STRATEGIES FOR THE ANALYSIS OF POLAR ANALYTES USING SOLID-PHASE MICROEXTRACTION

by

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ABSTRACT

In this thesis, new SPME and derivatization/SPME techniques were developed for the determination of two challenging groups of analytes, fatty acids (C_2-C_{22} acids) and amines (C_1-C_6 amines), in gaseous and aqueous matrices. The studies illustrated that derivatization coupled with SPME is a versatile technique that can be used for the selective and sensitive determination of fatty acids and amines in aqueous and gaseous matrices. Derivatization/SPME techniques were performed in three ways: derivatization in the sample matrix, derivatization in the SPME fiber coating, and derivatization in the GC injector port. Derivatization in sample matrices increased coating/gas and coating/water partition coefficients of analytes, hence increased the SPME extraction efficiency and method sensitivity. Derivatization in the SPME fiber coating showed potential to quantitatively derivatize and extract polar analytes from sample; therefore could be used as an integrating sampler. In-fiber derivatization and in-injector port derivatization decreased the polarity of target analytes, thus improving their GC separation, detection and quantitation.

Direct SPME was applied for the analysis of C_2-C_{10} fatty acids and amines in aqueous solutions. Polar fiber coatings, carbowax divinylbenzene [CAX(DVB)] and poly(acrylate) (PA), extracted polar analytes, C_2-C_{10} fatty acids and C_1-C_6 amines, in larger amounts than the non-polar poly(dimethylsiloxane) (PDMS) coating. For the analysis of C_2-C_{10} fatty acids, the addition of salt and a strong mineral acid to the matrix greatly enhanced the amounts of these fatty acids extracted into the fiber coatings. Under saturated salt at pH 1.5 conditions, direct SPME had limits of detection (LODs) of pg/mL for the analysis of less polar C_6-C_{10} fatty acids, and LODs in low ng/mL to the high ng/mL levels for more polar C_2-C_5 fatty acids, when GC/FID was used. For the analysis of amines in water, saturated salt at pH 10 conditions were used and the method had an LOD of μg/mL, while GC/FID was employed.

Headspace SPME was also applied for the analysis of C_2-C_{10} fatty acids in water and the results were similar to those with direct SPME.

Direct SPME was employed for the analysis of long-chain (C_{12}-C_{22}) fatty acids in aqueous solutions. The PDMS fiber coating was shown to extract these acids in higher amounts than the

polar PA coating. The optimum extraction efficiency was obtained when neutral matrix conditions were used.

Direct SPME was also used to extract volatile C_2-C_5 fatty acids and C_1-C_6 amines from air. The methods had LODs in the mid- to the high pg/mL levels using GC/FID.

Trace analysis of fatty acids and amines with high polarity or low volatility, in aqueous and gaseous matrices, was significantly improved when derivatization coupled with SPME was applied. For the analysis of volatile analytes in aqueous solutions, SPME/derivatization provided LODs ranging from low ng/mL to high pg/mL levels for C_2-C_5 fatty acids when GC/ECD was utilized, and low ng/mL to pg/mL levels for C_1-C_6 primary amines when GC/FID was employed. For air analysis, quantitative derivatization and extraction of C_2-C_5 fatty acids in the SPME fiber coating were achieved with LODs in fg/mL range using GC/ITMS. In-matrix derivatization of volatile amines in air was successful with LODs in low pg/mL level using GC/FID.

The kinetics of in-fiber derivatization for the analysis of C_2-C_5 fatty acids in air was theoretically modelled and the results from the experimental data were consistent with the theoretical estimates.

Derivatization of long-chain fatty acids in the SPME fiber coating as well as in the GC injector was found to considerably improve GC separation, detection and quantitation of these acids. These techniques presented a convenient way to improve the GC behaviour of long-chain fatty acids.

Potential applications of the derivatization/SPME techniques were demonstrated by analyzing a number of real samples.

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To

Jason and my family

ABBREVIATIONS

BSA	N,O-bis(trimethylsilyl)acetamide
BSTFA	N,O-bis-(trimethylsilyl)trifluoroacetamide
BTEX	Abbreviation for benzene, toluene, ethylbenzene, and three xylene isomers: *m*-xylene, *o*-xylene, and *p*-xylene
CAX(DVB)	Carbowax poly(divinylbenzene)
ECD	Electron capture detector
EPA	Environmental Protection Agency of the United States of America
FFA	Free fatty acid
FID	Flame ionization detector
GC	Gas chromatography, or gas chromatograph
HPLC	High performance liquid chromatography
ITMS	Ion trap mass spectrometer
LLE	Liquid-liquid extraction
LOD	Limit of detection
MNNG	1-Methyl-3-nitro-1-nitrosoguanidine
MS	Mass spectrometry or mass spectrometer
NPTFA	4-Nitrophenyl trifluoroacetate
NQS	1,2-Naphthoquinone-4-sulfonate
PA	Poly(acrylate)
PDAM	Pyrenyldiazomethane
PDMS	Poly(dimethylsiloxane)
PFBBr	Pentafluorobenzyl bromide
PFPDE	Pentafluorophenyldiazoethane
PFBAY	Pentafluorobenzylaldehyde
RSD	Relative standard deviation
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction

SPI	Septum equipped temperature programmable injector, used in Varian GCs
SPME	Solid-phase microextraction
TMAOH	Tetramethylammonium hydroxide
$TMAHSO_4$	Tetramethylammonium hydrogen sulphate
TMS	Trimethylsilyl
VOC	Volatile organic compound

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 General Considerations

An analytical method usually consists of several steps including sampling, sample preparation, separation, identification, quantitation and statistical evaluation. Each step is critical in obtaining an accurate and reproducible analytical results; however, sampling and sample preparation are often the steps where real problems begin. Errors or faults in the sampling protocol and preparation process cannot be corrected at any later point in the analysis, even with the most advanced methods and instrumentation.[1] In a sampling step, obtaining representative samples and sample transportation and storage can have significant impact on the final results of sample analysis. Sample preparation is often necessary to separate the analytes of interest from the sample matrix. In addition, sample preparation is needed to bring the analytes of interest into a suitable concentration and purity that are amenable to chromatographic analysis, since most of sample mixtures obtained from biological or environmental sources are too complex, too dilute, or are incompatible with the chromatographic system to permit analysis by direct injection. To date, with powerful analytical instruments such as gas chromatography/mass spectrometry (GC/MS) and with the aid of laboratory robots, the separation of sample mixtures, the identification of sample components, and the collection of quantitative data information are more rapid and efficient.[2] The incorporation of microprocessor control into analytical instruments and their interfacing with micro- and minicomputers has also brought improved instrument control, performance and easier routine maintenance.

Trace analysis of organic compounds in different sample matrices is often required. Usually, apolar organic compounds can easily be extracted from a sample matrix into an organic solvent and subsequently analysed by sensitive methods such as gas chromatography (GC).[3] However, the determination of relatively polar analytes, e.g. low molecular weight carboxylic acids and organic amines, by GC is rather difficult. This is because of their low extractablity into an organic phase, owing to their high solubility in water. Therefore, efficient methods are continually being sought. It is worth noting that the focus of research and development in trace analysis appears to have turned from analytical instrumentation to sample preparation.[4]

This thesis is concerned with the development of new strategies for the sample preparation of polar and/or less volatile analytes, particularly fatty acids and organic amines, for analytical purposes. Prior to discussing the objectives, it is appropriate to review, admittedly in a non-comprehensive fashion, the importance of the analysis of fatty acids and organic amines, conventional techniques of sample preparation for these organic compounds, techniques for the generation of gaseous samples, and the derivatization techniques used to derivatize fatty acids and amines for GC analysis. Given the enormous number of references in the literature, no attempt to be comprehensive will be made.

1.2 Analysis of Fatty Acids

The determination of fatty acids in water and biological matrices is becoming increasingly important in several fields such as the physiology and taxonomy of micro-organisms,[5,6,7] clinical chemistry,[8] dairy,[9] food and beverage[10,11,12] and environmental industries.[13,14] This has led to extensive method development for their measurement, the importance of which is reflected by the estimated 25% of GC publications devoted to these compounds.[15] Recently, a few review articles on the analysis of long-chain fatty acids,[16,17,18] fatty acids in lipid samples,[19,20] and physiologically important carboxylic acids[21] have been published.

Short-chain fatty acids are intermediates in many biological processes.[22] Evidence suggests that these acids might have beneficial physiological effects.[23] They may influence the differentiation and growth of colonic epithelial cells.[24] They play important roles in biological tissues. For example, fatty acids as constituents of lipids in biological membranes influence membrane properties such as fluidity, integrity, permeability, and the activities of membrane-bound enzymes.[25,26] Fatty acids as components of food serve as a source of energy for man and animals. The type of fatty acids consumed has important implications for human health, especially with respect to concentrations of serum lipids and the risk of coronary heart disease.[27,28]

The traditional U.S. Environmental Protection Agency (EPA) priority pollutant methods for organic constituents virtually ignore a sample's carboxylic acids content.[13] However, carboxylic acids can make up the majority of organic constituents present in water samples from

municipal landfills and their monitoring wells because they are produced by the bacterial activity that occurs in landfills.[29] Detection of carboxylic acids should therefore allow identification of a significantly larger fraction of the organic carbon.[30] In addition, short-chain fatty acids are often produced from humic substances during water treatment processes. These acids may play an important role as corrosive agents in water distribution nets, steam generation processes, etc.[31]

1.3 Analysis of Organic Amines

The determination of organic amines present in various liquids is an important problem. These compounds can be found in biological fluids,[32,33] environmental samples,[34] and industrial process streams, usually in aqueous solutions and often at trace levels.

Low molecular weight amines are important air pollutants due to their odorous and toxic characteristic.[35] Aliphatic amines are common components of biological systems and their accurate measurement is often a requirement for characterizing biological processes and for clinical purposes. For example, elevated urinary levels of aliphatic diamines have been associated with certain carcinomas.[36] The determination of amine drugs, such as amphetamines, in biological fluids, is also very important to toxicological and forensic fields, due to their abusive characteristic.[37]

1.4 Conventional Techniques and their Limitations for Extraction of Fatty Acids and Amines

In conventional methods, several extraction techniques such as liquid-liquid extraction, supercritical fluid extraction, solid-phase extraction and headspace extraction are often used to isolate fatty acids and amines from sample matrices into a gaseous phase or an organic phase prior to GC analysis. These techniques were extensively reviewed by Poole *et al.*[1] and Namiesnik *et al.*[38]

1.4.1 Liquid-Liquid Extraction

Liquid-liquid extraction (LLE) is one of the most popular methods used to isolate fatty acids[12,27,35,39,40,41] and organic amines[3] from the aqueous matrices. Partitioning of organic compounds into a suitable organic solvent from an aqueous mixture has long been known as a means of removing compounds from water. The partitioning efficiency of an extracting solvent depends primarily on the affinity of the solute towards the extracting solvent, K, and the number of extraction steps, n. For acidic and basic analytes, the values of K may be made more favourable by adjusting pH to prevent ionization of acids and bases, by forming ion pairs with ionizable solutes,[38] by forming hydrophobic complexes with metal ions, or by adding neutral salts to the aqueous phase to diminish the solubility of the analytes,[42,43,44] thus, it will be easier to isolate them into the organic phase.

Although LLE is simple and does not require complex equipment, it suffers from many practical problems. The formation of emulsions can be a problem if they cannot be readily broken up by conventional techniques, such as filtration, centrifugation and salting out. The other big problem of LLE is its non-selective characteristic. Since polar analytes have a high affinity towards water, fatty acids and amines are not effectively extracted in very low concentrations from water with LLE technique. Usually, it is necessary to repeat the extraction procedure a few times to ensure quantitative recovery, which is time consuming and tedious, so the compounds are diluted and further enrichment of the compounds is needed. Therefore, the solvent is usually evaporated under a gentle nitrogen stream. However, volatile substances like short-chain fatty acids or low molecular weight amines would be lost, hence another concentration procedure has to be found. Since relatively large solvent volumes are used in most extraction procedures, solvent impurities, contamination, etc., concentrated along with the sample during these sample enrichment steps, are always a common cause for concern.[45,46] In addition, using large volumes of high purity solvents may impose significant disposal costs associated with their use. Recent awareness of the pollution and hazard caused by halogenated solvents, including ozone depletion, has resulted in international initiatives to eliminate the production and the use of such organic solvents. The phasing out of organic solvents is poised to include a major change in analytical methodology in the near future.[47]

1.4.2 Supercritical Fluid extraction

Supercritical fluid extraction (SFE) was first introduced more than 40 years ago.[48] Only in recent years, has there been tremendous interest in this technique.[49] It is often used to isolate target analytes from solid matrices. Recently, a few applications on the analysis of fatty acid,[50,51,52,53] and amines[54] by SFE have also appeared.

The major advantages of SFE include low toxicity of supercritical fluids (one of the most often used is carbon dioxide), their high chemical inertness, selectivity of the process achievable by varying the fluid pressure, enhanced mass transfer of solutes because of low viscosity of fluids, and easy separation of the solute from the mixture by lowering the pressure. The diffusivities of supercritical fluids are much greater than those of liquids, but their viscosities are similar to those of gases. These properties frequently result in higher extraction efficiencies in SFE than obtained typically from LLE. In practice, the choice of the supercritical fluid depends on the polarity of the analytes, the solvent strength and selectivity required, the thermal stability of the analytes at the operating temperatures, and the instrumental limitations.[55] In food and clinical industries, the non-toxic properties of supercritical carbon dioxide and low temperatures have been recognized.[56]

SFE also has some disadvantages such as poor extraction of trace levels of analytes from neutral samples, and the safety concerns due to the requirement of high pressure operating conditions. SFE equipment is not portable and relatively expensive compared to other techniques. SFE has limited applications towards polar analytes since most of the supercritical fluids are relatively non-polar. Although the extraction of polar compounds can be enhanced by the addition of a polar solvent (e.g. acetonitrile, methanol or water) or a mixture of polar solvents as a modifier, the amount that can be used is limited. Also, adding a modifier will change the critical properties of the mixture.[53]

1.4.3 Solid-Phase Extraction

Solid-phase extraction (SPE) is also one of the most frequently used techniques for the isolation of fatty acids[57] and amines[33] from aqueous matrices. This technique was introduced in

the mid-1970s.[58,59] Using small cartridges or disks filled with sorbents of a small size packing material, SPE has been rapidly established as an important sample preparation technique for either matrix simplification or trace enrichment.[60,61] Compared to LLE, the most important feature of SPE is the concentration enrichment of the analytes by the sorbent. This technique also has the advantages of lower costs, reduced processing times, substantial solvent savings, flexibility in choosing mobile phases, ability to adsorb a wide variety of organic compounds, and simpler processing procedures. It can be automated and used in the field. It has been widely used for trace enrichment of very dilute solutions, such as natural water, where large sample volumes may have to be processed to yield concentrations of analytes sufficient for convenient detection. The efficiency of this technique depends on the relative importance of the analyte-matrix, sorbent-matrix and analyte-sorbent interactions. For effective sorption, the analyte-matrix and sorbent-matrix interactions should be weak and the analyte-sorbent interactions strong. By selecting the correct sorbent, semivolatile and non-volatile ionic species can be recovered in high yield from sample matrices. Because of the nature of the manipulation and sampling steps of SPE, the recovery of volatiles is generally poor. Desorption of accumulated organic compounds can be carried out by elution with a suitable solvent or solvent mixture, or by increasing temperature (thermal desorption).

One of the most applied SPE techniques for the analysis of carboxylic acids[9,13,62,63,64] and amines[65] is ion-exchange resin extraction. Ion-exchange resins can be used for the efficient isolation and concentration of ionic substances in the presence of complex matrices, e.g. biological fluids. Usually silica-based and macroreticular polymeric ion-exchangers are used for the isolation of low molecular weight carboxylic acids and organic amines. Sorption of basic compounds is done at low pH values, and the sorbed ions are subsequently desorbed at high pH values by a mixture of organic solvents with a suitable buffer. Acidic compounds are sorbed at high pH values and desorbed by acid-modified organic solvents.

