFAs. A fresh stock of *Pseudomonas fragi* (*P. fragi*) was cultured on brain heart infusion (BHI) agar and then used to inoculate skim milk. The milk was incubated and centrifuged for 48 hours at 15 °C and 130 rpm, and then a 0.2% by volume solution of ethanol was added to the mixture to derivatize the SCFAs produced by the *P. frag* to their ethyl ester analogs. The mixture was incubated and centrifuged for 48 hours at 15 °C and 130 rpm. The resulting suspension was divided into ten separate 10.00 mL aliquots, then frozen and kept at -60 °C in sealed ampules until time to purge. Before purging, all solutions were spiked to 1 ppm of the methyl ester of heptanoic acid as an internal standard, and then heated at 80 °C for 7 minutes with He (g) and purged for 3 minutes with He(g) at a

rate of 40 mL/minute. The purged volatiles were collected onto a graphitized carbon trap, and the trap was heated for 3 minutes at 200 °C to desorb all volatiles onto a GC column. An effluent splitter at the end of the column was used to direct half of the sample to a FID (flame ionization detector), and the other half to a mass spectrometer that used electron impact ionization. Separate standards were run of the SCFA esters that were expected to be present. Concentrations of the analytes ranged from 0.019 to 1.424 ppm. Important numerical figures of merit such as LOQs, LOLs, standard deviations, and % RSDs were not mentioned. There was no mention of calibration curves or a LOD. Although the quantitative part of this method is questionable, it did qualitatively determine the SCFAs present.

Campo *et al.*[16] attempted to use purge and trap with GC-GC-MS to identify the aroma compounds characteristic of 24 different wines to determine the specific processes characteristic in the production of a particular wine. Twenty mL of each wine were purged in a solution containing a mixture of NaCl, $NaHCO_3$, K_3HPO_4, KH_2PO_4 to form a "synthetic saliva." The solution was magnetically stirred and heated at 37 °C, and purged with $N_2(g)$ for 200 minutes at 100 mL/minute. Volatiles of the wines were extracted onto a trap containing LiChrolut EN resins, selected for their ability to extract various aroma compounds. The extracts were further dried using freezing at -30 °C for 2 hours, and then passing a $N_2(g)$ stream through each prior to loading. Each sample was loaded for 30 minute periods onto a main GC column connected to a Deans valve fitted with a GC-FID and a GC-MS, each with an olfactometric port. The data were analyzed using ANOVA to group the wines into different categories based on the number of aroma compounds each wine contained. The method mainly focused on qualitative analysis of

the wine and was only semi-quantitative. The concentrations of aroma compounds varied from less than 0.0001 to 150,995 μg/L. There was no mention of standard curves, LODs, LOQs, LOLs, or % RSDs. Variances of the concentrations that ranged from 0.0001 to 16,684 were listed.

c.) Preconcentration Methods: Multibed-Sorbent Traps

A study outlined by Sanchez and Sacks[12] detailed the development of an on-line method for analyzing large volume air samples using a multibed-sorbent trap. This involved making a multibed-sorbent trap that contained four different beds of sorbent materials of varying sorbent strength: Carboxen 1000 and Carbopack B, X, and Y. The trap was then mounted onto a GC-FID instrument, and standards of 31 different VOCs from gas sampling bags were drawn onto the trap by way of a sniffer line and a vacuum pump. The concentration of the VOC standards ranged from 8 to 35 ppmv. Computerized valve switching was used to direct sampling and carrier gas flows. In the analysis mode, a voltage signal from the computer caused the trap temperature to increase rapidly, causing desorption of analytes from the trap. A carrier gas passed though the trap during this heating cycle to push the desorbed analytes to a GC column where differential separation occurred. The analytes were detected by the FID. Calibration curves of the log of the peak area versus the log of sampling time were made to determine the optimum sampling time. Each curve was made at a particular desorbing temperature, 200, 250, 300, 350, and 385 °C. Sampling times varied from 2 to 20 s. The optimum sampling time was 12 s, and optimum desorbing temperature was 300 °C. The slopes of the curves for ethanol, hexane, 1-propanol, heptane, 1-butanol, octane, and

nonane ranged from 0.99 to 1.06 with R^2 values greater than 0.999. The important figures of merit are the % RSDs that typically ranged from 1 to 3%, but always below 5%. This indicates that multibed-sorbent traps have potential for the analysis of air samples.

Sanchez and Sacks[17] have taken multibed-sorbent traps even further to develop a method that can be used to monitor biomarkers representative of people who smoke. This method used two-dimensional GC with time-of-flight (TOF) mass spectrometry detection and was chosen because it uses two different GC columns to achieve tunable selectivity. While two or more analytes may have the same elution time on one particular column with one type of stationary phase, they are unlikely to have the same result on a different column with a different stationary phase. This provides better separating power that results in better resolution of analyte peaks upon elusion. The multibed-sorbent trap used was made the same way as the one used in the previous study by Sanchez and Sacks.[7] The instrument design was similar as well, except that two GC columns were connected by a thermal modulator, and a TOF mass spectrometer was used for detection in place of a FID. The first column was a dimethylsiloxane column temperature programmed from 35 to 185 °C at a temperature ramp of 10 °C/minute. The second was a poly(ethylene glycol) column contained in a separate oven, programmed at a positive 10 °C offset from the first column. The modulation time used for all experiments was 6 s. Two commercial volatile compound mixtures were used in calibrating the instrument, a 39 component-reagent-grade mixture and a 54 component-reagent-grade mixture. The standard curve range was from 2.7 to 80.0 ppb. Breath samples were obtained by having volunteers breathe into 1-L Tedlar gas sampling bags, and loading 560 cm^3 of sample onto the trap. The samples were taken from both smokers and non-smokers.

Interestingly, peak areas for 2,5-dimethylfuran, 2-methylfuran, and furan decreased for smokers at post-cigarette times of 30 minutes, 1 hour, and 2 hours. The measured amounts of VOCs in the samples ranged from 18.8 to 57.6 ppb. All standards used showed linearity, with R^2 values ranging from 0.9925 to 0.9999. LODs, LOQs, and LOLs were not provided, but the % RSDs ranged from 5 to 10%. This method shows potential for the trace analysis of VOCs not only for human breath samples but for air quality analysis.

d.) Derivatization Methods

There are many different methods and agents for the derivatization of SCFAs. Derivatization not only allows analytes to volatilize, but also adds C and H mass for a better FID signal. In addition, derivatization of SCFAs can be used to add fluorescent labels for improved detection in high-performance liquid chromatography. Nimura *et al.*[18] developed a method to prepare 1-pyrenyldiazomethane (PDAM) and used it to derivatize SCFAs for analysis by reverse-phase HPLC with fluorescence detection. Once the PDAM was prepared as outlined in the procedure, 100 μL solutions of different SCFAs ranging from 0.01 to 10 μg/mL were derivatized by adding 100 μL of 1 mg/mL of PDAM in ethyl acetate. After the solutions stood for 90 minutes at 25 °C, they were loaded individually into a HPLC instrument with a spectrofluorometric detector. The same procedure was tried on a long-chain fatty acid, palmitic acid, but MS detection was used in place of spectrofluorimetry. The standard curves for some of the SCFA derivatives were provided, but LODs, LOQs, LOLs, standard deviations, and % RSDs

were not provided. The only figure of merit mentioned was the coefficient of variation for palmitic acid being 1.1 %.

Schneede and Ueland[19] have investigated how higher yields can be obtained for the derivatizaion of fatty acids with PDAM for improved fluorescence detection in HPLC. In this experiment, concentrations of methylmalonic acid (MMA) and ethylmalonic acid (EMA) ranging from 0.1 to 300 μM in methanol were derivatized by adding PDAM and ethyl acetate. One set of each acid was kept at a pH between 5.5 and 9, and another set between 8 and 10.3 using a Tris-HCl buffer. Both sets of solutions were incubated in the dark at 25 and 50 °C for 24 hours. The solutions were analyzed using reversed-phase HPLC. The results showed linearity over two orders of magnitude, but the MMA solution that was maintained at a pH between 8 and 10.3 had the highest fluorescence intensity. GC-MS was performed on this same solution, indicating that the MMA derivative had the highest percent yield.

Yu and Sai Hang Ho[11] succeeded in developing a method for the analysis of carbonyl compounds in ambient air samples by collecting the samples in glass capillary tubes containing a sorbent material impregnated with a derivatizing agent. The derivatives were thermally desorbed into a GC column after a specific reaction time period. Sampling tubes of pyrex glass with an inner diameter of 4 mm and an outer diameter of 6 mm, and 7.8 cm long, were packed with 50 mg of Tenax TA 60/80 mesh that was pretreated with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA hydrochloride). Two methods were used to prepare the gaseous standards used for calibration. The permeation method was used to prepare the formaldehyde standards ranging from 13 to 50 ppbv, and liquid vaporization into a 100 L Tedlar bag was used to

prepare the standards of acetaldehyde, benzaldehyde, glycoxal, and methylgloxal ranging from 5 to 20 ppbv. The samples were collected at the main gate of the Hong Kong University bus stop into the previously prepared sampling tubes by using a constant-flow digital air sampling pump. Each individual sample was collected for a period of four hours at a flow rate of 20 mL/minute. The samples were analyzed by placing each sampling tube into the injection port of a gas chromatograph at 100 °C, and then increasing the temperature of the port to 250 °C over a period of 5.3 minutes. Temperature programming was carried out by starting at 30 °C for 2 minutes and gradually increased as follows: increased to 85 °C at a rate of 7 °C/min., increased to 150 °C at a rate of 3 °C/min., increased to 250 °C at a rate of 10 °C/min. and then held at 250 °C for 10 minutes. Detection was accomplished by mass spectrometry detector with electron impact ionization. Calibration curves were made for all individual carbonyls, ranging in mixing ratios from 5 to 10 ppbv, but the sampling durations were timed to give a loading range that varied from 0.1 to 9.6 nanomoles (nmol). The collection efficiencies were reported to be greater than 90% for each carbonyl. The precision in % RSD ranged from 0.7 to 45%, and the limits of detection ranged from 0.02 nmol (0.1 ppbv) to 0.20 nmol (1.0 ppbv).