Problems encountered when using SPE for analytical work are related to large variations in the physical and chemical properties of different sorbents. Therefore, analytical reproducibility is relatively poor.[1] Other limitations include low flow rates of 1-10 mL/min through a typical disposable cartridge, plugging of the cartridge by analyzing dirty samples, e.g. sample containing

suspended solids, and channelling of the sorbent bed.[66] The use of particle-loaded membranes (known as Empore extraction disks) introduced in 1990 further improved the extraction efficiency (higher mass transfer), lowered the time and cost of sample extraction, reduced the use of solvent, and decreased plugging in SPE.[13] However, matrix effects are always the biggest problem associated with SPE techniques.

1.4.4 Headspace Extraction

Headspace extraction coupled with GC have been employed to analyse volatile organics in clinical biochemistry[32] and the food and beverage industry.[67] It provides an indirect method of sample analysis suitable for the determination of organic volatiles.[1] The principles of the headspace technique are based on phase equilibration of the analytes between the vapour phase and liquid or solid phases.[68] Headspace analysis can be operated in two modes, the static mode or the dynamic mode. If the sample is in a closed thermostated system, then the method is referred to as static headspace. If a carrier gas is passed over the sample and the volatile analytes are accumulated in a cryogenic or sorbent trap, then the method is referred to as dynamic headspace. Analysis of headspace samples is simple, it can be performed manually using a gas-tight syringe or automatically using pneumatic headspace analyzer.

Headspace techniques are used predominantly for the determination of trace concentrations of volatile analytes in samples that are difficult to handle by conventional chromatographic means. Examples include dilute solutions where the matrix would obscure the components of interest, damage the column or require excessively long separation times owing to the presence of late eluting peaks, and heterogeneous mixtures, such as blood, sewage, etc., which require extensive sample cleanup prior to analysis. Headspace techniques are advantageous because they make use of the analytes that partition into the vapour phase without, usually, disturbing the actual sample. These techniques have the potential to reduce the use of pure organic solvents in extraction process, thus are economical. Also, they can reduce adverse effects from the sample matrix and minimize the problems associated with the chromatographic properties of the matrix. These advantages make these techniques very attractive for field and routine analysis.

However, there are disadvantages that limit the usefulness of headspace techniques. Generally, headspace sampling is not considered to be an extremely sensitive technique due to the lack of any concentration effect. All headspace techniques are usually limited to the analysis of volatile analytes. Heating the sample during the sampling process can help release less volatile analytes from the sample matrix. However, thermally labile analytes and the high moisture content of the desorbed gas mixture frequently prevent the use of the thermal desorption approach. Furthermore, the matrix from which the analytes are being extracted must be closely monitored so that the matrix effects on the partitioning of the analytes to the headspace are minimized.

1.5 Techniques for the Generation of Air Samples

The conventional techniques for the generation of standard gaseous mixtures were reviewed by Poole *et al.*[1] and Namiesnik *et al.*[69] The techniques can be broadly classified as static or dynamic.

Static methods include pressure, volumetric, gravimetric and pressureless methods. Static systems involve preparing and storing the mixture in a closed vessel, for example, a cylinder, flask or plastic bag. The sample volume is thus limited to that of the container, under pressure P and at temperature T (usually room temperature). Cylinders must be used to store mixtures at high pressure. Static systems are preferred when comparatively small volumes of mixtures are required at moderately high concentration levels, but losses of components to the vessel walls may occur.

Dynamic methods include the mixing of gas streams, exponential dilution, permeation, diffusion, evaporation, electrolytic and chemical reaction methods. Dynamic systems generate a continuous flow of sample mixture and can produce large volumes, with lower surface losses, owing to an equilibrium between the walls and the flowing gas stream.

Whether static or dynamic systems are employed, the methods used to create homogeneous mixing of the gas and vapour are the important consideration and some prevision for creating forced convection is incorporated into most devices.

1.6 Conventional Techniques for Air Sampling

Air can be sampled with a number of techniques depending on the analytes of interest.[1,70] Volatile compounds are usually collected by grab sampling or, in the case of reactive substances, by chemical trapping in bubblers. Solid-phase extraction techniques are less often used due to the low breakthrough volumes of many low boiling point organic compounds. Grab samples are taken by pulling air into an evacuated glass bulb, syringe, stainless steel canister or plastic bag. However, all such containers show different rates of adsorption. Extensive cleaning and testing of the container between use, particularly for trace analysis, detract from the apparent simplicity of the grab sampling methods.

Semivolatile organic compounds are usually collected by drawing filtered air through a bed of an appropriate sorbent. Porous polymers, various forms of carbon, such as charcoal and graphitized carbon blacks, or inorganic adsorbents, such as silica gel and alumina, are the most frequently used. No single sorbent is useful for all sample types. These sampling techniques are quite useful, but at the same time they are troublesome. For carbon based sorbents, not to mention that the chemical and physical properties of different types vary tremendously, they also tend to irreversibly adsorb polar analytes. Therefore the recovery of the polar compounds from these sorbents are poor. Although use of polar solvents can increase the recovery of the polar compounds from the carbon based sorbents, careful calibration is often needed when recovery from the sorbent is incomplete.

Passive sampling devices (usually containing charcoal as an adsorbent) for personal monitoring are being developed as a replacement for sorbent sampling using flow-through samplers to reduce the cost of large-scale screening studies and to make the monitor more convenient for the wearer. However, the analysis maintains the use of toxic solvents for chemical desorption and the system is limited by interfering organics from the charcoal bed holder which increases method detection limits.

1.7 Derivatization of Fatty Acids for GC Analysis

Acids are reactive substances and many are too polar to be chromatographed well. This is particularly true in gas chromatography. Underivatized acids tend to tail because of non-specific interaction with the 'inert' support. Due to associative effects, they are also not as volatile as their molecular weights might indicate. Esters, on the other hand, are less polar, more volatile, and can be chromatographed well. Esterification is therefore the obvious first choice for derivatization of acids, particularly for GC separation. A wide variety of post-extraction derivatization methods for fatty acids have been proposed to make them easier to separate and/or detect. These include alkylation and silylation. These methods were extensively reviewed in the *Handbook of Derivatives for Chromatography*.[71]

1.7.1 Alkylation

Methyl esters are the most often produced esters for fatty acids. The popular esterification techniques often use diazomethane;[72] boron trifluoride or boron trichloride/methanol,[73] acid-catalyzed methylations, such as hydrochloric acid/methanol,[74] sulphuric acid/methanol,[75] and perchloric acid/methanol,[76] or base-catalyzed esterification using sodium methoxide in methanol[77] to make methyl esters of the target fatty acids. An interesting variant is the use of the reagent trimethylsilyldiazomethane,[78] which is a safe and stable substitute for diazomethane and is used in the same way. It can also produce methyl esters of fatty acids when it is used in the presence of methanol. The pyrolysis of tetramethylammonium salts in the heated inlet of a GC is another popular method to produce methyl esters of fatty acids of interest. The quaternary ammonium salts are usually formed by mixing fatty acids together with tetramethylammonium ion in an organic or aqueous solution.[79] The advantage of this approach is that all is achieved with the microliter syringe for the injection.

The introduction of electron capturing moiety into the target analytes has been the subject of many investigations. Compounds of this kind have great selectivity and sensitivity towards electron capture detector (ECD) and negative-ion MS. The most often used reagents to derivatize fatty acids for GC analysis include pentafluorobenzyl bromide or pentafluorobenzyl

chloride with the presence of potassium or sodium salts (e.g. potassium carbonate or potassium bicarbonate), tertiary amines (e.g. di-isopropylethylamine[80]), or tetraalkylammonium salts (e.g. tetrabutylammonium hydrogen sulphate[81]).

The above listed methods are usually fast and near quantitative. However, the majority of these derivatization reactions require high temperatures, the presence of organic media, and/or the presence of a catalyst.

1.7.2 Silylation

Silylation is the most versatile technique currently available for enhancing GC performance by blocking protic sites, thereby reducing dipole-dipole interactions and increasing volatility of the target analytes. Nearly all functional groups which present a problem in GC analysis can be converted to silyl derivatives. The introduction of a silyl group can also serve to enhance MS properties by producing either (i) more favourable diagnostic fragmentation patterns of use in structure investigations, or (ii) characteristic ions of use in trace analysis employing selected ion monitoring and related techniques.

Numerous silylation reagents have been used to derivatize fatty acids into silyl esters in different kinds of sample matrices.[1,72] The most often used ones include N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA),[82] N,O-bis(trimethylsilyl)acetamide (BSA)[83] for GC analysis. For GC/MS applications, N-tert-butyl-dimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA)[84] is the best reagent to use. Overall, these reagents offer fast reactions (5-15 minutes) under mild conditions (room temperature) and produce stable esters for fatty acids in good yields. Since most of these reagents are water sensitive, these reactions often require the presence of an organic medium.

1.7.3 Other Derivatization Techniques

In addition to the derivatization techniques listed above, a number of other reagents, such as silver salts, methyl halides, etc., have been used for the derivatization of fatty acids for GC analysis.[72] Due to the limited applicability, they will not be covered in this thesis.

1.8 Derivatization of Amines for GC Analysis

Chromatographic separation and quantitation of free amines is hampered by their polarity, which can cause peak tailing and favours irreversible adsorption. The formation of less polar and more stable derivatives has therefore been the subject of many investigations. There are many derivatization methods available for the derivatization of amines.[72] The reagents and conditions used for derivatization reactions vary a lot. Therefore, only popular reagents and a few typical applications will be discussed.

Alkyl chlorides[85] are extensively used for the derivatization of primary, secondary and tertiary amines, whereas aryl-aldehydes (mainly benzaldehyde-type)[86] can only be used selectively to derivatize primary amines. Alkyl chloroformates, in general, are widely used for the derivatization of tertiary amines and are one of the few general reactions applicable for these compounds.[87] Recently, attention has been centred on the halogenated derivatives of amines. When the above reagents contain a pentafluorobenzyl moiety, they can be used to convert polar amine compounds into less polar derivatives for selective and sensitive electron capture detection, which is about 1000 times more sensitive than the flame ionization detection. Polymer (usually polystyrene resin) reagents containing a benzoyl- group, such as 3,5-dinitrobenzoyl[88] and pentafluorobenzoyl,[89] have also been used recently in the solid-phase derivatization of amines coupled with GC/MS.

1.9 Thesis Objectives

An ideal sample preparation technique should be simple, solvent free, time efficient, inexpensive, selective, portable, easily automated and used for field analysis, and independent of the instrumental design. It should be able to concentrate and extract the target analytes from the sample matrix simultaneously. Solid-phase microextraction (SPME) is a relatively new sample preparation technique that has the above advantages.

The aim of this thesis is to develop new strategies and principles for the analysis of fatty acids and amines, two groups of very polar and difficult to analyze compounds, using SPME. The flow chart of possible techniques to analyze polar compounds using SPME sampling coupled

with GC is shown in Figure 1-1. The techniques can be classified mainly into three categories: direct SPME, headspace SPME and SPME coupled with derivatization. Since the majority of the analytes studied so far with SPME were relatively non-polar, investigation of polar analytes is necessary to help fully understand the scope of the SPME technique. Therefore, the preliminary objective of this thesis is to establish the possibility of using direct SPME and headspace SPME for the analysis of fatty acids and amines in air and/or water. The main focus of this thesis is to develop new principles that combine derivatization coupled with SPME for the analysis of polar and/or less volatile analytes in aqueous and/or gaseous matrices. Three types of techniques: derivatization in the SPME fiber coating, derivatization in the sample matrix followed by SPME sampling and SPME followed by the derivatization of target analytes in the GC injector port are carried out and will be discussed extensively in Chapter 4 and Chapter 5. A mathematical model for the derivatization in the SPME coating is developed and will be discussed and compered with the experimental data.

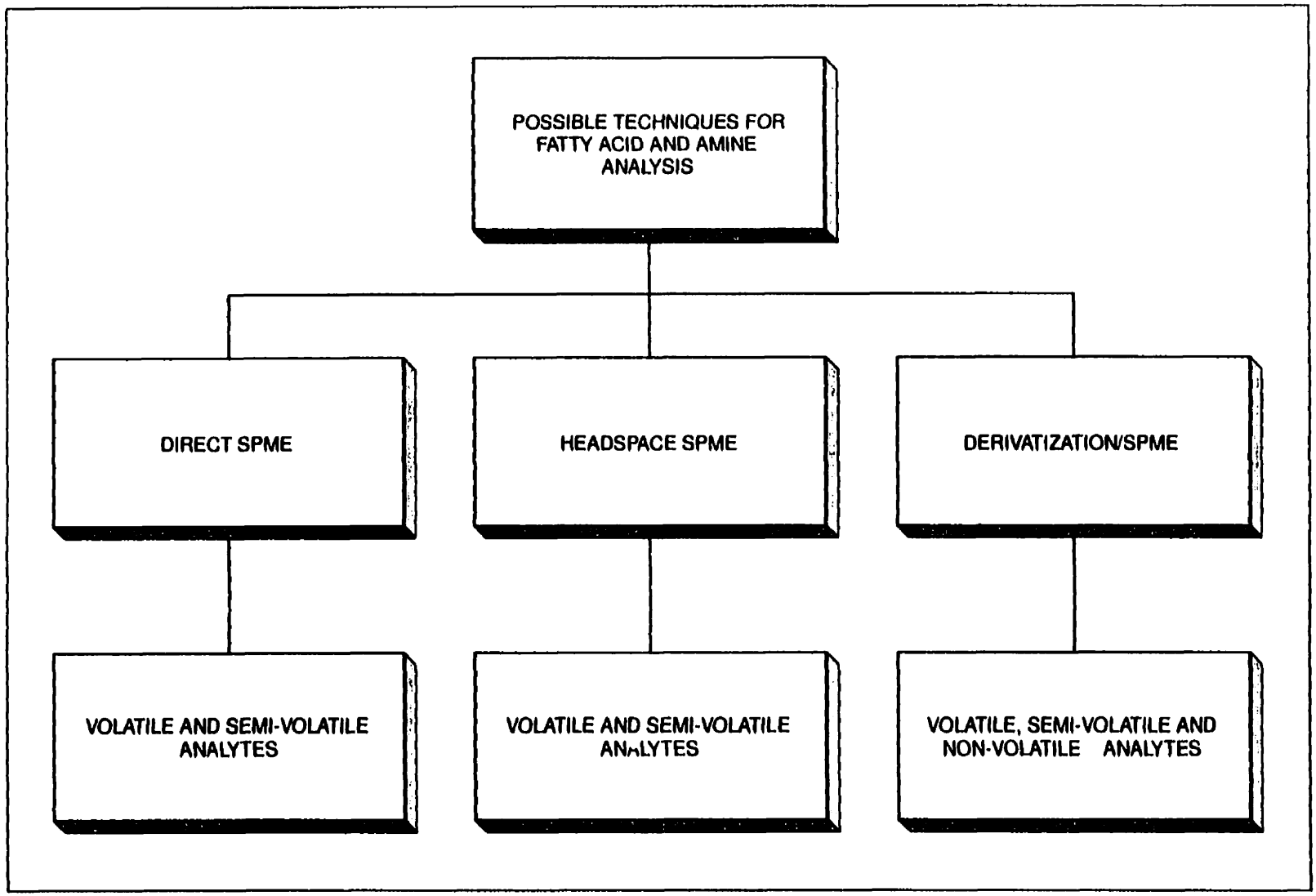

Figure 1-1. Possible techniques for GC analysis of fatty acids and amines using SPME sampling.

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CHAPTER 2

SOLID-PHASE MICROEXTRACTION

2.1 Introduction

Unlike SPE which uses cartridges or disks to enrich target analytes during sample preparation process, a typical SPME set-up contains a cylindrically shaped optical fiber coated with a polymeric coating similar to a GC stationary phase, and mounted in a syringe-like device. The SPME process consists of two steps: partitioning of analytes between the SPME fiber stationary phase and the sample matrix, and desorption of the concentrated analytes into an analytical instrument. In the first step, the SPME fiber is exposed to a sample and extracts target analytes from the sample matrix. After a proper extraction time is reached, the fiber concentrated with target analytes is transferred into an instrument where the analytes are either thermally desorbed from the coating when GC is used, or solvent desorbed when HPLC is employed, and subsequently separated and analyzed.

The SPME device is now commercially available from Supelco (Supelco Inc., Bellefonte, PA) and is illustrated in Figure 2-1. Usually, the syringe assembly contains a 1 cm long coated fiber which is glued into a 30-gauge stainless steel tubing with high-temperature epoxy resin. Then the stainless steel tubing is connected with the plunger for easy handling. Before sampling, the fiber is drawn back into the syringe needle. Once the needle penetrates the septum of the sample vial, the plunger is depressed and the fiber is exposed to the sample. After sampling, the fiber is withdrawn into the needle again and the needle is removed from the sample vial. The fiber is then ready for desorption.

The fiber stationary phases used are usually viscous liquids. The extraction step is, in effect, a non-exhaustive liquid-liquid extraction with the convenience that the "organic phase" is attached to the fiber. Because the fiber coating acts as an "organic phase", the use of organic solvents can be completely eliminated during the SPME sample preparation step. Optical fibers are used because they are inexpensive and are made of chemically inert fused silica, the same material used to make capillary GC columns. There are several SPME fiber coatings commercially available for the analysis of different types of analytes and for different kinds of applications. The design of the SPME device allows it to be used in the same manner as an

ordinary syringe. The small size of the fiber assembly can be easily accommodated in a chromatographic injector without any modification.

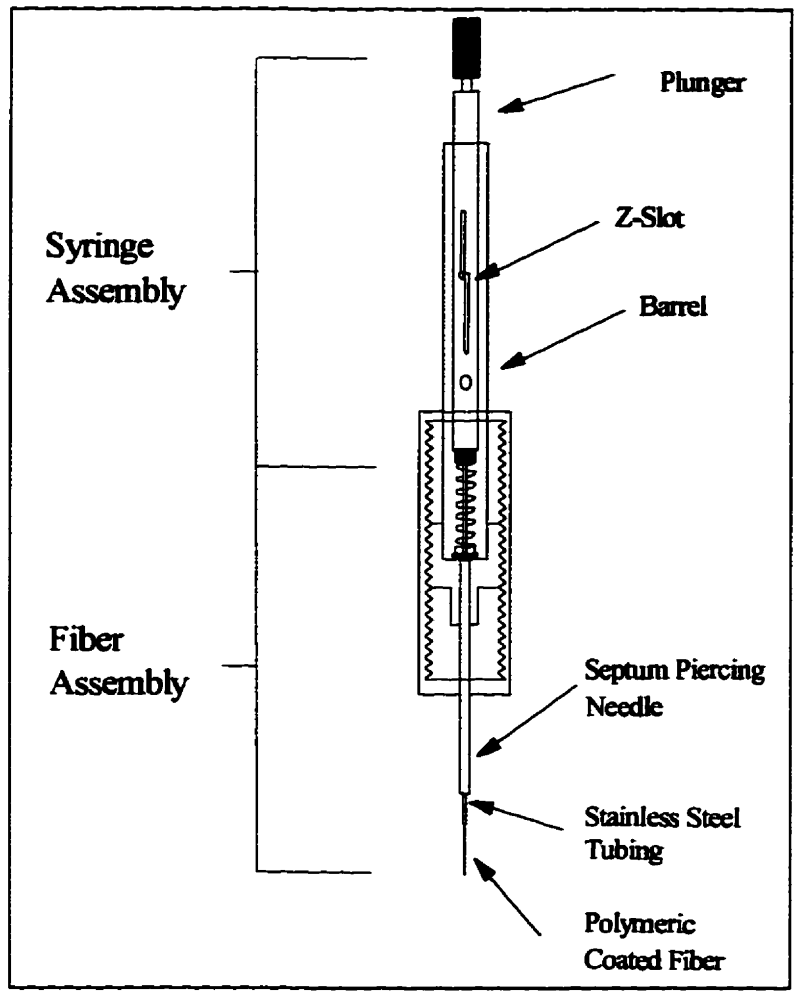

Figure 2-1. A commercially available SPME device.

The SPME device is portable and can be used for field analysis. When SPME is used to sample in the field, it is important to prevent losses of analytes during transportation back to the laboratory for analysis. This can be achieved by sealing the needle opening of the SPME device, e.g. by plugging the SPME needle with a piece of septum, or by cooling the needle with dry ice.[1]

SPME can be operated both manually and automatically. The automation of SPME is very simple. The only difference between a normal autosampler and a SPME autosampler is that the plunger movement and timing have to be carefully controlled to ensure proper absorption and desorption.[2]

2.2 Theoretical Considerations

SPME is an equilibrium process where the analytes partition between the fiber coating and the sample matrix. There are two types of sampling by SPME: direct SPME and headspace SPME. The schematic representation of direct SPME (two-phase system) and headspace SPME (three-phase system) is shown in Figure 2-2.

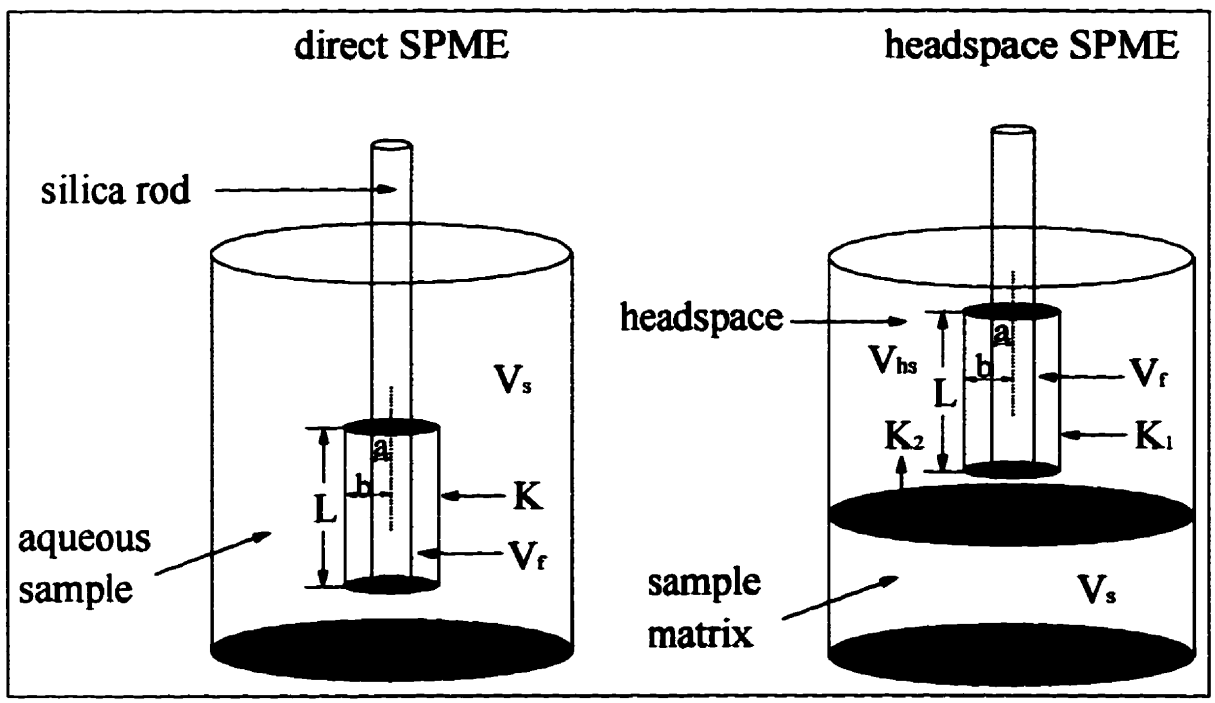

Figure 2-2. Schematic representation of direct SPME and headspace SPME systems (*a* and *b* are the radii of the silica core and the fiber coating, respectively; *L* is the length of the coated fiber; V_f, V_{hs} and V_s are the volumes of the stationary phase, the headspace and the sample, respectively; and K_1, K_2 and K are the coating/gas, gas/water and coating/water partition coefficients, respectively).

2.2.1 Direct SPME

In direct SPME, the fiber is immersed into the sample matrix to isolate target analytes. It is a two-phase system which contains fiber and sample matrix. For a two-phase system, at equilibrium, the mass balance of the system can be described as:

$$C_0 V_s = C_f^{\infty} V_f + C_s^{\infty} V_s \tag{2-1}$$

where C_0 is the initial concentration of the analyte in the aqueous phase; C_f^{∞} and C_s^{∞} are the concentrations of the analyte in the fiber coating and the aqueous solution, respectively, at equilibrium; and V_f and V_s are the volumes of the stationary phase and the sample, respectively. When the two-phase system reaches equilibrium, we also have:

$$K = \frac{C_f^{\infty}}{C_s^{\infty}} \tag{2-2}$$

where K is the partition coefficient of the analyte between the fiber coating and the aqueous phase. By combining Equations (2-1) and (2-2), the amount of analyte, n, absorbed by the fiber stationary phase after the system reaches equilibrium can be expressed as:

$$n = \frac{C_0 V_f V_s K}{K V_f + V_s} \tag{2-3}$$

Equation (2-3) clearly indicates the linear relationship between the mass of the analyte absorbed by the fiber coating and the initial sample concentration.

Typically, the volume of the fiber stationary phase V_f is very small. Equation (2-3) demonstrates that when KV_f is much smaller than V_s, it can be simplified to

$$n = K V_f C_0 \tag{2-4}$$

Equation (2-4) indicates that the sensitivity of the extraction (the amount of the analyte extracted by the fiber coating) depends mainly upon two factors: the volume of the stationary phase V_f and the partition coefficient K. For a given concentration of a sample, the thicker the coating (larger volume) on the fiber, the greater amount of the analyte will be absorbed into the film. The

polarity of the stationary phase is the predominant element which affects the partition coefficient K. When the affinity of the target analytes towards the fiber is large (large K), the analytes are more efficiently extracted. By choosing the correct type of coating, extraction of specific compounds can be enhanced. Equation (2-4) considers only the mass absorbed at equilibrium and cannot take into account the finite time needed for the coating to absorb the analyte.

In direct SPME, the speed of absorption is controlled by the mass transfer of the analytes from sample matrix to the fiber coating. This process involves diffusive and convective transport in an air or liquid sample and diffusion of the analytes in the fiber coating. The dynamics of this absorption process have been mathematically modelled for both stirred and unstirred solutions.[3] A few assumptions have also been made: since most of the polymer coatings used are normal liquid polymers, diffusion is the only mass transport mechanism which determines the migration of the analyte molecules in the system; there is no activation energy involved in the transfer of the analyte between the solution and the coating; as the concentration of an analyte in the solution is increased, the physical properties of the polymer do not change, i.e. the polymer neither swells nor does the diffusion coefficient of the analyte in the polymer depend on analyte concentration; the model does not account for the mass of analyte absorbed through the surface at the bottom of the coated fiber since the surface area at the bottom is a very small portion of the total surface area; the concentration profile in the polymer does not vary along the axis of the fiber, nor does it depend on the radial angle from the centre of the fiber. Therefore, the amount absorbed as a function of time (the extraction profile) can be determined using Fick's Second Law of Diffusion[4] for a one dimensional process, shown in the following expression:

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial^2 x} \tag{2-5}$$

where D is the diffusion coefficient of the analyte and $C(x,t)$ is the concentration of the analyte in the fiber at position x and time t. The mass absorbed into the fiber coating can be expressed as:[3]

$$M(t) = \int_a^b C_f(x,t)dx \tag{2-6}$$

C_f is the concentration of the analyte in the stationary phase, a and b are the radii of silica core and coated fiber, respectively, and $(b-a)$ is the film thickness of the fiber coating.

Without sample agitation, the mass transfer rate is determined by the diffusion of the analyte in water. The average time t of the diffusion through the aqueous layer can be expressed as:[3]

$$t = \frac{x^2}{2D} \tag{2-7}$$

where x is the migration distance of the analyte and D is the diffusion coefficient. When perfect agitation is obtained, the mass transfer rate is determined only by the diffusion of the analytes in the fiber coating. Approximately 90% of M^∞ is absorbed after a time corresponding to $Dt/(b-a)^2=0.5$.[3] Time t here is referred to as the equilibration time and it is proportional to the square of the coating thickness and inversely proportional to diffusion coefficient D. In this situation, rapid extraction can be reached when D is large, since the fiber coating is very thin, typically between 7-100 μm. In reality, this can be achieved by gas samples because analytes have large diffusion coefficients in the gaseous phase. However, for aqueous samples, this is only possible when very vigorous agitation, such as sonication, is used. For more practical agitation methods such as magnetic stirring, imperfect agitation is encountered. With this inefficient mixing of sample, an unstirred thin layer of water remains next to the fiber, which limits the rate of absorption since analytes must first diffuse across this static layer. The extraction is hindered and the equilibrium time is increased since the diffusion coefficients of analytes in water are very small compared to those in air. The equilibrium time depends on the partition coefficient K.[3] When K is large, more analyte has to diffuse through the static water layer, therefore, the equilibrium time will be even longer. This is particularly true when dealing with compounds of larger molecular weights in the homologous series.

The mass-transfer limitation caused by the diffusion of the analyte in the aqueous phase can be overcome by sampling analytes from the headspace above the aqueous solution since diffusion coefficients of molecules in the gaseous phase are about 4 orders of magnitude higher than in water.

2.2.2 Headspace SPME

In headspace SPME, the fiber is in the headspace above the sample matrix to extract the target analytes. Real samples tend to contain particles or materials that could contaminate or damage a fiber coating if it is directly exposed to the matrix, which would interfere with the extraction. Headspace SPME could avoid these adverse matrix effects. Headspace SPME extends the SPME technique to more complicated samples which contain solid or high molecular weight materials such as soil and sludge. Headspace sampling also enables faster equilibration than sampling from liquid.[5] In headspace SPME sampling, a rapid equilibrium between aqueous and vapour phases can be achieved by constantly stirring the aqueous sample to generate a continuously fresh surface.

Headspace analysis involves three phases: coating, gas and liquid. The amount of analyte absorbed by the liquid polymeric coating is related to the overall equilibrium of analytes in the three-phase system. There are two partition coefficients in this three-phase system, K_1 and K_2.

K_1 is the coating/gas partition coefficient and is expressed as:

$$K_1 = \frac{C_f^{\infty}}{C_{hs}^{\infty}} \tag{2-8}$$

and K_2 is the gas/water partition coefficient and is expressed as:

$$K_2 = \frac{C_{hs}^{\infty}}{C_{aq}^{\infty}} \tag{2-9}$$

By combining Equations (2-3), (2-8) and (2-9), the amount of analytes absorbed at equilibrium can then be described as:[5]

$$n = \frac{C_0 V_f V_s K_1 K_2}{K_1 K_2 V_f + K_2 V_{hs} + V_s} \tag{2-10}$$

where C_0 is the initial sample concentration; V_f, V_{hs} and V_s are the volumes of the fiber coating, headspace and sample, respectively; and K_1 and K_2 are the coating/gas and gas/water partition coefficients, respectively.