Pan and Pawliszyn[20] outlined their development of sensitive, selective, and solvent-free methods for GC analysis of SCFAs using SPME. The derivatization was carried out in the sample matrix, the headspace, and on the SPME fiber itself. Derivatization of long-chain fatty acids was carried out in the sample matrix and also in the injection port of the gas chromatograph. The derivatizing agents used for the short-chain acids were pentafluorobenzyl bromide (PFB-Br), (pentafluorophenyl)diazoethane (PFPDE), and

pyrenyldiazomethane (PDAM). The long-chain acids were derivatized using PDAM, tetramethylammonium hydroxide (TMAH) and tetramethylammonium hydrogen sulfate ($TMA\text{-}HSO_4$). The methods of detection used were flame ionization detection (FID), electron capture detection (ECD), and ion trap mass spectrometry (ITMS). As a control, all short and long-chain acids analyzed were run without derivatization. Dynamic ranges, limits of quantitation (LOQs), limits of linearity (LOL), and limits of detection (LODs) were the figures of merit provided for each short-chain acid analyte, as well as the method of detection used. However, these ranges were not provided for the long-chain acids. Pan and Pawliszyn[20] state that the limits of detection were calculated from the noise of the reagent blanks, but they never stated the numerical values for the noise or how the LODs were calculated. The LODs for the short-chain acids in air samples were in the low pg (picogram) to ng/mL (nanogram per milliliter) and low fg (femtogram) to pg/mL (picogram/milliliter) levels for the samples in solution, and the on-fiber derivatization yielded LODs 1 to 4 orders of magnitude less than that obtained for the non-derivatized. The on-fiber method appears to be the most sensitive of all the methods attempted.

Earlier, Pawliszyn *et al.*[21] did a study from more of a physical chemistry point of view, comparing the results of derivatizing SCFAs in a sample matrix to on-fiber derivatization. SPME-GC-FID was performed on the matrix of free (non-derivatized) SCFA standards and on headspace of derivitized SCFA standards that were derivatized in the solution matrix. Then SPME was attempted using on-fiber derivatization in the sample headspace. Two types of SPME fiber were used, poly(dimethylsiloxane) and poly(acrylate) coated fibers. The acids used were acetic through decanoic acids. The

calibration for the free fatty acids ranged from 0.2 to 200 ppm, and the solution matrix was saturated in NaCl (aq) and kept at a pH of 1.5. The LODs ranged from 0.02 to 760 ng/mL, the % RSDs from 2.7 to 5 %, and calculated partition coefficients ranged from 0.09 for acetic acid to 441 for decanoic acid. The same procedure was used for both the derivatization in the solution matrix, as well as for the on-fiber derivatization. For the on-fiber derivatization, the SPME fibers were kept in a vial containing PDAM in hexane solution to saturate the fiber with PDAM prior to sampling the headspace. In addition, ^{13}C-labeled acetic, propionic, and butyric acids were used as internal standards for the on-fiber derivatization. For the in-solution matrix and on-fiber derivatization, only calculated partition coefficients were provided for the two different SPME fibers for propionic and butyric acids. No calibration curves or figures of merit were provided. The partition coefficients for extraction using a poly(dimethylsiloxane) coated fiber were 5,000 for propionic acid, and 12,000 for butyric acid. For the poly(acrylate) coated fiber, the values were 81,000. This is important, because the partitioning between the gaseous headspace and the coating on the fiber limit the number of SCFA molecules that can react with the PDAM. Therefore, the poly(acrylate) coating provides the best medium for the derivatization reaction to take place. The authors also mentioned that the derivatization reaction takes five hours to complete.

Pre-column concentration and derivatization methods for the gas chromatographic analysis of VOCs have been active areas of research for a number of years. Purge and trap, SPME, on-sorbent derivatization with thermal desorption, and multibed-sorbent traps have significantly improved sensitivity and LODs for trace analysis. SPME has been used to detect VOCs in air from agricultural composting operations containing

swine remains, as well as additives in tobacco. The advantage of SPME is that it can be used to sample headspace and to sample matrix by direct immersion. Both purge and trap and SPME methods have been especially useful in the analysis of SCFAs. Purge and trap has been useful in detecting the odor-active compounds in milk and wines. Although the advantage of purge and trap is that most analytes can be quantitatively transferred to the trap, the time required for desorption is long, and on-trap decomposition is common during the heating cycle. On-sorbent derivatization with thermal desorption have proven to be effective in measuring trace amounts of carbonyl compounds in air. Multibed-sorbent traps have been useful in the detection of a large number of VOCs in large volume air samples, as well as human breath samples. The concentrations of such VOCs can range from the high ppb to the low ppm range. In spite of the advantage of rapid, on-line concentration, like the purge and trap, rapid resistive heating of the trap could cause decomposition of the analytes.

There are many different derivatization methods used in the analysis of SCFAs. The most recent development has been the incorporation of derivatizing agents onto a SPME fiber, causing derivatization and extraction to occur simultaneously. On-fiber derivatization incorporating PDAM onto a polyacrylate SPME fiber, resulted in fg/mL to low pg/mL LODs, which are four orders of magnitude lower than SPME used without derivatization. Kinetic studies have not been performed, and there are conflicting reports in the literature[18,19,21] regarding optimum reaction times. This makes it difficult to perform rapid analysis of SCFAs with SPME. Currently, no catalyst for this reaction is known. If a catalyst could be found, SPME performance would likely be enhanced due to lower effective sampling and reaction times. Also, it has been postulated that the

derivatization reaction proceeds best in the polymeric coating of the SPME fiber. This review of literature reveals that preconcentration coupled with the derivatization of SCFAs has the potential to achieve lower LODs in the GC analysis of SCFAs. The goal of this project was to achieve SCFA derivatization on a multibed-sorbent trap coupled to GC-FID in order to obtain LODs with GC-FID that are competitive with GC-MS.

Chapter III: MATERIALS AND METHODS

a.) Construction of the Instrument

Figure 1 shows a diagram of the instrument. The GC is a Shimadzu GC-14A gas chromatograph. A Gateway 2000 computer with Labview software was connected to the following system components: A Vici model number EHMA 6-port valve (CV), two Staco Energy Products type 3PN1010 variacs (PS1, PS2), and the sorbent trap by way of a NiDaq 16-bit analog to digital (A/D) converter and National Instruments 5CB-68 panel box and electrical breadboard with four 3-ampere, 125 volt alternating current, solid-state relays. Here, trap temperature was measured with a Micromega model CN77333 temperature controller used with a J-type thermocouple. The chromatograph was attached to a Shimadzu C-R8A Chromatopac integrator, which printed out chromatograms of the standards or samples under analysis. A Chromatofast brand vacuum pump (V) was attached to the 6-port valve in order to draw SCFA analytes onto the trap.

Labview software was used for programming the time settings for changing the valve position between sampling and analysis modes, and connecting the appropriate power supply for resistive heating of the trap to thermally desorb the analytes during analysis.

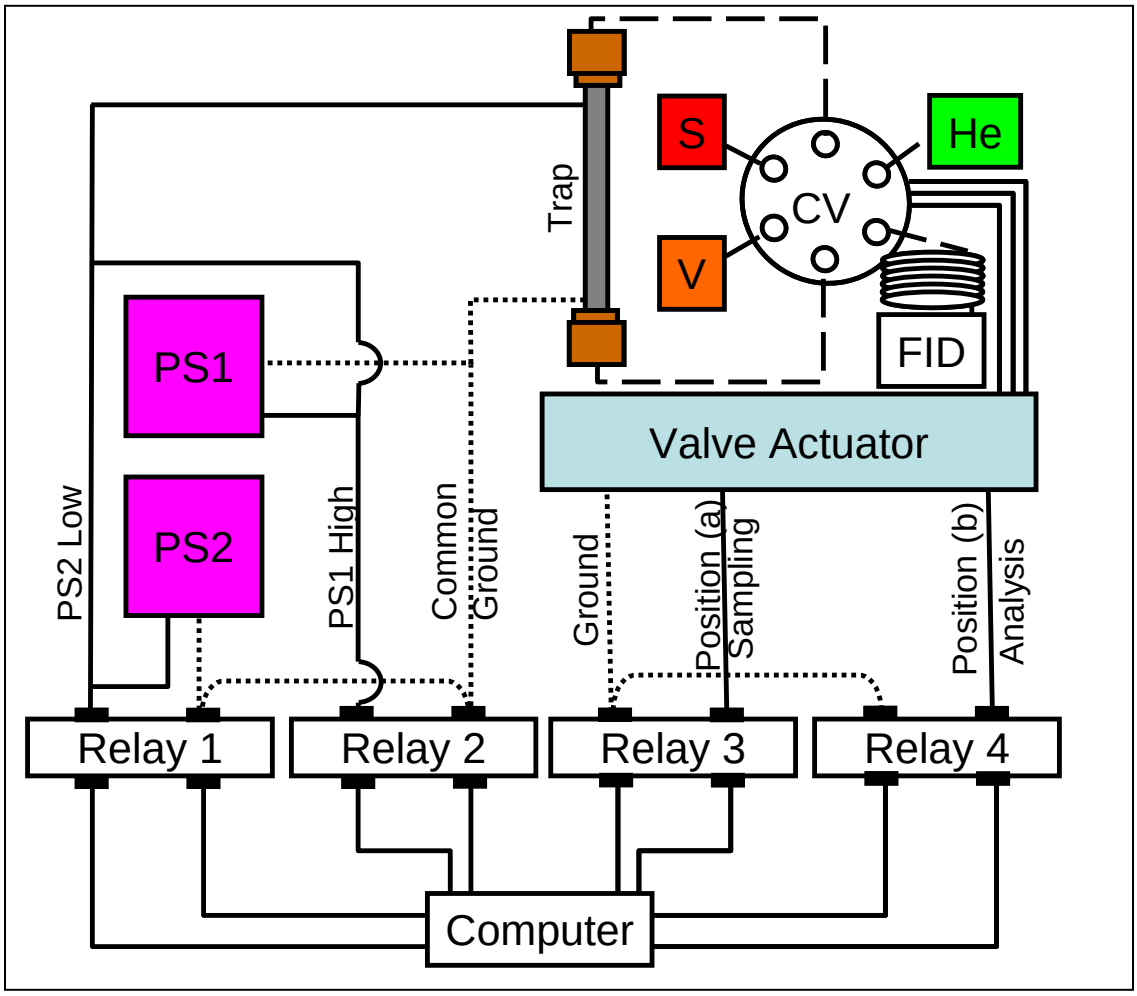

Figure 1: Instrument diagram of the sorbent-trap system

Figure 2 shows the configuration of the 6-port valve during sampling and analysis. In Figure 2a, the system is in sampling mode where the analytes are drawn into the trap by way of the vacuum pump. Once the sample is on the trap, the valve is switched to the configuration in 2b, and the carrier gas passes through the trap to the column. The carrier gas flow is allowed to equilibrate for several seconds prior to heating (firing) the trap to inject analyte to the column. The trap is heated using two variacs. The purpose of each variac is to serve as a power supply (PS) for a specific function: PS1 sends a high