The chemical potential of the analytes in fiber coating, headspace and sample matrix can be expressed as follows:

$$\mu_{hs} = \mu^0(T) + RT\ln(\frac{P_{hs}}{P^0}) \tag{2-11}$$

$$\mu_f = \mu^0(T) + RT\ln(\frac{P_f}{P^0}) \tag{2-12}$$

$$\mu_s = \mu^0(T) + RT\ln(\frac{P_s}{P^0}) \tag{2-13}$$

where μ_{hs}, μ_f and μ_s are the chemical potentials of the analyte in the headspace, fiber coating and sample matrix, respectively; $\mu^0(T)$ is the chemical potential of the analyte at standard pressure P^0 (usually set $P^0 = 1$ *atm*) and temperature T; and P_{hs}, P_f and P_s are the vapour pressures of the analyte in the headspace, fiber coating and sample matrix, respectively. According to Henry's law,[6]

$$P_f = K_F C_f^{\infty} \tag{2-14}$$

and

$$P_s = K_H C_s^{\infty} \tag{2-15}$$

where K_F and K_H are Henry's constants of the analyte in the liquid polymer coating and the aqueous solution, respectively. When the three-phase system reaches equilibrium, the chemical potentials of the analyte in three phases must be equal

$$\mu_{hs} = \mu_f = \mu_s \tag{2-16}$$

From Equations (2-11)-(2-13) and (2-16), we then have

$$P_{hs} = P_f = P_s \tag{2-17}$$

If we assume the ideal gas law $P_{hs}V_{hs}=n_{hs}RT$ (n_{hs} is the number of moles of the analyte in the headspace) is valid for the analyte vapour in the headspace, thus

$$P_{hs} = C_{hs}^{\infty}RT \tag{2-18}$$

From Equations (2-14), (2-15) and (2-18), the partition coefficients with Henry's law can easily be connected as:

$$K_1 = \frac{C_f^{\infty}}{C_{hs}^{\infty}} = \frac{RT}{K_F} \tag{2-19}$$

and

$$K_2 = \frac{C_{hs}^{\infty}}{C_s^{\infty}} = \frac{K_H}{RT} \tag{2-20}$$

In the case of direct SPME sampling, we have

$$K = \frac{C_f^{\infty}}{C_s^{\infty}} = \frac{K_H}{K_F} \tag{2-21}$$

From Equations (2-19)-(2-21), it is obvious that

$$K = K_1K_2 \tag{2-22}$$

Equation (2-10) can then be rewritten as[5]

$$n = \frac{C_0V_fV_sK}{KV_f+K_2V_{hs}+V_s} \tag{2-23}$$

Similar to Equation (2-4), Equation (2-23) considers only the mass absorbed at equilibrium and does not reflect the finite time needed for the coating to absorb the analyte.

In headspace SPME, the speed of absorption is controlled by the overall mass transfer of the analytes from sample matrix to the headspace and eventually to the fiber coating. Similar to the direct SPME, the dynamics of the headspace sampling has been theoretically investigated for

stirred and unstirred solutions in a three-phase system[5] instead of two-phase system in the direct SPME.

The results suggest that static headspace SPME can be used to differentiate volatile compounds from less volatile compounds. Volatile compounds usually have large Henry's constants (large K_H, thus large K_2) which result in high volatility and hydrophobicity. Also volatile compounds have relatively small partition coefficients between the fiber coating and the headspace, K_1, thus they reach equilibrium faster. Semivolatile compounds usually have large K_1 and smaller K_2, thus the equilibrium time tends to become longer. Therefore, rapid stirring is essential to facilitate the mass transfer of the analytes from the aqueous phase to the headspace for sampling of semivolatile compounds using headspace SPME. For compounds with high boiling points, sampling the compounds at higher temperature can reduce the equilibrium time since Henry's constants increase as temperature increases.[5] It has also been shown that the absorption of analytes into the fiber is an exothermic process and heating the sample and its surrounding may reduce the absorption of analytes into the fiber.[7] This can be overcome by creating a temperature gradient where the fiber is cooler than the sample, thus the mass transfer of the analytes from the sample matrix can be facilitated while the partitioning of the analytes to the fiber coating remains unchanged or increases.[7]

2.3 SPME Applications

SPME coupled with GC has been applied to the analysis of substituted benzene compounds,[8] polynuclear aromatic hydrocarbons and polychlorinated biphenyls,[9] chlorinated hydrocarbons,[10] phenols,[11] inorganic lead and tetraethyllead,[12] organochlorine and organophosphous containing pesticides[13] and nitrogen containing herbicides[14] in environmental samples, caffeine[15] and other flavour compounds[16] in food and beverages, drugs in biological fluid,[17] chlorinated hydrocarbons in indoor air,[10] as well as alcohol and acetone in breath analysis.[18] Phenols were the first group of polar compounds analyzed with the SPME technique in this research group.

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CHAPTER 3

ANALYSIS OF FREE FATTY ACIDS USING DIRECT SPME AND HEADSPACE SPME SAMPLING

3.1 Introduction

Direct SPME and headspace SPME sampling have been used to carry out the analysis of a variety of compounds presented in Chapter 2. The majority of the analytes studied were relatively non-polar compounds. The only group of polar compounds previously studied, using SPME techniques was phenols and their derivatives. Therefore, investigation of other polar analytes, such as fatty acids, using SPME is necessary to help fully understand the scope of the SPME technique. This chapter presents the use of direct SPME and headspace SPME sampling for the determination of both short-chain and long-chain fatty acids in aqueous and/or gaseous samples.

3.2 Experimental

The following acids were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received: acetic, propionic, butyric, valeric, hexanoic, heptanoic, octanoic, nonanoic, decanoic, palmitic and stearic. The silanizing reagent dimethyldichlorosilane was purchased from Supelco Canada (Mississauga, Ont.). All solvents used in this study were analytical-reagent grade. Deionized water (NANOpure, ultrapure water system, Barnstead Inc., Iowa) was used to prepare all aqueous samples.

The SPME device was purchased from Supelco Inc. (Bellefonte, PA). The detailed set-up and the manipulation of the SPME device were described in Chapter 2. Four types of fibers were used for this work: Carboxen™, carbowax divinylbenzene [CAX(DVB)], poly(dimethylsiloxane) (PDMS), and poly(acrylate) (PA). The PDMS and PA coated fibers with the coating thicknesses of 100 and 95 μm, respectively, were obtained from Polymicro Technologies (Tuscon, AZ). The CAX(DVB) coated fibers with the film thickness of 65 μm was obtained from Supelco Canada. The Carboxen™ coated fiber was also obtained from Supelco Canada. The exact coating thickness was unknown, but it was in the order of several micrometers. The Carboxen™ coating was made of graphitized carbon black. The detailed chemical structure of the CAX(DVB) coating was unknown, it contained cross-linked segments of poly(ethylene glycol) and poly(divinylbenzene). The detailed structures of the PA and PDMS fiber coatings are illustrated in Figure 3-1.

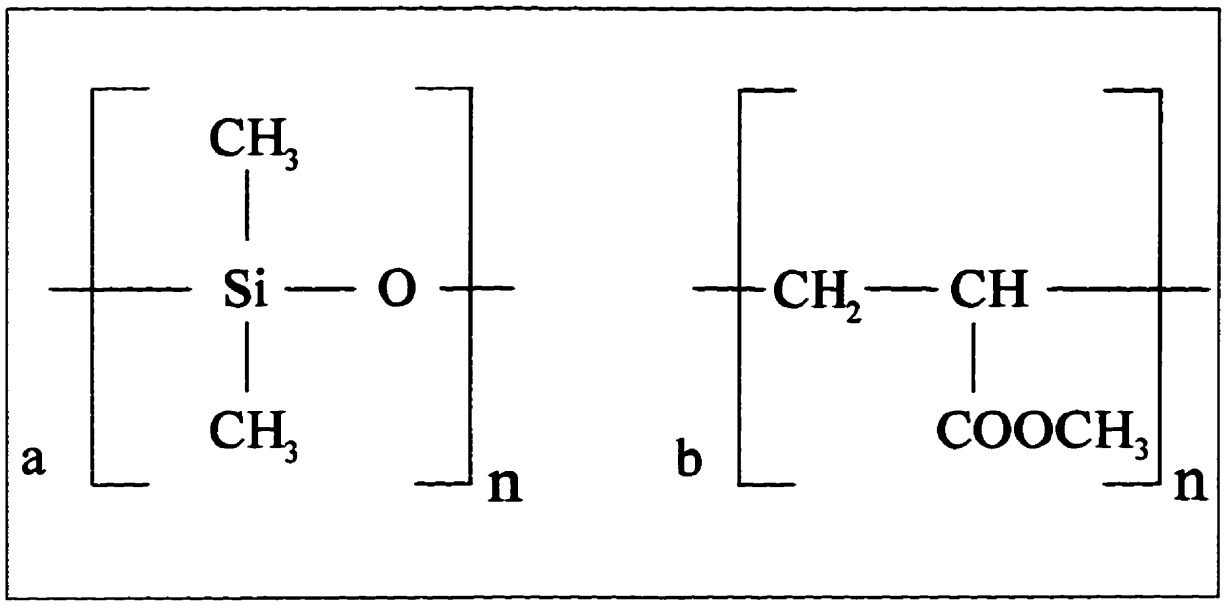

Figure 3-1. Detailed chemical structures of (a) PDMS and (b) PA coatings.

The CAX(DVB) coated fiber was conditioned at 270°C under helium for 30 minutes to 1 hour, whereas the PDMS and Carboxen™ coated fibers were conditioned at 250°C under helium for 4-5 hours, and the PA coated fiber was conditioned at 275°C for 6-7 hours prior to use.

The stock standard containing 1000 μg/mL each of acetic, propionic, butyric, and valeric acids was prepared with the pure analytes dissolved in methylene chloride. This standard was then diluted with methylene chloride by factors ranging from 10 to 100 in order to prepare gaseous samples. Similarly, for the aqueous samples, the stock solution containing C_2-C_{10} acids was prepared by dissolving 1000 μg/mL of pure fatty acids in deionized water. This stock solution was further diluted with deionized water to prepare the working aqueous solutions.

For both air and aqueous sample analysis, 40 mL amber vials were used along with 2.5 cm × 0.8 cm stir bars (Bel-Art products, Pequannock, NJ) to agitate the samples during extraction. The vials were sealed with Teflon® faced silicone septa and hole caps. For air analysis, 2 μL of

C_2-C_5 acids stock solution in methylene chloride was injected into the sample vial. Prior to the sampling, the sample vial was heated in a drying oven at 100°C to help fully vaporize the acids and then cooled to room temperature. For aqueous sample analysis, 35 mL of the aqueous solution was added into a 40 mL sample vial.

A Varian 3400 GC with a Septum-equipped Programmable Injector (SPI) and a flame ionization detector (FID) (Varian, Georgetown, ON) were used. The GC was also equipped with cryogenics in both the injector and the oven for temperature programming. The glass wool was removed from the 0.8 mm ID SPI insert (Varian) before use. The column used was a 30 m, 0.25 mm ID SPB-5 with a 1 μm stationary phase (Supelco Canada). LB-2 septa (Supelco Canada) were used in the injector.

For the analysis of short-chain C_2-C_5 fatty acids, the GC conditions were as follows: the injector was ramped from 40°C to 250°C at a rate of 250°C for solvent injections and was kept at 250°C for fiber desorption; the desorption time (the time that the fiber was in the injector) was 4 minutes; the initial oven temperature was 40°C for 4 minutes, then programmed to 250°C at a rate of 15°C/min; and the FID was held at 300°C. For the analysis of all the C_2-C_{10} fatty acids, the injector temperature was set up to 275°C and the desorption time was 4 minutes. All other GC conditions were the same as those used for the analysis of C_2-C_5 acids. For the analysis of long-chain fatty acids, the column temperature was ramped from 60°C to 280°C at a rate of 20°C/min and held at 280°C for 5 minutes; the injector temperature was ramped from 60°C to 275°C at a rate of 250°C for solvent injection and maintained at 275°C for fiber injection; the desorption time was 4 minutes; the FID was held at 300°C.

3.3 Analysis of Short-Chain C_2-C_5 Fatty Acids in Air

Although there is no evidence to show that short-chain fatty acids are hazardous to human health, the concentrations of these acids in air still have to be controlled. Because of their odour, such analysis is of great concern in the food and beverage industry.

Techniques for the generation of standard gaseous mixtures were introduced in Chapter 1. In this thesis, the static volumetric method was used to generate gaseous samples for the analysis of short-chain fatty acids.

The initial work for the analysis of fatty acids in air was performed with direct SPME sampling, since it is a simple and straightforward technique. Two kinds of fiber coatings were employed: PA and PDMS. Each coating offers particular advantages. The PDMS coating is a non-polar coating which has been known to work very effectively for a wide range of analytes, both polar and non-polar. In addition, this coating has great similarity to the stationary phase of the capillary column used in most of the experiments. On the contrary, the PA coating offers a polar phase by which the polar analytes can be more readily extracted.

As mentioned before, SPME is an equilibrium process of analytes partitioning between the fiber coating and the sample matrix. The equilibration time is determined by exposing the fiber to an aqueous phase spiked with the target analytes for a variety of time periods until the amount extracted remains constant. Fresh samples are used for each extraction time tested. Information can be obtained from the exposure time profiles by plotting the amount of the eluted analytes extracted by the SPME fiber versus the time that the fiber was exposed to the aqueous samples.

The exposure time profiles for the analysis of free fatty acids (FFAs) in the gaseous phase were examined with propionic (C_3) and butyric (C_4) acids as representatives. Both the PDMS and PA coatings were used. Initially, the exposure time profiles were examined with non-silanized sample vials. The acid concentration of 2.5 ng/mL (a total amount of 100 ng each of C_3 and C_4 acids in a 40 mL sample vial) was added to the vial when the PDMS coated fiber was used, whereas 0.5 ng/mL of the acid samples (a total amount of 20 ng each of C_3 and C_4 acids in a 40 mL sample vial) were spiked in the vial when the PA coated fiber was employed. Surprisingly, the equilibration times were 45 minutes with the PDMS coated fiber and even longer than 45 minutes with the PA coated fiber (Figure 3-2) for both of the acids tested.

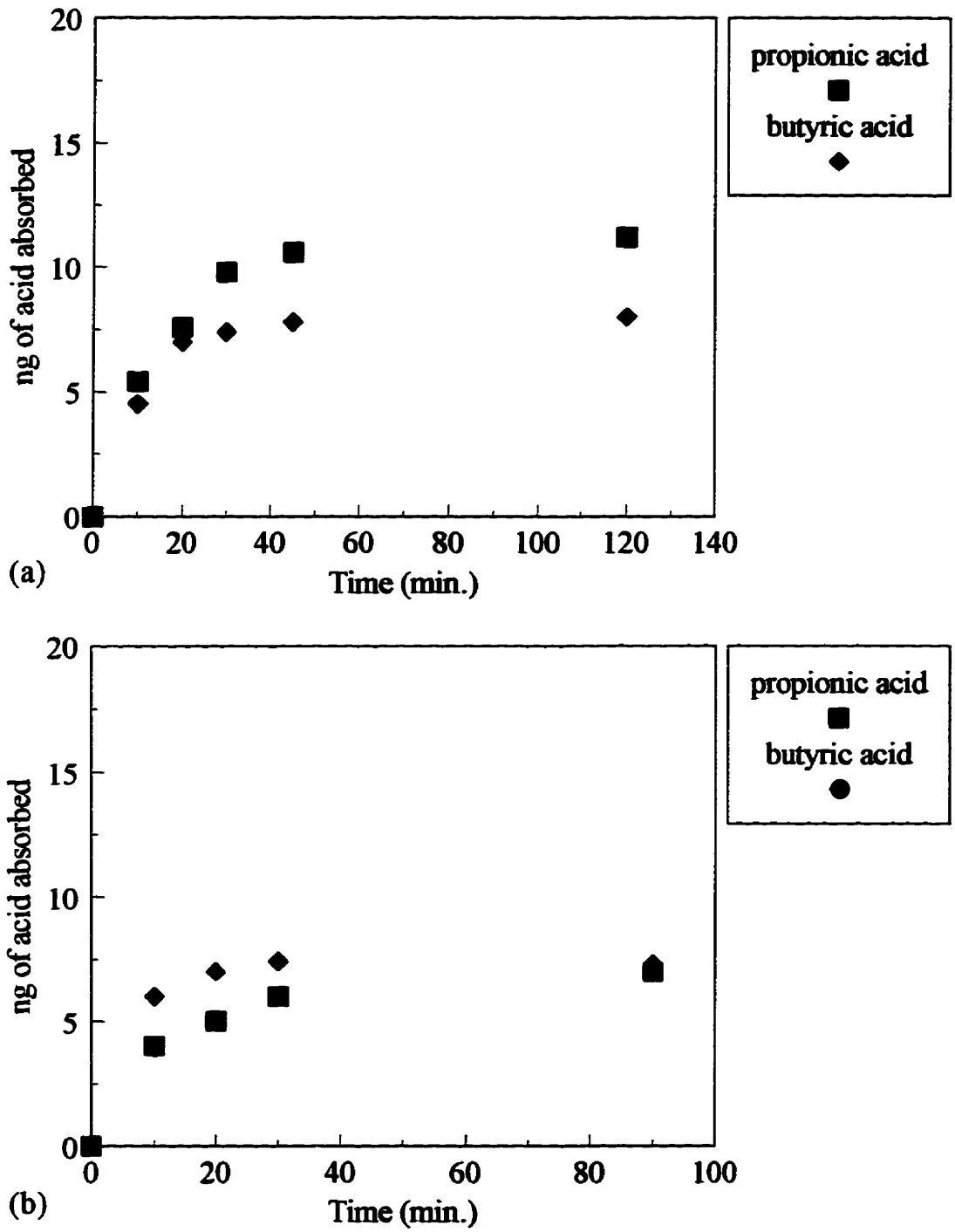

Figure 3-2. Exposure time profiles for the extraction of free gaseous propionic and butyric acids in the non-silanized vial using (a) PDMS and (b) PA coated fibers. Note: samples contained 100 ng each of C_3 and C_4 acids when the PDMS coated fiber was used, and 20 ng each of C_3 and C_4 acids when the PA coated fiber was used.