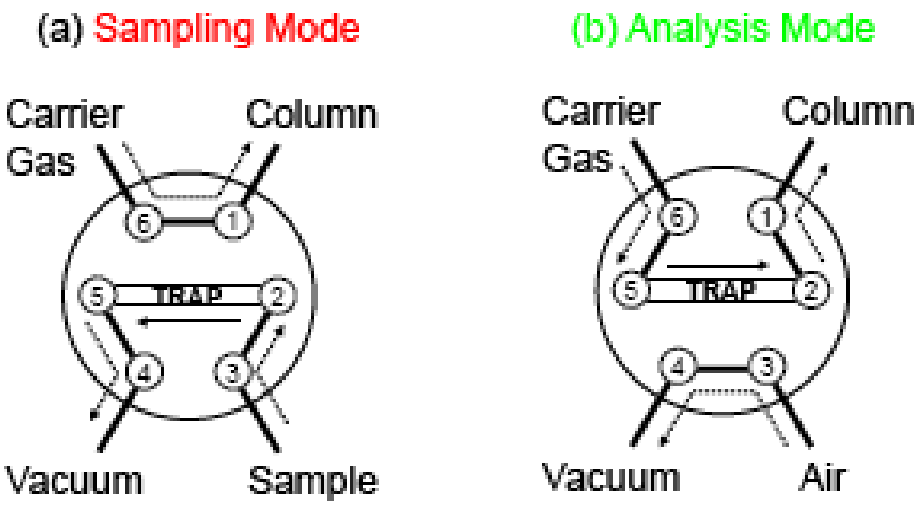

Figure 2: Valve configurations

voltage signal to rapidly heat the trap, and PS2 sends a low voltage signal to maintain the trap at a specific temperature. Testing of the relationship between time settings for the variacs and the resulting trap temperature was done by setting the high voltage variac, PS1, and the low voltage variac, PS2, at different voltages and measuring the trap temperature with the temperature controller and thermocouple. The high voltage pulse was given for 2.5 seconds (s), then after a 0.05 s pause in which no electrical signal was sent, the low voltage pulse was given for 5 seconds.

Chromatograms were obtained to determine the assembled instrument's ability to detect free fatty acids. Underivatized fatty acids yield a higher LOD than their derivatized analogs. Thus, if the instrument with the sorbent trap set-up could detect SCFAs in the high ppb range without derivatization, then low ppb detection of derivative analogs should be possible. Two solutions, 100 and 200 ppb solutions of Aldrich brand 99% isobutyric acid and double-deionized water, were prepared in two small GC vials for analysis with a graphitized carbon multibed-sorbent trap. The conditions used were oven

temperature 80 °C and FID 175 °C. These same conditions were used for all other tests throughout this project.

Tests in saturated headspace were elected to achieve large signals from which retention times (t_R) could be obtained. Underivatized acetic, propionic, butyric, and isobutyric acids were used to investigate the selectivity of the newly constructed instrument. The brands and grades of acids used were Fisher brand certified A.C.S. (American Chemical Society) glacial acetic, and Aldrich brand 99% for the other three acids. The column was changed to a J & W, 15 m long with the first 3 m 5%-dimethylsioxane, and the remaining 12 m carbowax. The inner diameter of the column was 0.25 mm and the film thickness was 0.5 μm. The acetic acid used was Fisher brand, certified A.C.S. glacial acetic acid; the propionic acid used was Fisher brand, class II certified. One drop of pure acetic acid was placed into a GC vial and loaded into the instrument. Five chromatograms were collected. The same tests were performed for propionic, butyric, and isobutyric acids. Retention times were obtained from the five chromatograms for each acid in order to calculate average retention times, standard deviations, retention factors (k), and selectivity coefficients (α) for each pure acid. An additional test was also done using a 100 ppm butyric acid solution that was 5 g/L in KCl. The conditions were the same as used previously, but a different trap was used. A single vial of the test solution yielded nine chromatograms, and an average retention time, average peak area, and standard deviations for the set were determined.

b.) Graphitized Carbon Multibed-Sorbent Traps

The first traps used were constructed using beds of graphitized carbon for sorbent material, as outlined by Sacks[12] and Sanchez. Supelco supplied all three graphitized carbon sorbents, and Alltech supplied the quartz wool that acts as a partition. Approximately 2.2 mg of each sorbent was weighed-out on a Mettler AE50 electronic analytical balance, then transferred into the trap tube. Figure 3 shows all of the components used in constructing the traps and the order of the beds with respect to analyte flow.

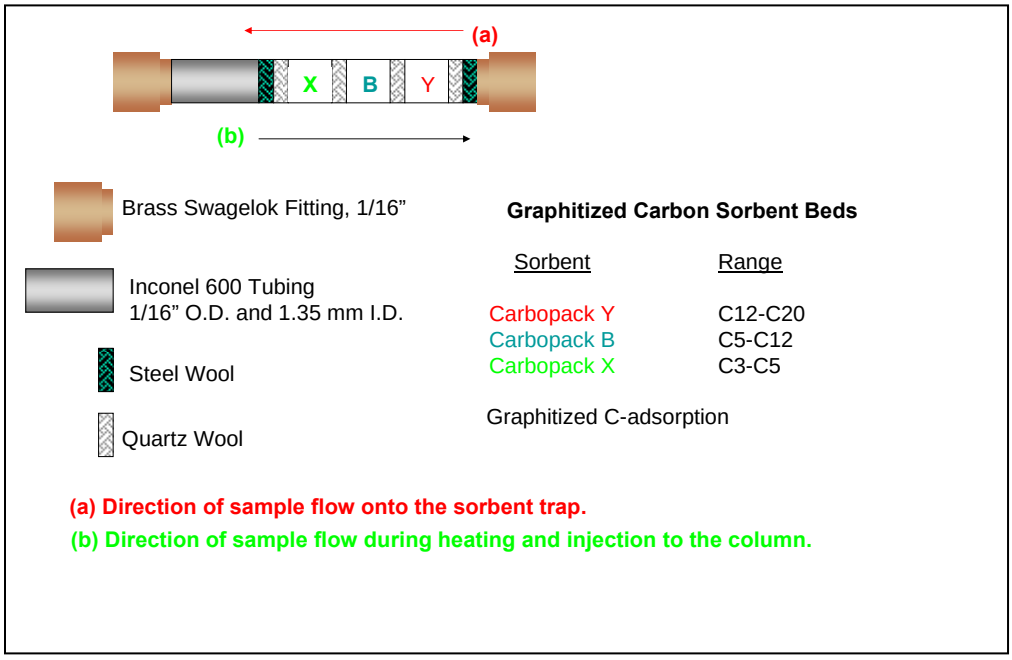

Figure 3: Graphitized Carbon Multibed-Sorbent Trap

Arrows (a) and (b) indicate direction of flow through the trap during sampling and analysis. The strength of the beds increases from Y, B, and X, so large molecules are retained on the weakest bed and do not come into contact with the stronger beds from which they might not desorb quantitatively. Initial traps were constructed using steel

wool, as shown in Figure 3. Later, the steel wool was replaced with Red Devil brand super-fine steel wool that was rolled up tightly to fit within the trap tube. The wool was cleaned in Fisher brand certified ACS spectrophotometric grade cyclohexane, then allowed to dry for a few hours prior to trap construction.

Flow through the trap was measured using a soap-bubble meter constructed using a 1.0 mL volumetric pipet, a Pasteur pipet bulb, alconox solution, polymer tubing, and brass fittings. Carrier gas pressure was varied until the flow exiting the trap (entering the column) was 1.0 mL/min.

c.) Poly(acrylate) Sorbent Traps

Sigma brand poly(acrylate) beads were baked for 8 hours at 210 °C in an oven to dry. Impregnation of the poly(acrylate) with PDAM was achieved by adding a known mass of Invitrogen Molecular Probes brand PDAM to a dark-tinted gas chromatography vial, and then 1.00 mL of Aldrich brand anhydrous 99% diethyl ether was added to the vial. Next, poly(acrylate) was added to the vial until only a small amount of solution was just over the surface of the poly(acrylate). The vial was placed in a dark cupboard for approximately 24 hours to allow all of the ether to evaporate and avoid explosion upon firing of the traps that would be made using the treated poly(acrylate). Masses between approximately 6 and 6.6 mg were used for making the traps, which were constructed using the same method as was used for the multibed-sorbent traps, but this time a single bed of PDAM impregnated poly(acrylate) was used.

Extractions were performed by placing a small amount of PDAM treated poly(acrylate) into a clear, screw-cap vial, then allowing it to sit for approximately 1 hour

in Fisher brand, HPLC grade acetonitrile. The chemical state of the PDAM impregnated poly(acrylate) was monitored on a daily basis by performing extractions in acetonitrile, followed by UV/VIS spectrophotometry on the extractions.

d.) Validation of Derivatization on Sorbent Material

An experiment was conducted to determine if derivatization could occur within a sorbent trap containing PDAM impregnated sorbent material. Individual UV/VIS absorbance spectra of PDAM in acetonitrile and ethyl acetate were taken to identify the absorbance peaks indicative of PDAM. A mix of different graphitized carbons (X,B, and Y) and poly(acrylate) was impregnated with PDAM using the method previously stated, distributed over a watchglass, and placed in two separate sealed beakers above the surface of 12.1 M glacial acetic acid. After the beakers remained in a dark cupboard for twenty-four hours, extractions were performed on both sorbent materials using ethyl acetate and Aldrich brand 99% octane, followed by UV/VIS spectrophotometry.

PDAM impregnated poly(acrylate) was used to construct three separate traps. To identify the peaks resulting from PDAM, one trap was connected to the GC in analysis mode. It was then fired, and a chromatogram obtained following injection. The two remaining traps were loaded with headspace from a 100 ppm standard of butyric acid, that was 0.1 M in HCl(aq) and 5 g/L in KCl(aq), and allowed to react for 30 minutes with their ends sealed with tape. After the 30-minute reaction time, one trap was fired, and two chromatograms were obtained. The other trap was not fired. Both traps that had been loaded with sample were then dissected, and extractions in acetonitrile were

performed on the removed sorbent material, followed by UV/VIS spectrophotometry of the extractions.

e.) Sampling Time

An experiment was conducted to determine the optimum sampling time for the headspace of the standards. A graphitized carbon multibed sorbent trap was mounted onto the instrument, and five separate 100 ppm acetic acid solutions of 0.1 M in HCl(aq) and 5 g/L in KCl(aq) were volumetrically prepared. A 1 mL aliquot of each solution was transferred to a GC vial and allowed to equilibrate for one hour. The trap was loaded for a pre-determined sampling time and then placed in a 50 mL beaker containing water at 100 ˚C for 30 s. Each solution was individually sampled for 30, 40, 50, 60, and 70 seconds. The log of each peak area was plotted as a function of time.

f.) Tests of Linearity

An experiment was conducted to see if the instrument utilizing the sorbent traps could produce peak areas that would linearly increase as a function of concentration. For the first test, five solutions of acetic acid ranging from 0.1 to 1,000 ppb in 0.1 M HCl(aq) and 5 g/L KCl(aq) were used. The solutions were allowed to equilibrate for one hour. Five separate single-bed traps made with PDAM impregnated poly(acrylate) were loaded with the acetic acid using a 70 s sampling time and then allowed to sit for 2 hours with their ends sealed with tape. Chromatograms were obtained using a 1.0 mL flowrate with a J & W brand, 0.25 μm (50% phenyl)-methylpolysiloxane, 15 m long, and 0.25 mm inner diameter column. The oven temperature was 80 ˚C. On test completion, the traps that

produced quality signals were dissected and extractions performed in acetonitrile in order to perform UV/VIS spectrophotometry to confirm that derivatization did take place. A second test was conducted under the same conditions, using butyric acid at concentrations ranging from 0.01 to 100 ppb. Once all acetic and butyric acid traps were fired and chromatograms obtained, peak areas were plotted as a function of SCFA concentration.

Calibration was performed to determine the detection limit of the of the Perkin Elmer Lambda 20 brand multichannel spectrophotometer. A reaction mixture of PDAM and acetic acid was prepared to yield a 10 ppb solution of PDAM/acetic acid derivative. The solvent used was acetonitrile, and the mixture was prepared and allowed to sit for 24 hrs in a red-tinted volumetric flask. The 10 ppb solution was used to volumetrically prepare four additional solutions of the derivative in 1, 0.1, 0.01, and 0.001 ppb concentrations in acetontitrile. Finally, UV/VIS spectra were obtained for all five solutions by scanning from 700 to 200 nm at a 60 nm/minute scanning rate. A calibration curve was prepared, plotting the absorbance of each solution as a function of the derivative concentration.

CHAPTER IV: RESULTS AND DISCUSSION

Statistical and other relevant mathematical equations are defined in the appendix.

a.) Trap Heating

To see what variac settings were necessary to achieve linear increase in temperature for desorption, trap temperature as a function of the voltage settings of power supplies 1 and 2 (PS1, PS2) was investigated. Numerical data are shown in Table 1a. The heating

curve in Figure 4 shows the trap temperature with respect to the voltage setting of PS1, the high voltage variac while the voltage of PS2 was held constant at 2V.

Table 1a: Voltage settings and initial and final measurements

PS2	PS1	Initial T (°C)	Final T (°C)
2	2	23.2	39.7
2	4	23.2	47.2
2	6	23.1	60.3
2	8	23.4	84.6
2	10	23.1	102.8
2	12	23.3	128.6
2	14	23.1	153.6
2	16	23.4	183.1
2	18	23.1	209.1
2	20	23.6	239.2
2	22	23.6	261.1
2	24	23.6	290.3

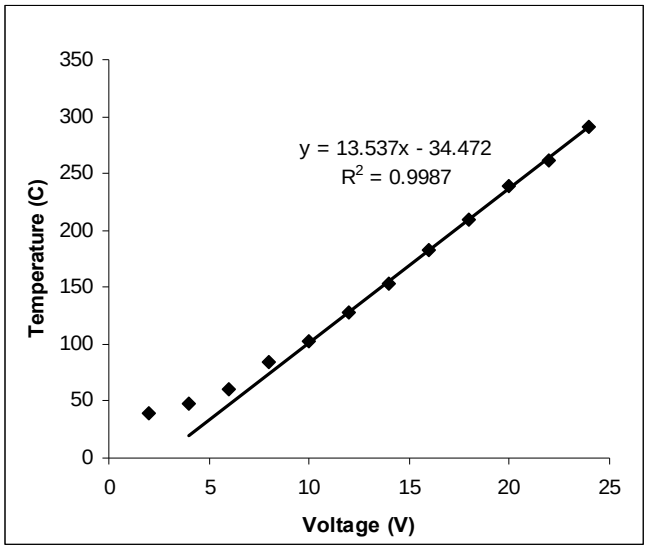

Figure 4: Trap temperature with respect to PS1 voltage

When linear regression with error analysis (as defined in the appendix) is applied to the data-range encompassing the minimum useful to the maximum attainable temperature without trap combustion (approximately 150 to 300 °C), the equation of the line is $y(\pm 2._9) = 13.5_{37}(\pm 0.3_{45})x - 34._{47}(\pm 6._{67})$. The slope (m) is 13.5 ◦C/V, with a standard deviation

(s_m) of $\pm$0.3 ˚C/V, the y-intercept is 34 ˚C with a standard deviation (s_b) of $\pm$7 ˚C, and the standard deviation of the y-values (s_y) is $\pm$3 ˚C. This plot with its representative equation shows that in this temperature range, for every 1 V increase in voltage of PS1, the resulting temperature should be within 3.81 ˚C of the theoretical temperature. Temperatures below 150 ˚C are generally not useful for thermal desorption of trapped components.

Table 1b: Data reflecting the relationship between variac settings and trap temperature

PS1	PS2	Initial T (˚C)	Final T (˚C)	t (s above 250 ˚C)
12	2	19.9	97	0
12	3	20.2	123.2	0
12	4	20.2	152.6	0
12	5	20.6	220.3	0
13	2	20.4	127.2	0
13	3	20.5	150.4	0
13	4	19.8	186.2	0
13	5	19.9	202.6	0
14	2	20.1	131.2	0
14	3	19.4	170.2	0
14	4	20.2	215.3	0
14	5	20.1	270.3	4
14	6	20	283.1	4

Table 1b contains additional data regarding the relationship between power supply voltage settings and trap temperature. The first consideration for quantitative desorption is the maximum temperature reached by the trap. The length of time for which the trap remains within 10 ˚C of the maximum temperature is also a significant criterion, as components often exhibit slow desorption. PS1 is used primarily to bring the trap to its maximum temperature, while PS2 is set at a lower voltage in order to maintain the maximum temperature. The data reflect that settings of PS1 at 14 V and PS2 at 5-6 V

result in a maximum temperature between 250 and 300 ˚C with 4 s hold time within 10 ˚C of the maximum.

b.) Free Fatty Acids Using Graphitized Carbon Traps

The instrument was successful in detecting a 100 and 200 ppb solution of isobutyric acid. The chromatograms are shown in Figure 5. The time settings were a sampling time of 45 s (0.75 minutes), an equilibration time of 0.05 minutes, and a high voltage signal for 0.35 minutes. Shortly after, a peak elutes. The peak areas increased from 9053 for 100 ppb (A) to 12,930 units for 200 ppb (B), indicating that analyte peaks are being exhibited.

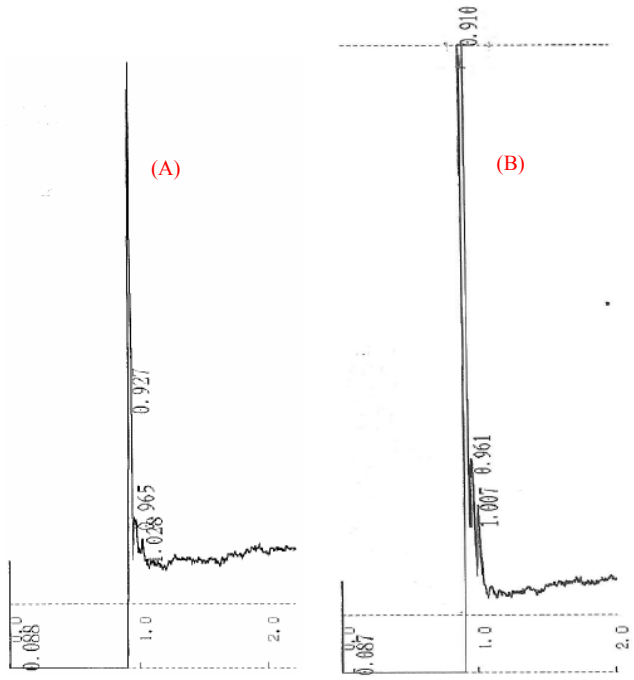

Figure 5: Chromatograms of 100 (A) and 200 (B) ppb isobutyric acid. The x-axis shows time in minutes, with 0 min corresponding to the beginning of sampling. The trap is fired for sample injection to the column at 0.90 minutes.