Since the diffusion coefficients of FFAs in the gaseous phase are relatively large and the sample was stirred vigorously with a magnetic stir bar, the mass transfer of the analytes from the gaseous phase to the fiber stationary phase would be expected to be very fast. For VOCs, they should reach extraction equilibrium within a few minutes with good mixing.[1] However, this was not what was found. Consequently, the sample vials were acid washed and silanized with 10% of dimethyldichlorosilane/toluene solution before being spiked with the sample. The concentrations of the fatty acid were the same as in the previous experiments in which the sample vials were not silanized. The equilibration times were significantly reduced after incorporating this process (Figure 3-3). Only 5 minutes were required for both C_3 and C_4 acids to reach equilibrium in the silanized vials when the PDMS coating was used. A similar trend was also observed for the PA coating and equilibrium was established in 5 and 10 minutes for C3 and C_4 acids, respectively. The longer equilibration times of the C_3 and C_4 acids in the non-acid washed and non-silanized vials may be due to the initial adsorption of the acids onto the glass wall of the sample vials and then their gradual release to the gaseous phase during the fiber absorption process. It was also observed that the amounts of propionic and butyric acids extracted by both fibers in the silanized vials also increased compared to the extraction performed in the non-silanized vials. Therefore, acid washed and silanized sample vials were used for the remainder of the experiments.

For the PDMS coated fiber, it was necessary to have 2.5 ng/mL each of C_3 and C_4 acids in the sample vial so that the PDMS coating could extract enough of the acids for GC/FID detection. Approximately 7% and 16% of C_3 and C_4 acids were absorbed by the PDMS coated fiber, respectively. On the other hand, 0.5 ng/mL each of C_3 and C_4 acids was more than enough for the PA coated fiber to extract them for GC/FID detection as 50% of propionic acid and 65% of butyric acid were extracted. The data presented in Figure 3-3 shows that the mass loadings of C_3 and C_4 acids with both fiber coatings were similar, even though the acid concentration used for the PDMS coating was 5 times higher than that used with the PA coating. Therefore, it would be expected that the polar PA coated fiber would extract larger amounts of fatty acids than the non-polar PDMS coating when the sample concentration is the same. The above experiments demonstrate that fatty acids have a larger affinity towards the polar PA coating than the non-polar PDMS.

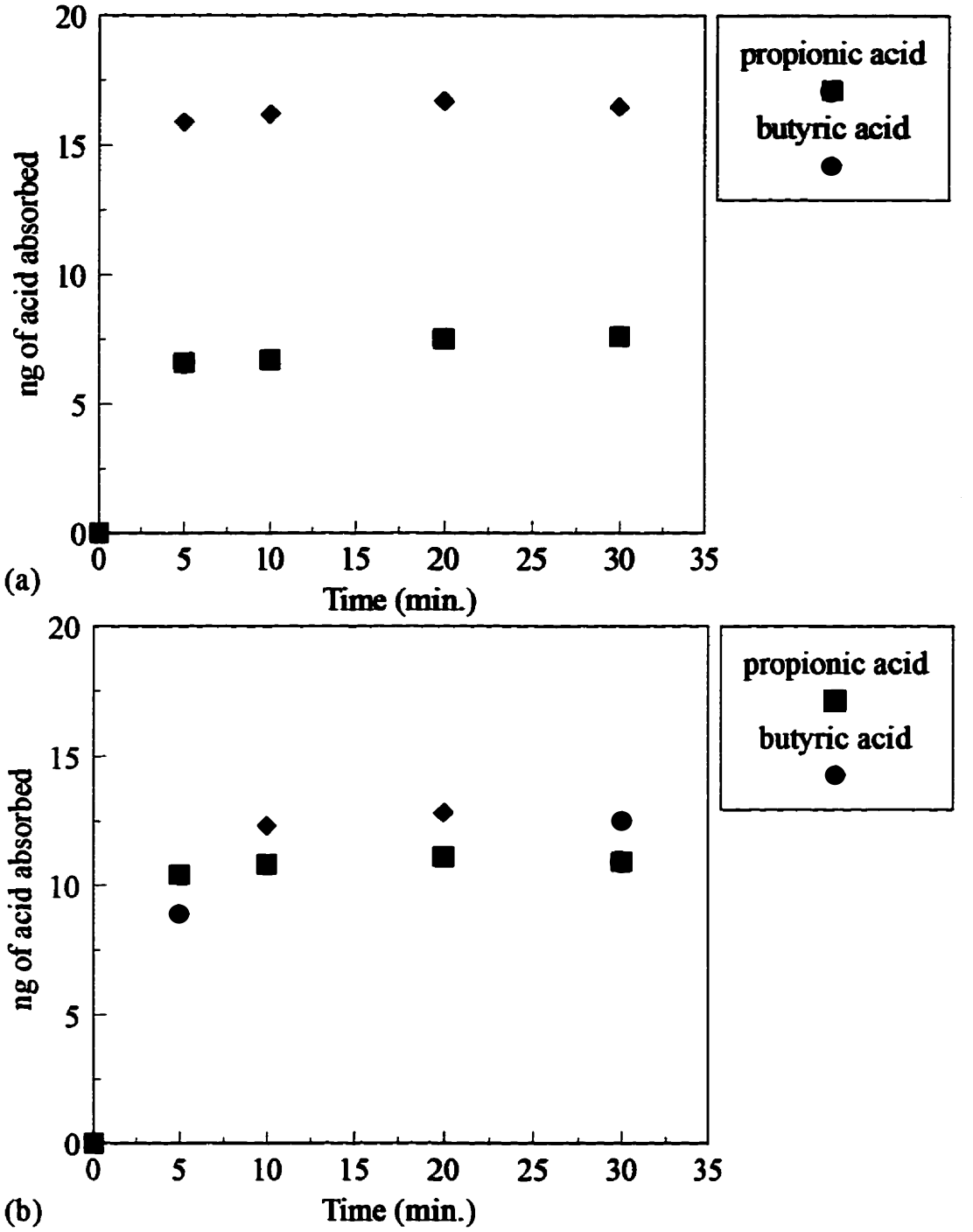

Figure 3-3. Exposure time profiles for the absorption of free propionic and butyric acids in the silanized vial using (a) PDMS and (b) PA coated fibers. Note: samples contained 100 ng of C_3 and C_4 acids when the PDMS coated fiber was used, and 20 ng of C_3 and C_4 acids while the PA coated fiber was used.

The effect of stirring versus non-stirring of gaseous samples was also investigated using the PA coated fiber. Samples contained 0.5 ng/mL each of C_2-C_5 acids and the fiber extraction time was 10 minutes. Figure 3-4 shows that the amounts of FFAs extracted by the PA coating in the stirred sample vials were higher than those in the unstirred ones. This was because with stirring, the PA coating reached extraction equilibration in 10 minutes. Without stirring, the extraction process could not reach equilibrium after a 10 minute sampling time, and therefore, the amounts of acids extracted were lower. Given sufficient sampling time, the extraction in the unstirred vials would reach equilibrium and the mass loading in the PA coating would be the same as with stirring. This indicates that stirring of the gaseous samples during SPME sampling process facilitates the absorption of all the short-chain C_2-C_5 fatty acids into the PA fiber coating. *Chai et al.*[1] also found that stirring of gaseous samples shortened the absorption time of VOCs into the SPME fiber coating. Although stirring of gaseous samples would not change the diffusion of volatile compounds in the fiber coating, it provides good bulk mixing which facilitates convection of gaseous sample in air and shortens their absorption time into the fiber coating. Therefore, stirring of gaseous samples was used for subsequent experiments.

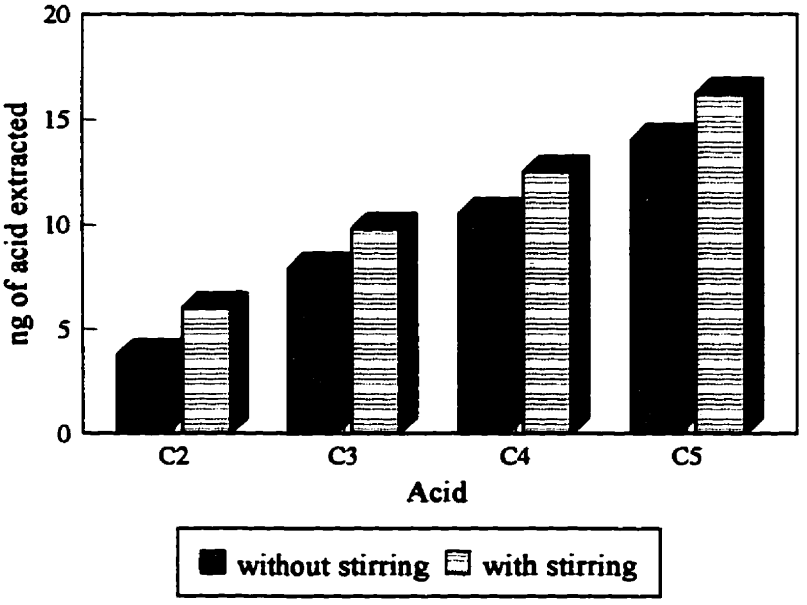

Figure 3-4. The effect of stirring versus non-stirring of gaseous samples for the analysis of acetic (C_2), propionic (C_3), butyric (C_4) and valeric (C_5) acids using the PA coated fiber.

The fiber desorption efficiency is a very important factor that affects the accuracy of results. It is necessary to maximize the amount of the analytes desorbed from the fiber so that analytes do not remain in the fiber coating and are carried over to subsequent samples. Carryover was determined by running a fiber blank immediately after the initial desorption of the analytes from the fiber. It is expressed as the percentage of the peak area of the initial desorption peak. Minimal carryover, less than 1%, was observed when both fibers with fatty acids were desorbed at 250°C for 3 minutes. To ensure the completion of fiber desorption, the desorption time of 4 minutes at 250°C was used for the experiments regarding the analysis of short-chain FFAs in air.

The coating/gas partition coefficient (K_1) of the analytes with different fibers can be calculated as follows:

$$K_1 = \frac{nV_s}{C_o V_f V_s - nV_f} \tag{3-1}$$

where V_f and V_s are the volumes of the fiber coating and gas sample, respectively; C_o is the initial sample concentration; and n is the amount of the analytes extracted by the fiber after the extraction reaches equilibrium. The calculated coating/gas partition coefficients, K_1, for C_2-C_5 acids, using both fibers, are listed in Table 3-1. Since FFAs have a larger affinity towards the polar PA coating than the non-polar PDMS coating, the K_1 values for all the acids using the PA coating are larger than those with the PDMS. The data in this table indicate that K_1 values are also associated with the polarity of the analyte; the lower the polarity (the longer the carbon chain) of the fatty acids, the larger the K_1 values. The procedure for the calculation of K_1 values is illustrated in Appendix A.

Precision describes the reproducibility of results determined by the technique. The experiments performed to estimate precision of this method used both the PDMS and PA coatings. Precision was estimated by performing 7 replicate extractions. The corresponding relative standard deviation (RSD) was then calculated for these extractions and expressed as a percent. The precisions for C_2-C_5 acids are illustrated in Table 3-2. The RSD for both fibers all fell below 8.5%, which was considered to be satisfactory. As well, the results showed good consistency between the various target compounds.

Table 3-1. **The gas/coating partition coefficient (K_f) of C_2-C_5 acids with the PDMS and the PA coated fibers.**

Compound	K_f (PDMS)	K_f (PA)
Acetic acid	NT	30,000
Propionic acid	5,000	81,000
Butyric acid	12,000	115,000
Valeric acid	NT	294,000

NT = not tested

Table 3-2. RSDs (%), fiber linear ranges and LODs for the analysis of short-chain C_2-C_5 fatty acids in air, using both the PA and PDMS coated fibers.

Compound	PA fiber linear range (ng/mL)	r^2	LOD (pg/mL) PA, GC/FID	RSD (%), n=7, 0.25 ng/mL	PDMS fiber linear range (ng/mL)	r^2	LOD (pg/mL) PDMS, GC/FID	RSD (%), n=7, 2.5 ng/mL
Acetic acid	0.25-5	0.99433	170	4.1	NT	0.99352	NT	NT
Propionic acid	0.1-5	0.99584	60	4.7	1-10	0.99491	250	3.5
Butyric acid	0.05-5	0.99889	33	5.3	0.5-10	0.99811	180	3.2
Valeric acid	0.05-5	0.99999	18	6.2	NT	0.99895	NT	NT

NT = not tested

The linearity for the isolation of C_2-C_5 acids from air using the above two fiber coatings, was also established. The results shown in Table 3-2 indicate that the linearity with both fibers was narrow. The PDMS coating had a linear range between 0.5 and 10 ng/mL, only 2 orders of magnitude for the C_3-C_4 acids. Similarly, for the PA coating, the linearity was found to cover 2 orders of magnitude, between 0.25 and 10 ng/mL for C_2-C_3 acids, and between 0.05 and 10 ng/mL for C_4-C_5 acids. The correlation coefficients were between 0.99352 and 0.99994 for all the acids tested with both fibers. As C_2-C_5 acids are very polar, at concentrations lower than 0.25 or 0.05 ng/mL, their amounts extracted by the current existing fiber coatings could not be detected by GC/FID. This difficulty may be overcome when a more appropriate sampling method is used. On the other hand, when sample concentrations were higher than 10 ng/mL, the relatively lower vapour pressures of these fatty acids resulted in acid condensation in the sample vial. Therefore, the amounts of the acids extracted by the fiber were no longer within the linear ranges. This problem may be overcome by using a more proper method of standard gaseous generation.

The limit of detection (LOD) is the minimum concentration that can be detected at a pre-set confidence level. This limit should be the minimum distinguishable analytical signal that is at least three times that of the standard deviation of the background signal.[2] The LODs for the analysis of C_2-C_5 acids using both the PDMS and PA coatings were estimated to be the concentration of the analyte which produces a signal three times that of the background noise. The estimated LODs are listed in Table 3-2. It can be seen that the PA fiber coating yielded a lower LOD than the PDMS coating for all the FFAs tested. This is expected since the PA coating, as a polar coating, extracts larger amounts of fatty acids than the non-polar PDMS fiber does, when the sample concentration is the same. The LODs with the PDMS coating were in the high pg/mL levels, whereas they were in the mid- to low pg/mL levels when the PA coating was used.