Tests in saturated head space successfully showed selectivity of the instrument for SCFAs. Figure 6 shows separation of acetic and propionic acids from saturated

headspace of the pure liquids. Peak A is the result of carryover from a prior loading that remained and was injected after sampling propionic acid. Tables 2 and 3 contain the data resulting from multiple injections of acetic and propionic acids. Acetic acid had an average retention time (t_R) of 1.444 minutes with a standard deviation of $\pm 0.04_4$ minutes (equations 2.1 and 5.1), and propionic acid had an average retention time of 2.01_2

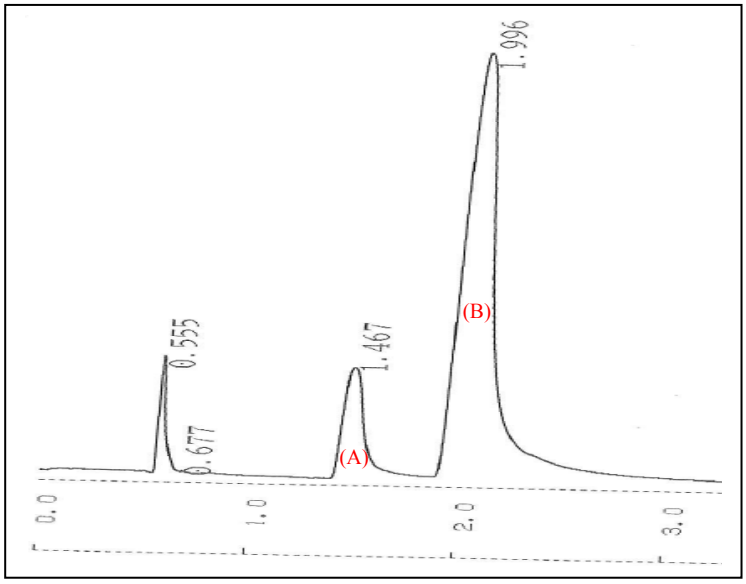

Figure 6: Chromatogram of acetic (A) and propionic (B) acids from saturated headspace of the pure liquids. The peak at 0.555 minutes is the result of organic vapors presently in the laboratory air.

Table 2: Retention times and peak areas for the headspace of pure acetic acid

Chromatogram	t_R (min.)	Peak Area
1	1.523	1274
2	1.423	284,904
3	1.429	323,682
4	1.423	349,656
5	1.425	343,166

Mean t_R (min.):	1.4446
Std. Dev. (min.):	0.0438953

Table 3: Retention times and peak areas for the headspace of pure propionic acid

Chromatogram	t_R (min.)	Peak Area
1	2.041	23,216
2	2.012	55,277
3	1.996	105,525
4	2.014	96,263
5	1.999	139,215
Mean t_R (min.):	2.0124	
Std. Dev. (min.):	0.0178129	

minutes with a standard deviation of $\pm 0.01_8$ minutes. The dead time (t_M, or hold-up time) used in calculating the retention factors (k) was 0.5 minutes, which is the mobile phase transport time. The retention factors (k) were 1.8892 (k_A) for the acetic acid and 3.028 (k_B) for the propionic acid. Both of these values are greater than one and different enough in magnitude to indicate that the two analytes are intermolecularly binding with the stationary phase of the GC column with sufficient differential to allow the detector to differentiate between the two analytes. The retention factors were then used to calculate the selectivity coefficients (α), which were 0.624 for the acetic acid and 1.603 for the propionic acid. Note that the initial peak areas for both acids are smaller than for subsequent runs, particularly for acetic acid. However, upon subsequent runs, the peak areas for the acetic acid seem to reach a consistent value, perhaps indicating trap saturation. For this qualitative experiment, run-to-run carryover on the trap was not considered, only selectivity. The potential indication of trap saturation highlights the necessity for subsequent studies of trap capacity. Table 4 contains data from chromatograms of 100 ppm butyric acid solution with an aqueous matrix containing 5 g/L in KCl. The purpose of treating the solution matrix with KCl was to promote more

fatty acid vapor into the headspace. *Pawlyszyn et al.*[21] state that sodium chloride (NaCl) was used to saturate the solution matrices in their study. In the present study, KCl was chosen because the potassium ion would have a larger ionic radius than the sodium ion, which would enable the potassium ions to attract more water molecules and leave the free fatty acid to volatilize and enter the headspace. The average retention time for the 100 ppm butyric acid in the KCl matrix was 1.91_8 minutes with a standard deviation of about $\pm 0.015_6$ minutes. The average peak area was about 230 units, with a standard deviation of about $\pm$29 units, and a % relative standard deviation (%RSD) of 13%. The chromatograms of butyric acid were obtained using a different trap than that used for the pure acetic and propionic acids; the retention time of butyric acid was very similar to that of the propionic acid (Table 3).

Table 4: Retention times and peak areas for 100 ppm butyric acid in a 5 g/L matrix

Chromatogram	t_R (min.)	Peak Area
1	1.928	213
2	1.926	303
3	1.922	245
4	1.912	245
5	1.91	241
6	1.895	240
7	1.919	212
8	1.904	217
9	1.949	212

Mean t_R (min.):	1.918333
Std dev (min.):	0.015676
Mean Peak Area:	230.2857
Std. dev:	29.0091

The overlap in retention times is likely due to the difference in carrier gas flow between the two traps. The flow though the trap will directly affect the retention time, because it can affect the amount of time needed for the butyric acid to reach the column where differential separation occurs. The retention time is directly proportional to the mobile phase velocity, thus flowrate. This can be shown by Equation 3 in the appendix.

The traps are made by hand and involve packing the steel wool, quartz wool, and graphitized carbon beds. Originally, the traps were constructed using fine steel mesh, as outlined by Sanchez and Sacks,[12,17] but this resulted in flow obstruction that in turn resulted in repeated malfunction of the 6-port valve repeatedly coming out of alignment. Later on, traps were constructed using super-fine steel wool that resulted in a differing number of porous holes of varying size in both of the steel wool ends, providing a more even gas flow with less restriction. With steel mesh or wool, not all sorbent traps can be constructed the same and have the same flow through them, resulting in varying flow of both carrier gas and analytes into and out of the trap. As a result, the peak areas may vary significantly from one run to the next due to the limit of the number of analyte molecules entering the trap during the sampling cycle, as well as leaving the trap during desorption to reach the column during the analysis cycle. In addition, if the carrier gas and analyte flow rate through the column will differ, so will the retention time.

According to Nimura *et al.*[18], SCFA derivatives of PDAM should yield absorbance maxima around 240 and 340 nm, as well as fluorescence excitation maxima around 270 and 340 nm. According to Schneede and Ueland,[19] such derivatives should yield fluorescence excitation maxima around 272, 324, and 340 nm. Because fluorescence

excitation maxima tend to be about the same as absorption maxima, ultraviolet/visible spectrophotometry was performed on acetonitrile extractions of the poly(acrylate). Pan and Pawliszyn[20] state that the SPME fibers used in their study were heated at 275 °C for 6-7 hours in helium. The purpose was to dry the fiber as well as drive off any possible interferants. For the present study, the poly(acrylate) that was later impregnated with PDAM was baked for about 8 hours at 210 °C to achieve the same purpose.

c.) Validation of Derivatization on Sorbent Material

The test of derivatization taking place on sorbent material shows that it is possible to derivatize SCFAs on sorbent material impregnated with PDAM. The UV/VIS spectra shown in Figures 7 and 8 contain the absorbance peaks that are indicative of PDAM derivatives of SCFAs. The spectrum resulting from the derivatization test with graphitized carbon and extraction with ethyl acetate (Figure 7) shows a very large peak at 241 nm, and small peaks at 275 and 341 nm. The spectrum from the test using poly(acrylate) and extraction with octane (Figure 8) shows 3 significant peaks at these wavelengths. It should be mentioned that the extraction for the graphitized carbon was done using ethyl acetate, and the extraction for the poly(acrylate) was done using octane. Pawliszyn et al.[21] suggest that the polar poly(acrylate) provides a medium in which the reaction can take place through stabilization of the transition states. Graphitized carbon, a nonpolar medium, does not provide the same stabilization effect. Thus, the size of the peaks in Figures 7 and 8 is likely due to the effectiveness of the sorbents at providing a reaction medium. Poly(acrylate) is a polymer containing polar functional groups with which analytes bind by way of intermolecular forces of varying strength. The

interactions between poly(acrylate) and analytes occur by way of absorption, in which analyte molecules partition into the poly(acrylate) so that they are surrounded by this polymer phase. The interactions between graphitized carbon and analytes occur by way of adsorption, or surface interactions. Poly(acrylate) was chosen as an alternative to the graphitized carbon for two reasons. First, the SPME fibers used in the on-fiber[20,21] technique were composed of poly(acrylate). Second, the UV/VIS spectra obtained from the extractions from the poly(acrylate) yielded the highest absorbance peaks. The test with poly(acrylate) produced larger, more significant peaks at 275 and 341 nm compared to the test on graphitized carbon.