In the *National Institute for Occupational Safety and Health* (NIOSH) guide,[3] the exposure limits for the short-chain fatty acids in industrial air are at 25-30 ng/mL. The limits for indoor air exposure are usually 100 to 1000 times lower (indoor air limits of 25-300 pg/mL) than that for the occupational exposure. Direct SPME extraction of FFAs in the gaseous phase indicates that the LODs were almost all higher than the lowest required concentration levels for

monitoring indoor air exposure, but within the limits for monitoring occupational exposure, with the above two fibers. Therefore, direct SPME sampling is suitable for the analysis of short-chain fatty acids in industrial air. More sensitive methods are needed for monitoring the concentration levels of these acids in indoor air.

3.4 Analysis of Free Fatty Acids in Aqueous Solutions using Direct SPME

3.4.1 Determination of C_2-C_{10} Fatty Acids

Three fiber coatings were examined for the analysis of free C_2-C_{10} acids in aqueous solutions: PDMS, PA and Carboxen™. The Carboxen™ coated fiber was a new type of fiber coating available to our research group at the time this project started. As a porous coating containing graphitized carbon black, the Carboxen™ coating was supposed to be better for isolating polar analytes. Therefore, it was introduced for this work. As mentioned before, for a given concentration of a sample, the thicker the coating (larger volume) on the fiber the greater amounts of the analyte can be absorbed into the film. Thus, the 95 μm PA and 100 μm PDMS coated fibers, which are the thickest fibers available in the laboratory, were employed.

As mentioned before, SPME is an equilibrium process of the analyte partitioning between the fiber coating and the sample matrix. Since both matrix and coating are competing for analytes, the affinity of coatings for target analytes is crucial in SPME sampling. Therefore, coating evaluation of the above three fiber coatings was first examined. Table 3-3 compares the amounts of fatty acids extracted into the fiber coating when each of the three fibers were exposed to a solution containing 50 ppm each of C_2-C_3 acids, 20 ppm each of C_4-C_5 acids and 5 ppm each of C_6-C_{10} acids, at pH 7 condition, after the system reached equilibrium. The data demonstrates that the polarity of the fiber coating is the dominant factor that affects the SPME extraction efficiency of the polar acids. Since the PDMS coated fiber is a non-polar fiber, the acids have the least affinity towards it than to the other fibers; thus it did not extract these acids effectively. Although the Carboxen™ coated fiber was better than the PDMS coating in the extraction of polar fatty acids from the aqueous solution, it still did not provide good extraction efficiency. The

PA, as a polar coating, extracted much more of the analytes under the same conditions than the other two fibers. Therefore it was selected for the remainder of this study.

Figure 3-5 shows the exposure time profiles for the extraction of 50 ppm of C_2-C_3, 20 ppm of C_4-C_5 and 5 ppm of C_6-C_{10} acids in an aqueous solution with the PA coated fiber at pH 7 conditions. Experiments illustrated that all the C_2-C_{10} acids reached equilibration within 30 minutes under these conditions. For C_2-C_4 acids, equilibrium was reached in less than 20 minutes. It can be seen that the amounts of the short-chain C_2-C_5 FFAs extracted by the PA coating were much smaller than those of the longer chain C_6-C_{10} acids.

Table 3-3. Comparison of the PDMS, PA and Carboxen™ coated fibers in the extraction of free fatty acids: acetic, propionic, butyric, valeric, hexanoic, heptanoic, octanoic, nonanoic, and decanoic, under pH 7 condition.

Compound	total amount (ng) of acids in the sample vial	ng extracted		
		Carboxen™	PA	PDMS
Acetic acid	1500,000	ND	1	ND
Propionic acid	1500,000	ND	2	ND
Butyric acid	600,000	ND	1	ND
Valeric acid	600,000	ND	2	ND
Hexanoic acid	150,000	ND	2	ND
Heptanoic acid	150,000	1	8	ND
Octanoic acid	150,000	3	40	1
Nonanoic acid	150,000	10	148	2
Decanoic acid	150,000	51	504	5

ND = not detected

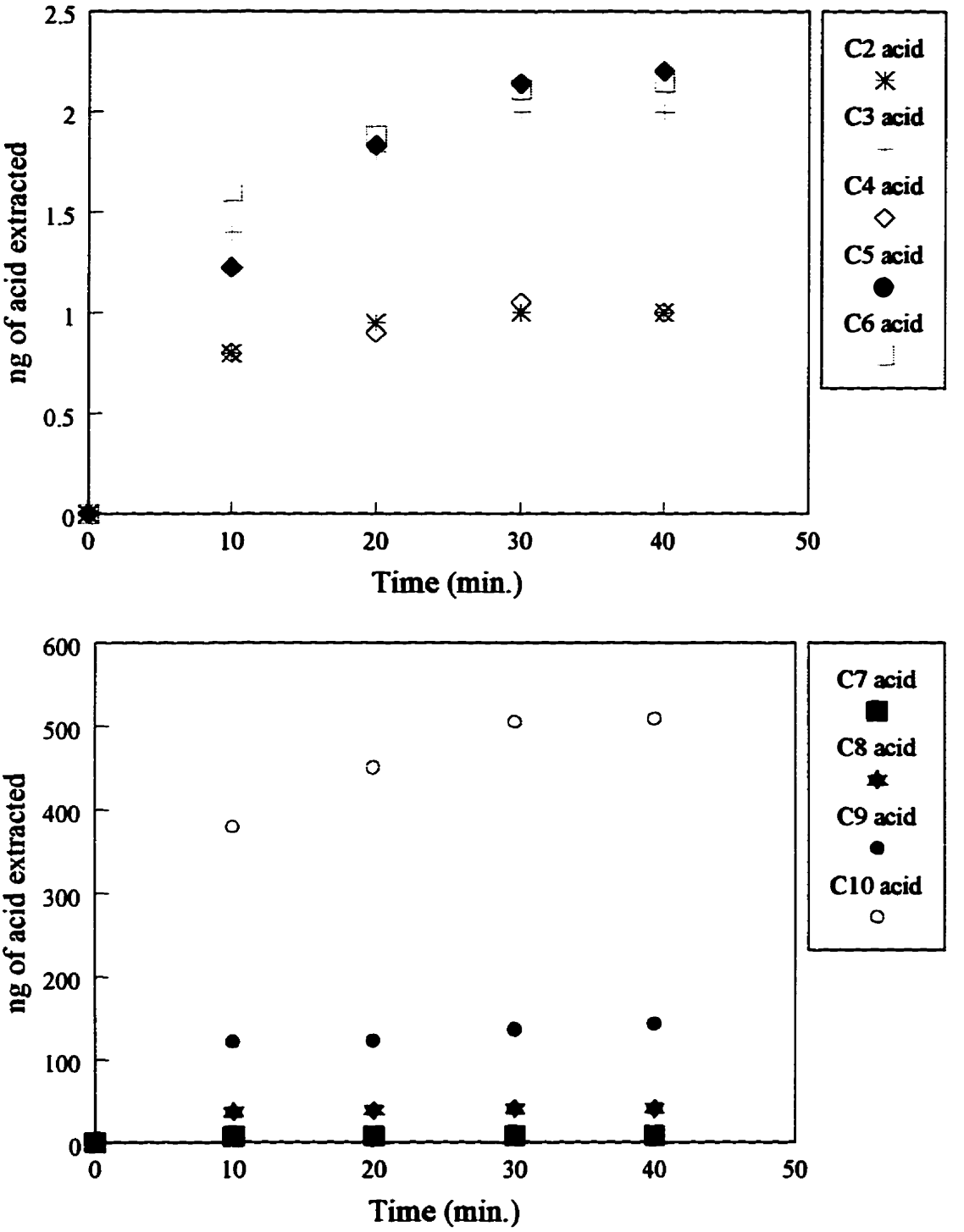

Figure 3-5. Exposure time profiles for the analysis of 50 ppm each of C_2-C_3, 20 ppm each of C_4-C_5 and 5 ppm each of C_6-C_{10} acids in aqueous solutions using the PA coated fiber.

The fiber desorption efficiency was then examined. The PA coated fiber was used to isolate 50 ppm each of C_2-C_3 acids, 20 ppm each of C_4-C_5 acids and 5 ppm each of C_6-C_{10} acids from water at pH 7 conditions. Figure 3-6 compares the amounts of C_2-C_{10} fatty acids desorbed at 250°C and 275°C with desorption times of 3-4 minutes. It shows that volatile C_2-C_5 acids can be fully desorbed at 250°C. However, for C_6-C_{10} acids, the amounts desorbed at 250°C were 30-40% smaller than those at 275°C. Therefore, a higher desorption temperature is necessary to ensure complete fiber desorption for the longer chain acids. The results demonstrate that when the fiber desorption was done at 275°C for 4 minutes, a maximum desorption efficiency was reached. Under these optimum desorption conditions, less than 0.5% carryover was found for all the acids examined.

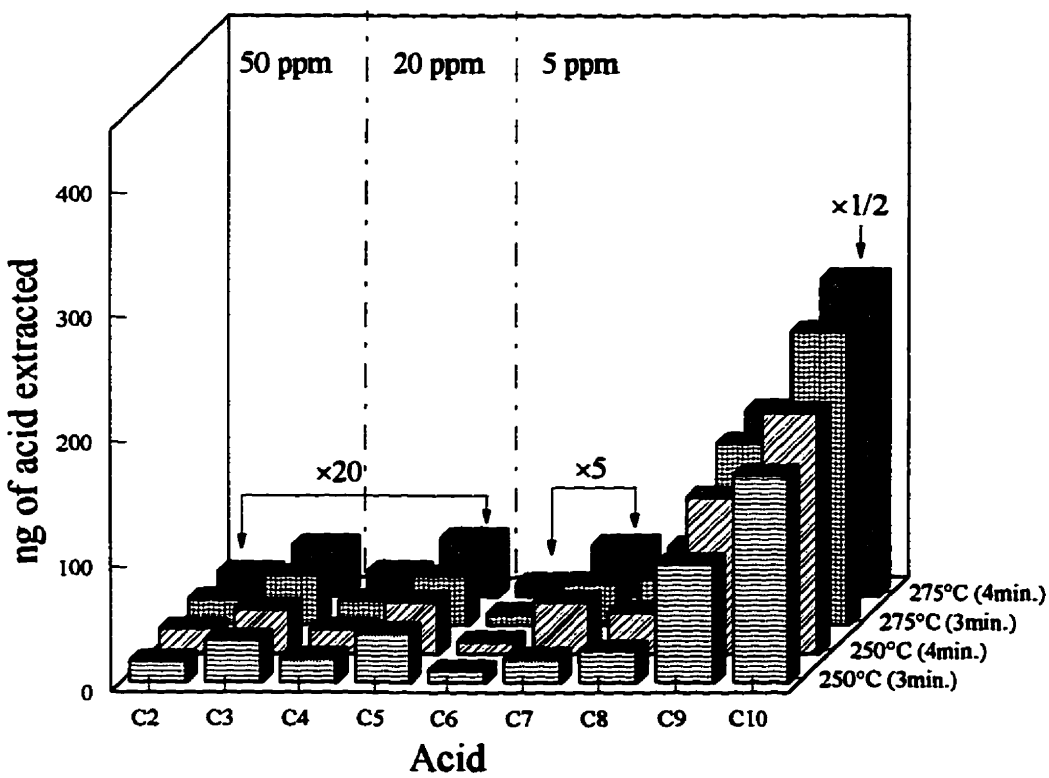

Figure 3-6. Effects of desorption temperatures and desorption times on the analysis of C_2-C_{10} acids using the PA coated fiber. Note: the data for C_2-C_5 acids, C_6-C_7 acids, and C_{10} acid were multiplied by 20, 5 and 0.5, respectively.

The addition of a strong mineral acid and NaCl to the sample matrices individually or in combination was examined as a means of increasing the amounts of acids extracted with the SPME fiber. Samples contained 20 ppm each of C_2-C_4 acids and 1 ppm each of C_5-C_{10} acids in water. Table 3-4 compares the increase factor of the amounts of fatty acids extracted under saturated salt and saturated salt at pH 1.5 conditions, to the extraction performed under pH 7 condition (control condition), using the PA coated fiber. The increase factor was calculated by comparing the amounts of acids extracted under saturated salt and saturated salt at pH 1.5 conditions with those obtained under the control conditions. A 50 minutes extraction time was used for all these tests.

Table 3-4. Matrix effect enhancements for the extraction of 20 ppm each of C_2-C_4 acids and 1 ppm each of C_5-C_{10} acids from water with the PA coated fiber.

	increase factor		
Compound	with salt	with pH 1.5	with pH 1.5 & salt
Acetic acid	2	6	80
Propionic acid	1.5	7	25
Butyric acid	11	22	306
Valeric acid	25	55	360
Hexanoic acid	9.4	15	120
Heptanoic acid	9.1	11	119
Octanoic acid	5.4	8.4	64
Nonanoic acid	2.3	7.4	24
Decanoic acid	1	6.7	8.3

The "salting out" effect achieved by the addition of saturated salt to the sample matrix increased the amounts of acids extracted by SPME. Since the water molecules prefer to solvate the salt ions,[4] the addition of salt in the sample matrix would decrease the solubility of the acids in the neutral form, which results in an increase in the amounts extracted by the fiber. The magnitude of the increase depends on the solubility of the acids. The solubilities of each of the C_2-C_{10} acids in water are listed in Table 3-5.[5,6] As the solubility of the acids in water decreases from C_2-C_{10}, the amount extracted resulting from the salt solution increases. For decanoic acid, the addition of salt produced no significant increase when compared to the pH 7 extraction conditions. This is likely due to its relatively low solubility in water.

Table 3-5. Dissociation constants of C_2-C_{10} acids and their solubilities in water.

Compound	Solubility (mg/L, 20°C)[5,6]
Acetic acid	∞
Propionic acid	∞
Butyric acid	∞
Valeric acid	37,000
Hexanoic acid	9,700
Heptanoic acid	2,400
Octanoic acid	680
Nonanoic acid	260
Decanoic acid	150

Since free C_2-C_{10} acids are polar, at pH 7, many of them are still in the ionic form, and therefore they are more soluble in water than in the fiber coating (the non-ionic polymer coating only extracts polar analytes in their non-dissociated form). By lowering the pH of the sample matrix, the acid-base equilibrium shifts towards the neutral forms of the acids which have greater affinity to the fiber and the amount extracted increases. Table 3-4 shows that at pH 1.5, the amounts of fatty acids extracted by the PA coating increased by factors ranging from 6 to 55. It is believed that the lower the pH of the matrix the higher the amount of acids is extracted by the fiber. However, the PA coating is not stable at pH < 1; therefore the pH value cannot be lower without destroying the fiber coating in the sample solution.

When both pH 1.5 and saturated salt conditions were used, the optimum enhancement of the extraction was achieved. The amounts of fatty acids extracted were increased by factors ranging from 8 to 360 fold when compared to the neutral conditions. Under these combined conditions, an equilibrium time of 50 minutes was observed for all the acids tested, compared to 30 minutes under the pH 7 control condition. This is because the sample agitation in these cases was non-ideal; therefore a static aqueous layer close to the fiber coating was present. As a result, a larger amounts of analyte had to diffuse through this static aqueous layer to reach the fiber coating, which led to a longer time for the fiber to reach the equilibrium.[5]

The calculated *K* values, method linear ranges, SPME detection limits and precisions for the analysis of C_2-C_{10} acids under the saturated salt at pH 1.5 conditions, using the PA coated fiber, are listed in Table 3-6. The *K* values were calculated using Equation 3-1. The results indicate that the *K* value is also associated with the solubility of the acids. When the solubility is decreased, the *K* value is increased.