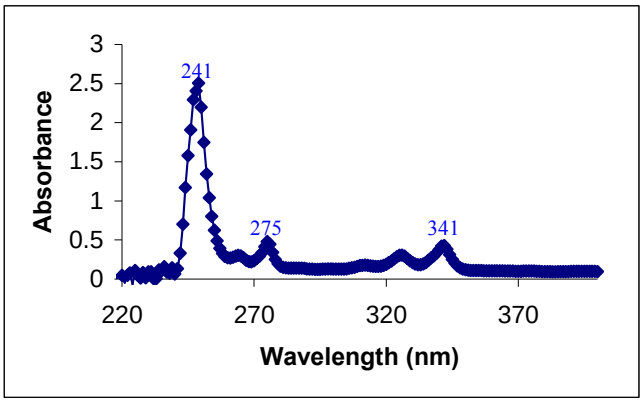

Figure 7: UV/VIS spectrum of the extraction of PDAM-derivatized fatty acids from a graphitized carbon trap using ethyl acetate

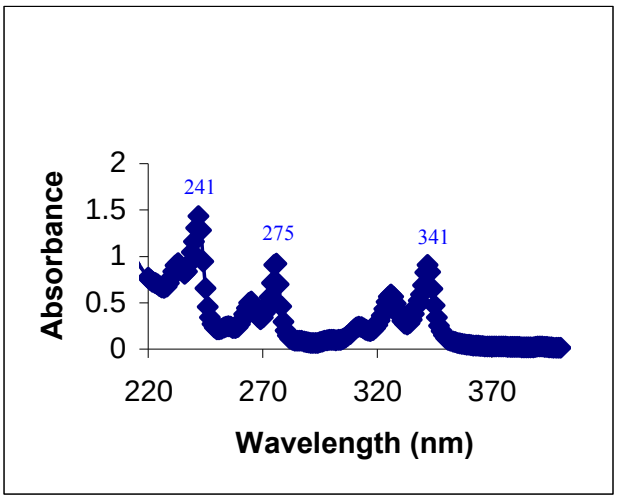

Figure 8: UV/VIS spectrum of the extraction of poly(acrylate) in octane

The chromatogram, shown in Figure 9 (a), was obtained from a PDAM-loaded poly(acrylate) trap prior to any sample loading. The resulting peak is very large (307,478 area units), with a retention time of 0.412 minutes. Chromatogram (b) was obtained from a second PDAM-loaded poly(acrylate) trap used to sample 100 ppm butyric acid. As in (a), the large peak attributed to PDAM with a retention time of 0.417 minutes appears though the area is approximately half of that obtained in the previous chromatogram (160,400 units). Because both traps were initially loaded with the same amount of PDAM, the significant decrease in FID response after derivatization on-trap indicates that less PDAM remained on the trap corresponding to chromatogram (b). The corresponding FID signal was 20 millivolts (mV). An FID signal is dependent on the mass of both carbon and hydrogen that is present. The more carbon and hydrogen present, the more intense the signal will be, as seen in the 0.563 peak. In derivatization of a SCFA, more carbon and hydrogen atoms are added to the molecule. Therefore, the peak at 0.563

minutes with an area and signal intensity approximately four times as great as that of the PDAM peak would suggest that derivatization has taken place.

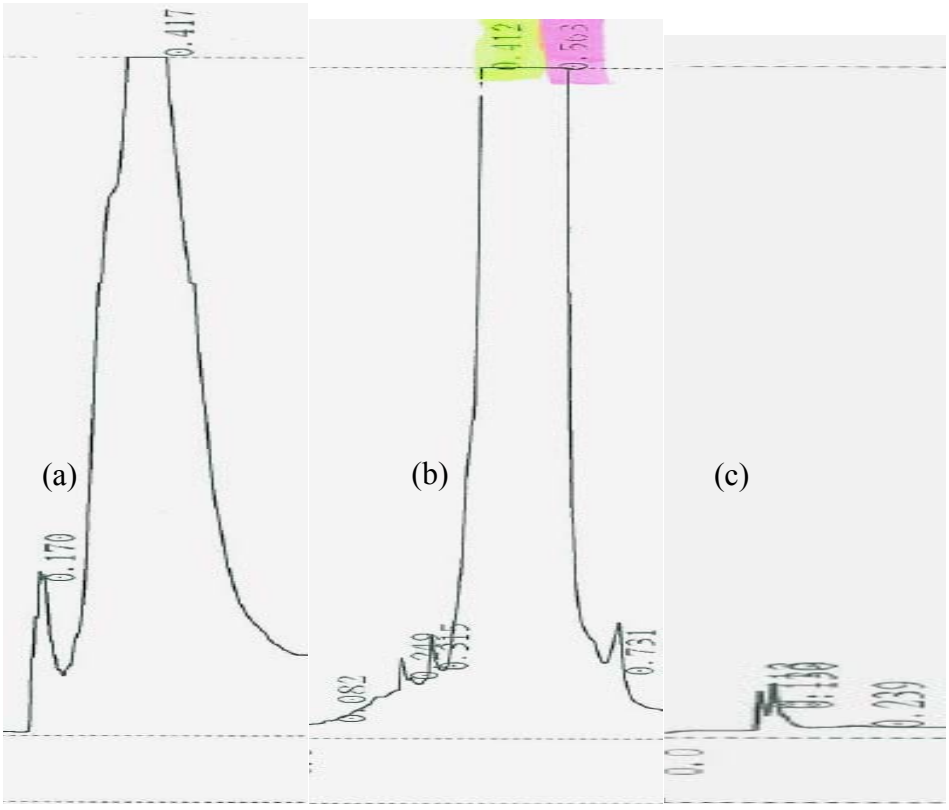

Figure 9: Chromatograms obtained from the dissections of three traps used in on-trap derivatization tests. The y-scale is adjusted in order to reveal the small response in chromatogram (c).

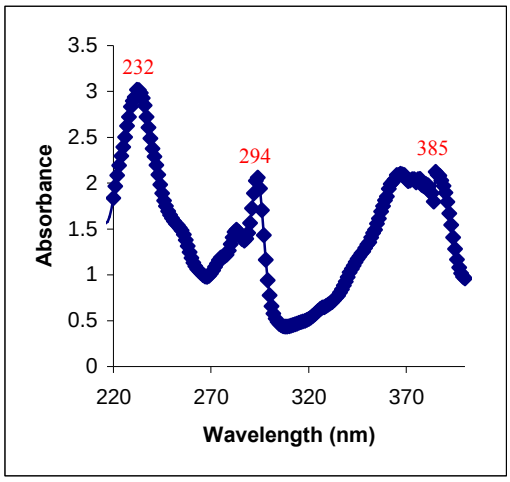

Figure 10: UV/VIS spectrum of PDAM in acetonitrile

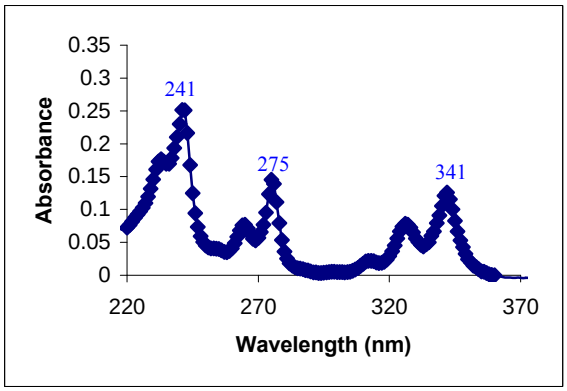

Figure 11: UV/VIS spectrum of the extraction of the fired trap

This is further suggested by the reduced peak area for PDAM. The chromatogram shown in Figure 9 (c) resulted from the same trap used in (b). The trap was not loaded with additional sample, but fired a second time to determine whether residual PDAM or derivative material was present. In the chromatogram, there are no peaks indicative of PDAM or the possible derivative.

Additional validation regarding on-trap derivatization was obtained using UV/VIS spectrophotometry. Figure 10 shows the spectrum of pure PDAM dissolved in

acetonitrile with characteristic absorbance peaks at 232, 293, and 385 nm. Figure 11 shows the spectrum resulting from an extraction of the trap used to collect the chromatograms in Figure 9 (b) and (c). There are absorbance peaks indicative of a PDAM/SCFA derivative being present although there was not a detectable signal from the FID (Figure 9 (b)). An amount of derivative not detectable by FID remained after the two consecutive firings. The smaller absorbance peak at 0.417 minutes is indicative of PDAM, which was likely present on the trap in excess of the butyric acid, and therefore was enough to produce a signal. The spectrum obtained from the extraction from the trap that was not fired shows absorbance peaks indicative of both PDAM and a PDAM/SCFA derivative, as shown in Figure 12. This indicates that SCFA derivatization can be achieved on a poly(acrylate) single bed sorbent trap.

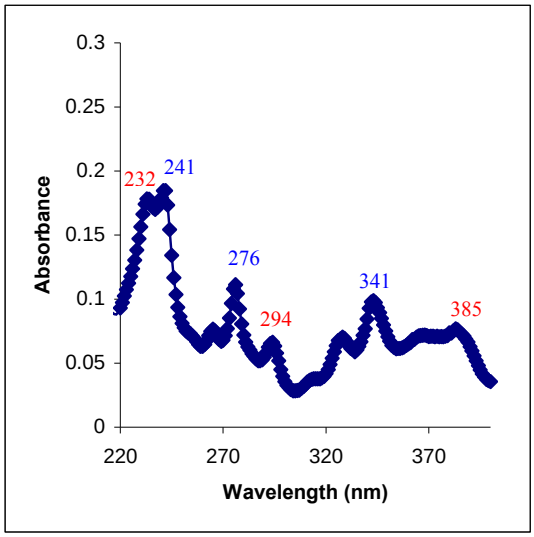

Figure 12: UV/VIS spectrum from the extraction of a non-fired trap.

d.) Sampling Time Experiment

The sampling time test indicates that 70 seconds was the optimal time to sample standards. Figure 13 and Table 5 contain data obtained in order to investigate the correlation between sampling time and peak area. Peak area appears to increase logarithmically with time for a given concentration of acetic acid. The tests for 30-, 40-, and 50-second measurements were taken sequentially under the same conditions, though a peak was not observed for the 60 s sampling time. The 70 s measurement was obtained earlier in the day to test flow conditions, with the same trap and under the same conditions. The plot has a correlation coefficient of 0.9985, which confirms its good linearity.

Table 5: Sampling time experiment

Chromatogram	Time (s)	t_R (min.)	Peak Area(A)	$Log_{10}(A)$
1	30	0.739	884	2.946452265
2	40	0.717	1797	3.254548077
3	50	0.693	4599	3.66266341
4	70	0.661	23067	4.362991116

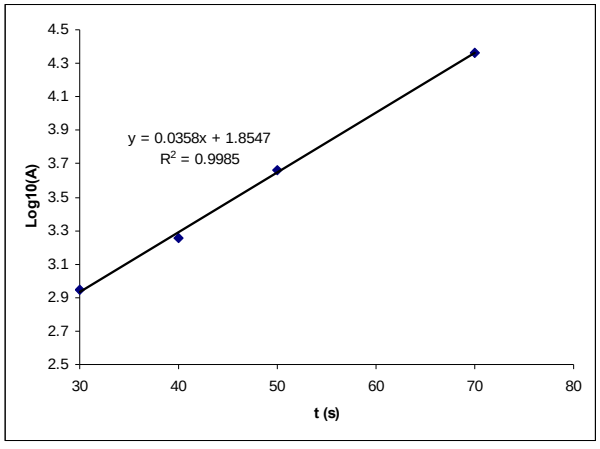

Figure 13: The plot resulting from the sampling time experiment using acetic acid

It would be expected that increasing sampling time over 70 s would result in further increase in peak areas (and correspondingly lower LOD) until reaching the saturation limit of the sorbent.

e.) Tests of Linearity

The test of linearity for acetic acid, as shown in Table 6 and Figure 14, resulted in a correlation coefficient of 0.9953, which suggests that a linear relationship may exist between the peak area and the concentration of derivative that forms within the trap. The solution matrices were spiked to give an HCl concentration of 0.1 M, which would keep the pH around 1 in order to keep the fatty acids in the solution in the protonated state.