The method's linear ranges using the PA fiber were examined. The response was determined to be linear in the entire range examined between 5 and 50 μg/mL for C_2-C_3 acids, 1 and 50 μg/mL for C_4-C_5 acids, 0.01 and 5 μg/mL for C_6 acid, and 0.001 and 5 μg/mL for C_7-C_{10} acids under the optimized matrix conditions. The correlation coefficients ranged from 0.99485 to 0.99989. Sample concentrations higher than 50 μg/mL was not tested. It was found that for C_6-C_{10} acids, when the concentration was higher than 5 μg/mL, the amounts extracted by the PA

coating under the saturated salt at pH 1.5 conditions already exceeded the FID linear response. Therefore, they were no longer within the method's linear range.

Table 3-6. **Partition coefficients *K*, method's linear ranges, SPME LODs and RSDs (%) for the extraction of C_2-C_{10} acids from water using the PA coated fiber, under the saturated salt at pH 1.5 conditions with direct SPME sampling.**

Compound	K value	method linear range (μg/mL, PA)	r^2	LOD (ng/mL), GC/FID	%RSD (n=10, 5 μg/mL)
Acetic acid	0.09	5-50	0.99485	760	5
Propionic acid	0.1	5-50	0.99714	280	4
Butyric acid	1.3	1-50	0.99928	122	3.1
Valeric acid	3.7	1-50	0.99989	3.1	2.9
Hexanoic acid	21	0.01-5	0.99982	0.5	3
Heptanoic acid	100	0.001-5	0.99791	0.11	2.7
Octanoic acid	263	0.001-5	0.99979	0.04	3.4
Nonanoic acid	376	0.001-5	0.99889	0.03	3.5
Decanoic acid	441	0.001-5	0.99676	0.02	4.5

The precision of the method was estimated by performing 10 consecutive fiber extractions with the same fiber under the same conditions. All of the analytes had a precision of 5% RSD or less, which was considered to be satisfactory.

The detection limits were calculated according to the signal to noise ratio of 5. The data demonstrates that the determination of FFAs in aqueous samples provided low ppb to ppt detection limits for C_5-C_{10} fatty acids, whereas for C_2-C_4 fatty acids the detection limits were in the high ppb level even under the optimized matrix conditions. When the required detection limits for the analysis of C_2-C_5 fatty acids in water are lower than the current technique can offer, alternative methods should be developed.

3.4.2 Determination of Long-Chain Fatty Acids

Since long-chain FFAs are biologically and clinically important, they were also included in this project. The initial experiments were carried out on the isolation of free palmitic and stearic acids in water. Palmitic and stearic acids were used as representatives since they are the most often encountered long-chain acids in biological and clinical samples.

Coating evaluation for the overnight extraction of 500 ppb each of palmitic and stearic acids in a pH 7 aqueous solution was first performed, using both the PA (85 μm) and the PDMS (100 μm) coated fibers. Figure 3-7 indicates that the PDMS coating extracted larger amounts of the acids than the PA coated fiber at pH 7. The pK_a values of these long-chain fatty acids in water are around 6.5 which is close to pH 7. Therefore, these acids have a larger affinity to the non-polar PDMS coating than the polar PA coating. This resulted in larger amounts of acids extracted by the PDMS coating compared to the PA coating. Therefore, subsequent experiments used the PDMS coating.

Figure 3-8 illustrates the exposure time profiles for the extraction of C_{16} and C_{18} acids in pH 7 solutions using the PDMS coated fiber. Samples contained 250 ppb each of C_{16} and C_{18} acids. The results indicate that the equilibration time was reached in 3 hours.

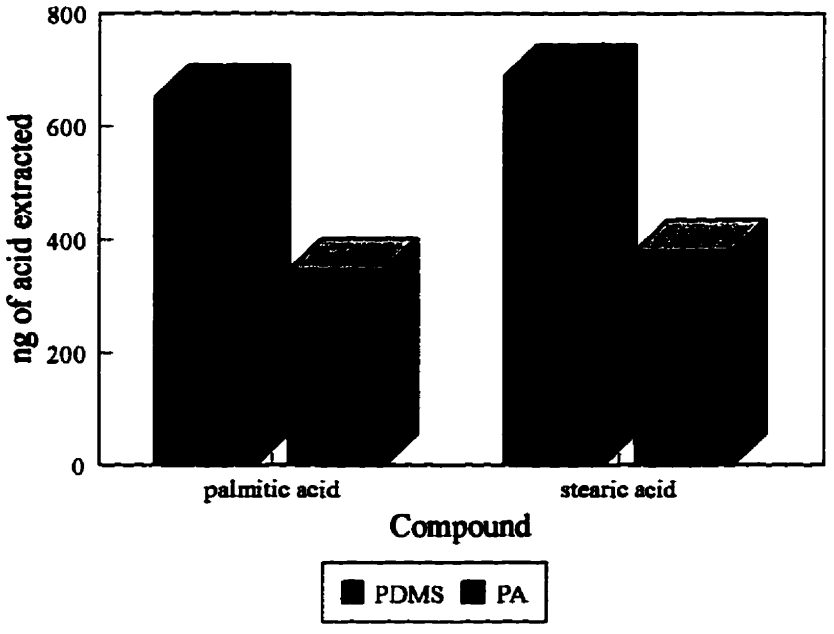

Figure 3-7. Coating evaluation for the extraction of 500 ppb each of C_{16} and C_{18} acids from water, using both the PA and PDMS coated fibers, at pH 7 conditions.

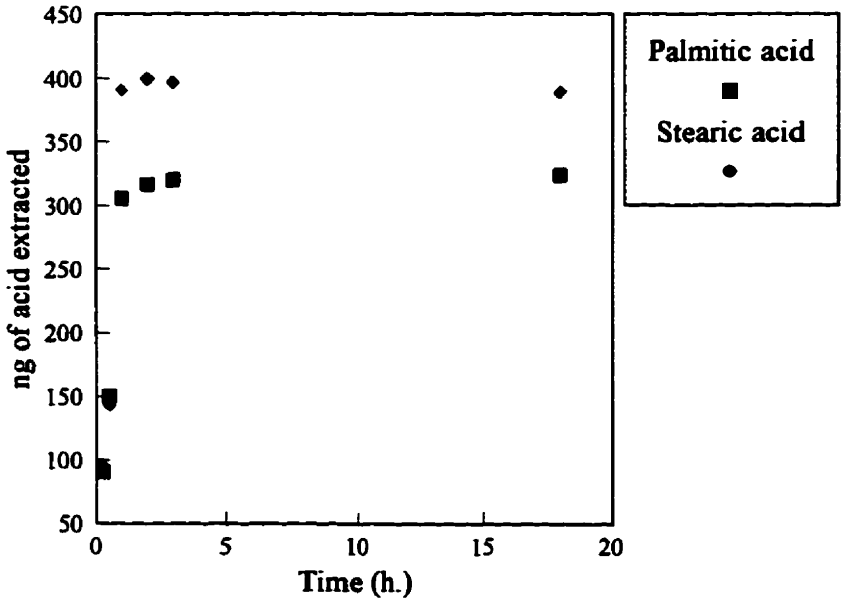

Figure 3-8. Exposure time profiles for the extraction of 250 ppb each of palmitic and stearic acids from neutral aqueous solution with the PDMS coated fiber.

The pH effect is an important factor to monitor since most of the biological and clinical samples containing fatty acids tend to be in the pH range between acidic and neutral conditions. Figure 3-9 compares the amount of C_{16} and C_{18} acids extracted using the PDMS coated fiber, under pH 7 and pH 4 conditions. Samples contained 250 ppb each of palmitic and stearic acids. It can be seen that lowering the pH from 7 to 4 resulted in a decrease in the amounts of FFAs extracted by the PDMS coating. Similar trends were also observed with the PA coating.

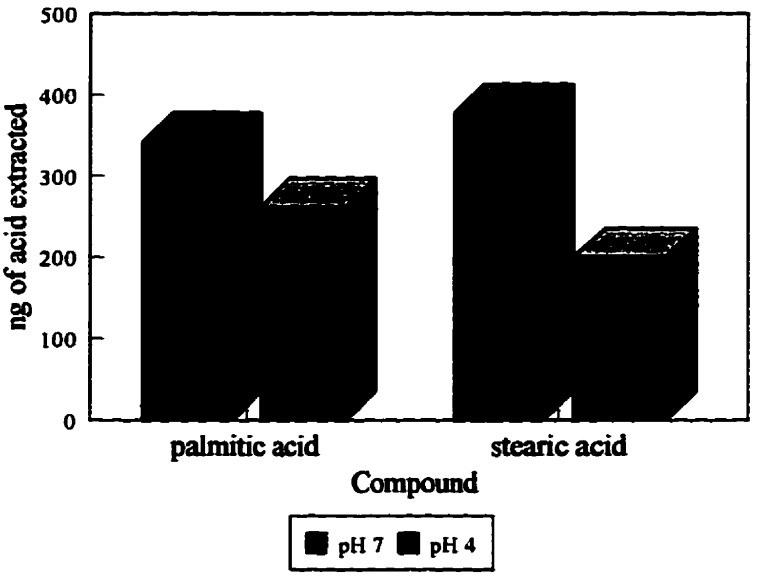

Figure 3-9. The effect of pH on the extraction of 250 ppb each of C_{16} and C_{18} acids from water using the PDMS coated fiber.

As mentioned before, the PDMS as a non-polar fiber coating extracts the polar analytes in their non-dissociated form from water. Although the pK_a values for palmitic and stearic acids in water are both very close to pH 7, lowering the pH of aqueous solutions from 7 to 4 would still increase the fraction of the acids in their non-dissociated form slightly. Therefore, the mass loading of FFAs in the PDMS coating would be expected to increase rather than decrease, which was observed for this experiment, after incorporating this process. However, lowering the pH would increase the ionic strength of the samples which would influence the activity coefficients and changing the activity coefficients would affect the activity of the analytes. In this experiment,

the lower pH of the sample matrix caused the activity coefficients to decrease for both acids, which lead to a decrease of the activity of the analyte. This resulted in a lower amount of the analyte extracted by the fiber coating. Theoretically, the partition coefficient K is described as $K=A_f/A_s$, where A is the activity of the analyte and $A=\gamma C$, where γ is the activity coefficient and C is the concentration of the analyte. In the ideal solution (dilute sample), $\gamma \approx 1$, therefore, the partition coefficient can be described as $K=C_f/Cs$. However, in this case, the sample solution can no longer be considered as ideal because of the presence of a strong acid, thus higher ionic strength of the solution. Therefore, the partition coefficient should be described as $K=A_f/A_s$. Assuming $\gamma_f = 1$, where γ_f is the analyte activity coefficient in the fiber coating, the concentration of the analyte in the fiber can be described as $C_f=K\gamma_s C_s$, where γ_s is the analyte activity coefficient in the sample solution. Decreasing the pH value of sample solution from 7 to 4 caused γ_s to decrease, thus $\gamma_s < 1$. Therefore, a decreased amount of the analyte extracted by the fiber was observed.

Figure 3-10 compares the amounts of acids extracted by the PDMS coating at saturated salt and pH 7. Since C_{16} and C_{18} acids are almost insoluble in water, adding salt into water would not be expected to increase the amounts of these acids extracted. However, adding salt again increased the ionic strength of the samples, which also resulted in a decrease of the mass loading in the PDMS coating. Therefore, this experiment showed agreement with that performed by lowering pH of the sample solution from 7 to 4. This result confirmed the previous assumption that increasing the ionic strength of sample solution would decrease the mass loading of acids in the PDMS coating. Therefore, the pH 7 condition was chosen for the remaining experiments.

The above results illustrate that direct SPME sampling could be very conveniently used to extract long-chain fatty acids from the aqueous matrix. However, the experiments indicated that the GC separation of these acids was very poor. This was due to their relatively low volatilities and incompatibilities with GC stationary phases. As a result, quantitation of the long-chain FFAs with GC was not accurate. Therefore, other feasible techniques which can improve GC properties of long-chain fatty acids are needed.

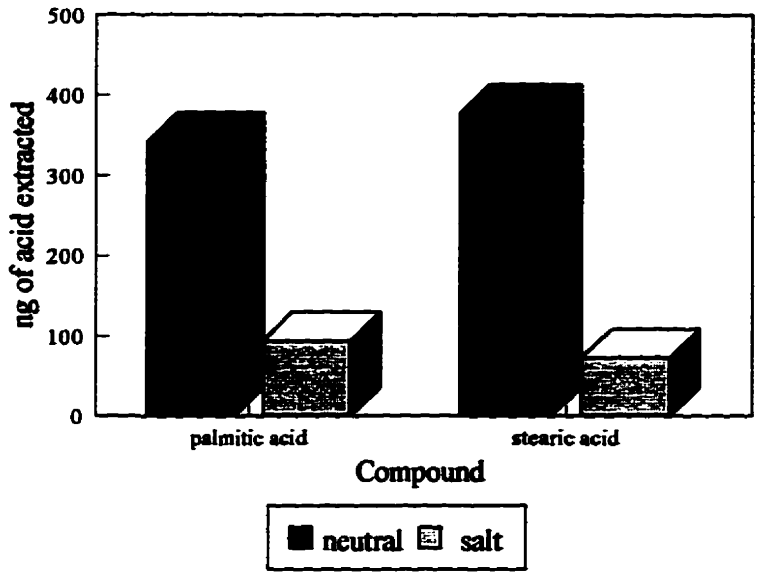

Figure 3-10. The effect of salt on the extraction of 250 ppb each of C_{16} and C_{18} acids from water with the PDMS coated fiber.

3.5 Analysis of Free C_2-C_{10} Fatty Acids in Water using Headspace SPME

Since real samples tend to contain particulate matter in relatively high concentrations, it would be undesirable to directly sample the aqueous solution. Materials from the sample matrix could coat the fiber and interfere with the extraction. To avoid this, the headspace above the sample solution can be used to sample analytes with the SPME fiber. Headspace SPME has already been used for a tremendous number of applications, including the analysis of BTEX, PAHs and petroleum hydrocarbons in aqueous and soil matrices; flavours in food and beverages; illegal drugs in urine and blood. Yet, it has not been extensively studied for polar analytes. Therefore, this technique was investigated for the analysis of C_2-C_{10} acids in water. Three fiber coatings were used: PA, PDMS and CAX(DVB) with coating thicknesses of 95, 100 and 65 μm, respectively. The CAX(DVB) coated fiber was a new type of fiber coating commercially available from Supelco. Since it contains polar OH functional groups, it would be suitable for the isolation of the target polar analytes.

Coating evaluation was first performed with the above three fiber coatings. A 40 mL vial containing 35 mL of aqueous sample, the same sample size used for direct SPME experiments,

was employed. Samples contained 50 ppm each of C_2-C_4 acids and 1 ppm each of C_5-C_{10} acids under saturated salt conditions. Figure 3-11 shows that both the CAX(DVB) and PA coatings extracted larger amounts of fatty acids than the PDMS coating using headspace SPME sampling. The experiments also indicated that the CAX(DVB) coated fiber extracted larger amounts of the shorter chain C_2-C_3 fatty acids than the PA coating. For the longer chain C_4-C_{10} acids, the PA coating extracted higher amounts than the CAX(DVB) coating. Since the PA coating extracted the majority of the target acids in larger amounts than CAX(DVB) coating, it was selected to carry out subsequent experiments.