Table 6: Data resulting from the acetic acid test for linearity

[AA], (ppb)	t_R (min.)	Peak Area
1000	0.332	740783
100	0.323	243997
10	0.315	148604

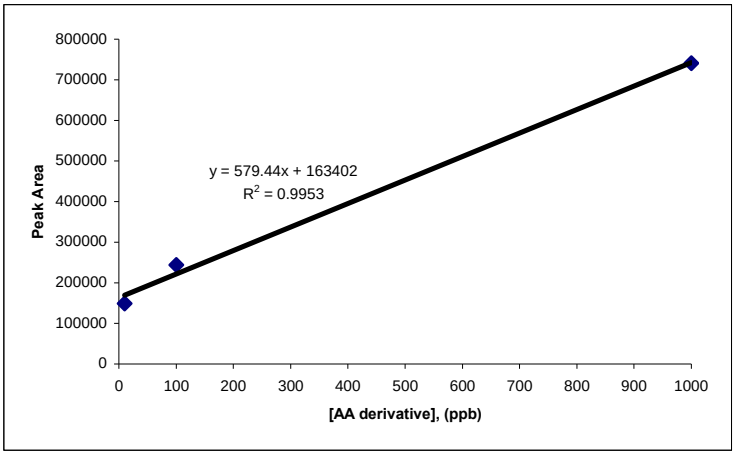

Figure 14: The plot resulting from the linearity test for acetic acid

UV/VIS spectra of the three acetonitrile extractions of the dissected traps are shown in Figures 15-17. Pawliszyn *et al.*[21] state that they used a pH of 1.5. No signals were

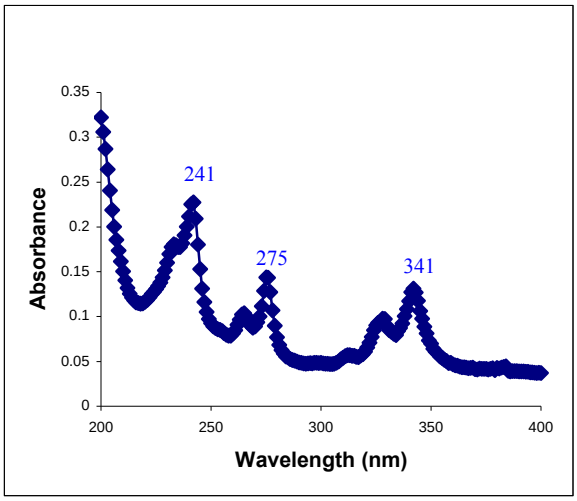

Figure 15: UV/VIS spectrum of the acetonitrile extraction of the dissected trap loaded with 10 ppb acetic acid solution

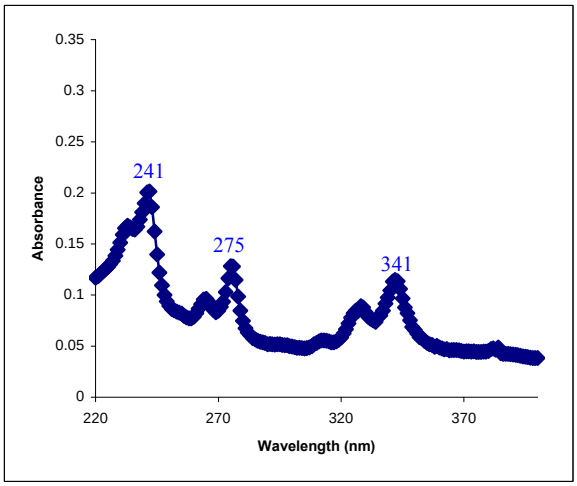

Figure 16: UV/VIS spectrum of the acetonitrile extraction of the dissected trap loaded with 100 ppb acetic acid solution

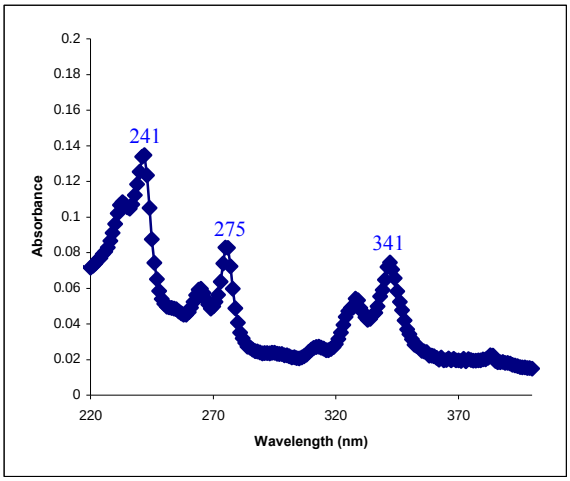

Figure 17: UV/VIS spectrum of the acetonitrile extraction of the dissected trap loaded with 1000 ppb acetic acid solution

obtained for 0.1 and 1 ppb. It is unclear whether this is due to the flow issues, as suggested earlier, or if these concentrations did not meet the LOD for the FID. The test with butyric acid had an interesting result. Signals were obtained for 0.01, 0.1, and 100 ppb, but not for 1 and 10 ppb. As shown in Figure 18, the peak area as a function of the natural log (ln) of the concentration of butyric acid derivative did show a linear relationship over two orders of magnitude, with a correlation coefficient of 0.9996. Peaks were obtained for concentrations of 0.01 and 0.1 ppb. The absence of the signals for 1 and 10 ppb could again be explained by flow issues through the trap. It should be noted that detection of the 0.01 ppb standard falls within part of the dynamic range for the on-fiber derivatization experiment conducted by Pan and Pawliszyn,[20] which gave a linear range of 0.005 to 5 μg/L (ppb) for butyric acid using ion trap mass spectrometry detection for the headspace analysis and utilized PDAM. For acetic acid, the group[20] obtained a linear range of 0.005 to 5 μg/L for ITMS detection. The lowest concentration of acetic acid derivative seen in these experiments was 10 ppb (10 μg/L), a value very

close to overlap. They also obtained 0.25 to 10 μg/L for direct SPME analysis without derivatization. This is important because it shows that FID detection, even in the absence of significant preconcentration, has the potential to compete with mass spectrometry detection in the low ppb range. A distinct advantage of the sorbent trap is the ability to extensively preconcentrate analytes. The peak areas obtained in the linearity studies could have been increased with longer sampling times. PDAM interfering with the PDAM/SCFA derivative signal peaks was not an issue in these calibration tests because as a precaution the poly(acrylate) was treated with a 1 mg/mL PDAM in diethyl ether solution. This was just enough PDAM to derivatize the SCFA that reached the trap, but not enough to interfere with the derivative signal peaks. This was the PDAM concentration used by two of the research groups[20,21] to treat the SPME fibers for the on-fiber derivatization.

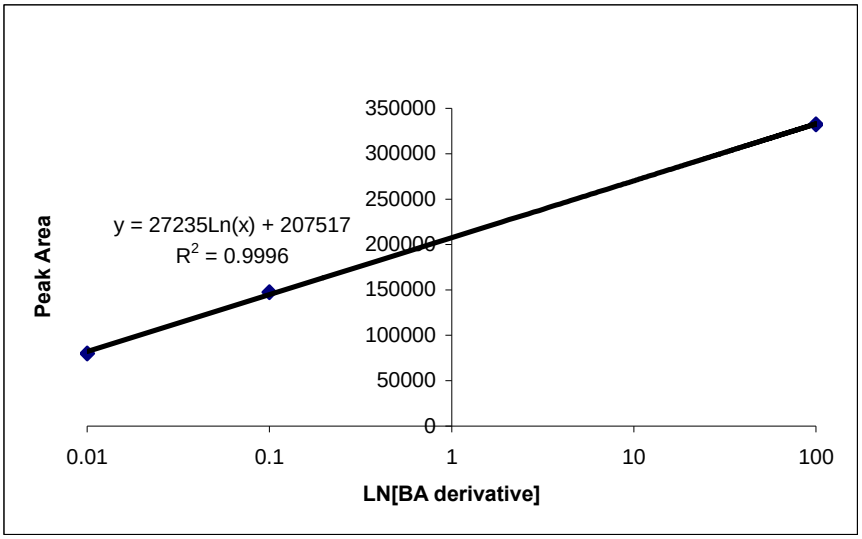

Figure 18: Plot resulting from the linearity test for butyric acid

Table 7: Data resulting from the butyric acid test for linearity

[BA], (ppb)	t_R (min.)	Peak Area
0.01	0.533	79970
0.1	0.542	147640
100	0.583	332231

The standard curve that was made to confirm that the spectrophotometer was able to detect PDAM/SCFA derivatives yielded a dynamic range of 0.085 ppb to at least 10 ppb. The data used to produce the curve in Figure 19 are shown in Table 8. This curve is also known as a Beer's Law Plot. The LOD was 0.02 ppb and the LOQ was 0.085 ppb. The correlation coefficient was exactly 1, and the standard deviations were low because the solution matrix did not produce a very high noise. The standard deviation of the blank (s_{blank}) was approximated as the standard deviation of the y-intercept because that is where the analyte concentration was zero. The standard deviations were ±0.00158 for the signal (s_y), ±0.00018 for the slope (s_m), and ±0.00080 for the y-intercept (s_b).

Table 8: Calibration data from the Lambda 20 UV/VIS spectrophotometer

λ (nm)	[AA Derivative], (ppb)	Absorbance
350	0.001	0.00096
347	0.01	0.00143
345	0.1	0.01142
345	1	0.09784
345	10	0.94023

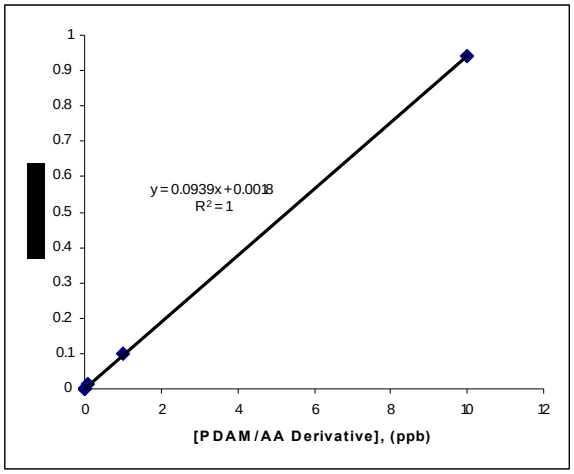

Figure 19: Beer's Law plot of PDAM/butyric acid derivative

f.) PDAM Stability

What made this project especially interesting is that more knowledge of the stability of PDAM in poly(acrylate) was gained, particularly after obtaining the results of the linearity tests for acetic and butyric acids. The tests were attempted only if the morning extractions of the PDAM impregnated poly(acrylate) showed absolutely no absorbance peaks indicative of a PDAM/SCFA derivative. The impregnated poly(acrylate) had to be absolutely pure. The PDAM is highly reactive and will react with the SCFAs produced by bacteria that get into the storage vial either during the treatment of the polyacrylate or from air that can diffuse in during storage. At the time of these two linearity tests, the UV/VIS spectra of the daily extractions of the poly(acrylate) did not show any signs of the derivatives for at least a week. Figure 20 shows the spectrum from an extraction of PDAM impregnated poly(acrylate) that was at least one week old, although some lasted

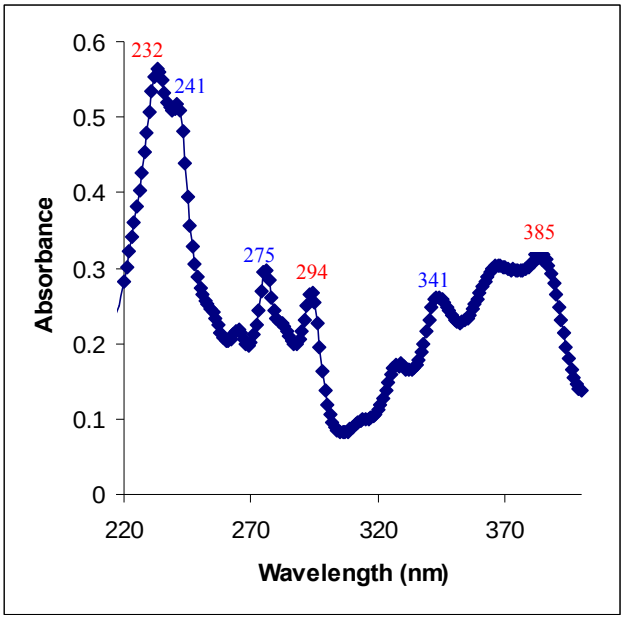

Figure 20: UV/VIS spectrum of the acetonitrile extraction of PDAM impregnated poly(acrylate) 10 days after preparation

as long as ten days. This spectrum shows the absorbance peaks indicative of both PDAM and the PDAM/SCFA derivative. Because this material had not been used, the presence of derivative peaks indicates a reaction with fatty acids in the ambient surroundings. However, as the weather became warmer and more humid, the impregnated poly(acrylate) could not last 24 hours after preparation, as shown in Figure 21. It is interesting that Nimura *et al.*[18] state that they were able to keep PDAM in solid phase stable at room temperature for 20 days. But as a solution in ethyl acetate, it was stable for only 48 hours at room temperature, and at least one week at -20 ˚C. Schneede and Ueland[19] state that they were able to keep the PDAM stable in ethyl acetate for 14 days when they stored it at -20 ˚C. The temperature in the laboratory of the present study was about 25 ˚C (or 77 ˚F) throughout the time that the linearity tests were conducted, but the

air became warmer and more humid shortly after, leading to spoilage of the PDAM with ambient acids.

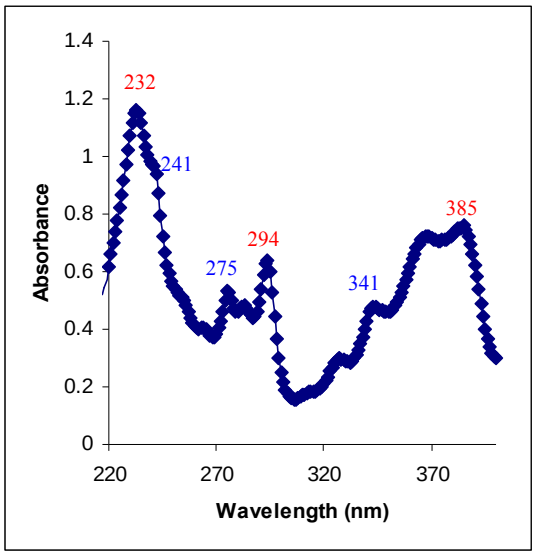

Figure 21: UV/VIS spectrum of the acetonitrile extraction of PDAM impregnated poly(acrylate) 24 hours after preparation

The warmer, humid air promotes bacterial growth. Therefore, the warmer and more humid the laboratory conditions, the faster the rate of spoilage of the impregnated poly(acrylate) because more bacteria grows which can produce more SCFAs. This would account for the spoilage of PDAM impregnated poly(acrylate) shortly after the linearity tests and should be considered whenever using PDAM.

CHAPTER V: CONCLUSIONS

Pre-column concentration and derivatization techniques have been successful in the gas-chromatographic analysis of VOCs, especially SCFAs. SCFAs are important VOCs because they are bacterial biomarkers which can be used in monitoring disease state. SPME, on-fiber derivatization with SPME, purge and trap, on-sorbent derivatization with

thermal desorption, and multibed-sorbent traps have been especially useful in achieving low ppb level detection of VOCs.

This project was successful in two areas. First, derivatization and detection of acetic and butyric acids in the low ppb range was achieved on a single-bed PDAM impregnated poly(acrylate) sorbent trap using GC with flame ionization detection. In addition, the lower concentrations used in the tests of linearity for the acetic and butyric acids were competitive with those obtained by SPME on-fiber derivatization with ion-trap mass spectrometry detection. The concentration range for butyric acid used in this study (0.01 through 100 ppb) overlapped part of the dynamic range that was achieved by Pan and Pawliszyn.[20] This is important because preconcentration and derivatization of the SCFA analytes by the sorbent trap could be increased for future work, thus providing lower LODs than represented in the present study. The present study revealed that traps have flow issues due to manual construction, resulting in differences in the retention time and peak area for a particular species not only from run to run but also from trap to trap. This significantly affects the precision when performing gas chromatographic analysis. To address this issue, research is currently underway involving the feasibility of polymer frits for use with the traps. Following characterization of the system, SCFAs in biological samples will be attempted using the standard addition method with an internal standard and compared to the LODs, LOQs, and the dynamic ranges reported in the chemical literature. Flame ionization detectors are less expensive than mass spectrometry detectors. If the same level of detection can be achieved using derivatizing agent impregnated traps that can be achieved using a mass spectrometry detector, it would

enable the analysis of biological samples for not only SCFAs, but also other VOCs that exist in such samples that could be used as biomarkers for disease.

The project's second success area is that it provided information on the stability of PDAM when it is impregnated into poly(acrylate). It was determined that the PDAM impregnated poly(acrylate) will react with the SCFAs from bacteria in the air. The warmer and more humid the laboratory conditions, the faster the impregnated PDAM spoils.

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APPENDIX

Mathematical equations and explanations:

1. Linear regression and error analysis was conducted using the 2003 version of Microsoft Excel to make the following table for a data set:

x_i	y_i	y	$(x_i)^2$	d_i	$(d_i)^2$

x_i = the value of an independent variable, which in the present study was voltage, time, and concentration.

y_i = the value of the dependent variable, such as temperature, retention time, peak area, and the log of peak area. In other words, an individual measurement.

y = the theoretical value according to the point-slope equation of the line computed by the Microsoft Excel program

deviation $(d_i)=(y_i-y)$

2.) General statistics

2.1: Average $= (y_1 + y_2 + \ldots + y_n)/n$
n = the number of individual measurements (y_i) in the data set

2.2: $\Sigma x_i = (x_1 + x_2 + x_3 + \ldots + x_n)$

2.3: $\Sigma x_i^2 = (x_1^2 + x_2^2 + x_3^2 + \ldots + x_n^2)$

2.4: $(\Sigma x_i)^2 = (x_1 + x_2 + x_3 + \ldots + x_n)^2$

2.5: $\Sigma d_i^2 = (d_1 + d_2 + d_3 + \ldots + d_n)$

2.6: D = a denominator used in equations 5.1, 5.2, and 5.3

$D = n\Sigma x_i^2 - (\Sigma x_i)^2$

3.) Retention factor (k)

$k = (t_R - t_m)/t_m$

t_R = retention time of analyte

t_m = mobile phase transport time (or hold-up time)

4.) Selectivity Coefficient (α)

$\alpha = k_B/k_A$

k_A = retention factor for first compound to elute

k_B = retention factor for the longer retained compound

5.) Standard deviation equations

5.1: Standard deviation of an individual data set (sy):

$s_y=\sqrt{((\Sigma d_i^2)/(n-1))}$

5.2: Standard deviation of the calculated slope (m) of an equation of a line (s_m):

$s_m = s_y\sqrt{(n/D)}$

5.3: Standard deviation of the calculated y-intercept (b) of a line (s_b):

$s_b = s_y\sqrt{((\Sigma x_i^2)/(D))}$

6.) % relative standard deviation for a individual data set (%RSD)

$\%RSD = (s_y/Average)\times 100$

7.) Minimal detectable signal (MDS), LOD, and LOQ

LOD = limit of detection

LOQ = limit of quantitation

7.1: $MDS = (S_{Blank} + 3s_y)$

7.2: $LOQ = 3s_y/m$, where "m" is the slope of the equation of the line.

7.3: $LOQ = (10s_y)$