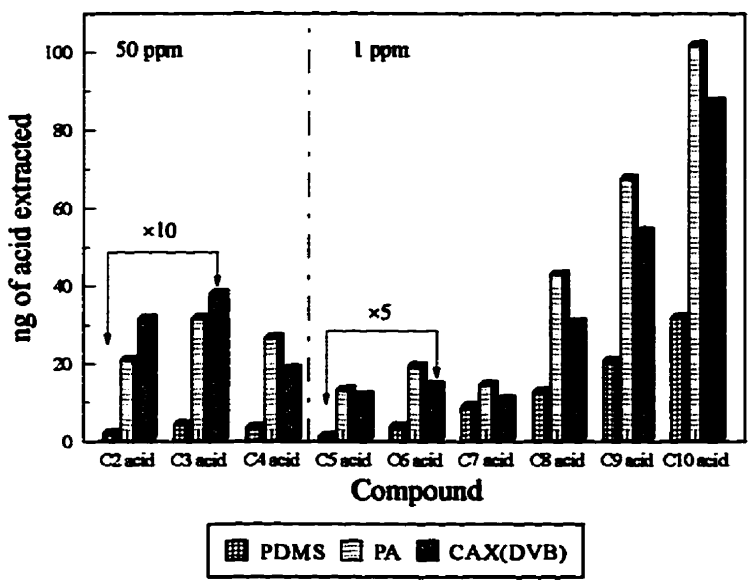

Figure 3-11. Coating evaluation for the extraction of 50 ppm each of C_2-C_4 and 1 ppm each of C_5-C_{10} acids using headspace sampling, under saturated salt conditions. Note: the data for C_2-C_3 acids and C_5-C_6 acids were multiplied by 10 and 5, respectively.

Exposure time profiles were determined for the PA fiber coating, under saturated salt conditions. The sample contained 50 ppm of C_2-C_4 acids and 5 ppm of C_5-C_{10} acids. Figure 3-12 illustrates that the equilibration time for the shorter chain C_2-C_4 acids was reached within 120 minutes. However, the equilibrium time was very long (between 6 and 13 hours) for the longer chain C_5-C_{10} acids. In fact, the equilibration times for C_2-C_{10} acids, using headspace sampling, were all longer than those for the direct SPME analysis.

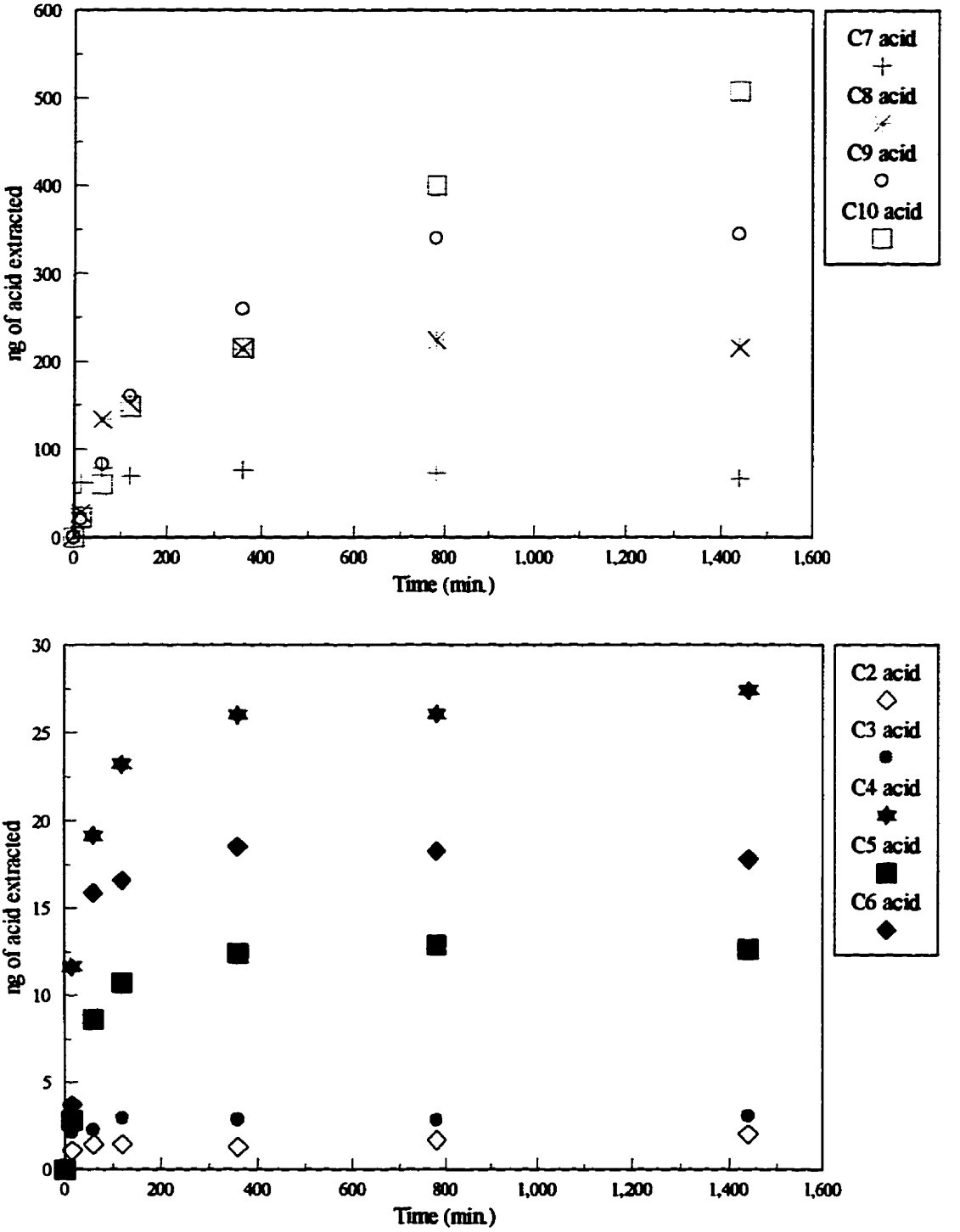

Figure 3-12. Exposure time profiles for the extraction of 50 ppm each of C_2-C_4 and 5 ppm each of C_5-C_{10} acids with the PA coating, using headspace sampling, under saturated salt conditions.

The success of the headspace SPME technique depends strongly on the mass transfer of the analytes from the aqueous phase to the headspace. As mentioned before, in a three-phase system, the distribution coefficient (K) can be defined in terms of the coating/gas partition coefficient (K_1) and gas/water partition coefficient (K_2), where $K=K_1K_2$. The shorter chain acids are expected to have larger K_2 values due to their high volatility so their mass transfer from water to the headspace would be faster. As demonstrated in Table 3-1, K_1 decreases as the carbon chain becomes shorter. Therefore, the shorter chain acids have smaller K_1 values (compared to the longer chain acids). As a result, smaller amounts of these acids would be extracted by the fiber coating, which would lead to a faster equilibrium. For the less volatile, longer chain fatty acids, they are expected to have smaller K_2 values which exemplifies a slower mass transfer from water to the headspace. As their K_1 values are relatively large, larger amounts of these acids would diffuse into the fiber coating. Therefore, the above combination effects resulted in a longer equilibration time for headspace extraction. For decanoic acid, it can be seen that even after an exposure time of 24 hours, it still has not reached equilibrium. Overall, these acids have low vapour pressures above water, thus their K_2 values are small, which leads to longer equilibration times for headspace sampling than for direct sampling.

As discussed before, the solubility of fatty acids in aqueous solution can be decreased by increasing the acidity of the sample solution. In the direct SPME method, this was used to force the fatty acids out of the water and into the fiber coating. It was believed that the lower the pH value of the sample solution, the higher amount of fatty acids can be extracted by the SPME fiber coating. Since the PA fiber coating cannot be stable in a water solution with pH < 1, the lowest pH value tested was 1.5 for direct SPME sampling. Headspace SPME sampling allows the fiber to isolate analytes in the headspace without contacting the aqueous phase. Therefore, the sample solution can be acidified even further. Figure 3-13 compares the amounts of FFAs extracted by the PA coating under the matrix conditions: pH 7, pH 4 and 1M HCl. Samples contained 50 ppm of C_2-C_4 acids and 1 ppm of C_5-C_{10} acids. At pH 7, the amounts of the acids extracted by headspace SPME method are very close to those obtained by directly extracting the aqueous phase of a 35 mL control sample. This is expected since the overall partition coefficients of the acids should be the same with the above two techniques at equilibrium, when the sample

concentrations and size, and the matrix conditions are the same. With headspace sampling the amounts of acids extracted under pH 4 and 1M HCl conditions were higher than those extracted at pH 7. Also, by acidifying the aqueous solution to 1M HCl the amounts of the fatty acids extracted by the PA coating (using headspace sampling) were higher than those obtained by direct sampling (35 mL aqueous sample) at pH 1.5. This confirmed the previous hypothesis that the more acidic the sample matrix, the more acids would be extracted by the SPME fiber coating. Acidic concentrations higher than 1M HCl were not tested since the higher acidity of the sample solution, would produce more acidic vapour in the headspace which could damage the fiber coating as well as the stainless steel tubing attached to it; thus shorten the life-time of the fiber. Higher acidic vapour concentrations could also destroy the epoxy glue used to connect the fiber to the stainless steel tubing, which could cause the fiber to fall off the SPME syringe assembly during the sampling process.

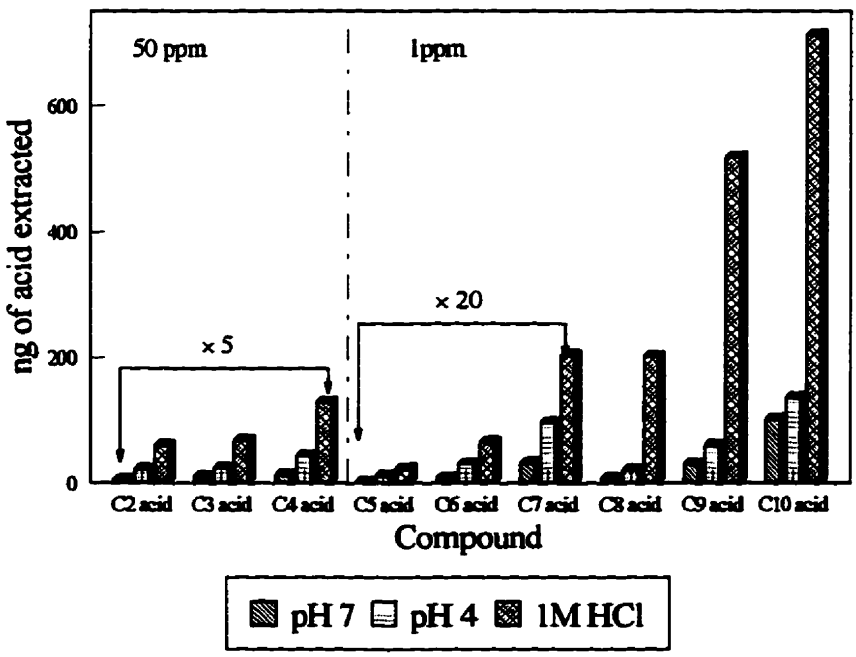

Figure 3-13. The effect of pH on the extraction of 50 ppm each of C_2-C_4 acids and 1 ppm each of C_5-C_{10} acids from water with the PA coated fiber using headspace SPME sampling. Note: the data for C_2-C_4 acids and C_5-C_7 acids were multiplied by 5 and 20, respectively, under all the above three conditions tested.

The combination of adding saturated salt and lowering the pH of sample solution could further decrease the solubility and polarity of the fatty acids in water and increase their mass transfer to the fiber coating. Figure 3-14 compares the amounts of fatty acids extracted by the PA coating under pH 7 conditions and the saturated salt with 1M HCl conditions using headspace sampling to that obtained by the direct extraction of the aqueous phase of a 35 mL sample under saturated salt at pH 1.5 conditions. It shows that the amounts of fatty acids extracted under the saturated salt with 1M HCl conditions using the headspace sampling were 1.5-5 times greater than those extracted using direct sampling under the saturated salt at pH 1.5 conditions, for most of the fatty acids tested. Headspace SPME would be useful when the target analytes have to be sampled from a very complex aqueous matrix.

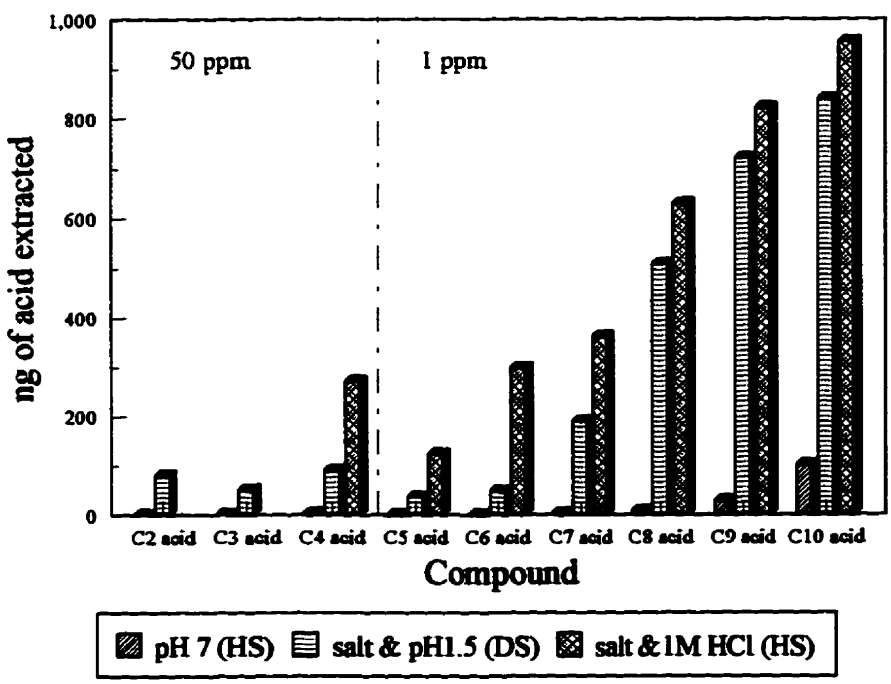

Figure 3-14. Comparison of the amounts of fatty acids extracted by the PA coating using the headspace sampling (HS), under pH 7 and saturated salt with 1M HCl conditions, to that using direct sampling (DS) under saturated salt at pH 1.5 conditions. Samples contained 50 ppm each of C_2-C_4 acids and 1 ppm each of C_5-C_{10} acids.

For C_2-C_3 acids, headspace sampling under the saturated salt with 1M HCl conditions, resulted in peaks interfering with the target area which hindered data collection for these two acids. Therefore, the comparison of the extraction efficiencies for C_2-C_3 acids, under the above experimental conditions, was not possible. Since this technique cannot be used for the analysis of very polar C_2-C_3 acids and produced no significant improvement for the analysis of C_4-C_5 acids, therefore the development of other sensitive techniques is necessary.

3.6 Comparison of Direct SPME and Headspace SPME Sampling for the Analysis of C_2-C_{10} Acids in Aqueous Solutions

Direct SPME sampling provided shorter equilibration time for all the C_2-C_{10} acids tested than the headspace SPME sampling. Since the PA coating would not be stable in the solution with pH < 1, the optimum conditions to isolate fatty acids with the direct SPME sampling was saturated salt with pH 1.5. Headspace SPME can avoid this adverse matrix effect to the fiber coating. Therefore, more acidified matrix conditions can be used, and saturated salt with 1M HCl was found to be the optimum conditions for the extraction of the above acids from water. At these optimized conditions, the amounts of the fatty acids extracted by the PA coating with headspace SPME sampling for C_4-C_{10} acids were 1.5-5 times larger than those obtained with direct SPME sampling at the optimized saturated salt at pH 1.5 conditions, when the sample concentrations tested were the same.

With direct SPME sampling, the LODs for the longer chain C_6-C_{10} acids were in the sub ppb levels, whereas for the shorter chain C_2-C_4 acids, the LODs were in the high ppb levels. Although the LODs using headspace SPME sampling have not been tested, they are expected to be similar to those for direct SPME sampling.

3.7 Summary

This chapter has presented new SPME methods for the analysis of FFAs in the aqueous and/or the gaseous phases. Direct SPME and headspace SPME sampling successfully extracted and quantified C_6-C_{10} acids in aqueous solutions with the PA coated fiber, under saturated salt and

lower pH conditions. With direct SPME, the fiber linear range covered 3 orders of magnitude with LODs in the sub ppb levels.

However, the SPME technique was not sensitive enough for the analysis of the volatile C_2-C_5 acids in aqueous and gaseous matrices because these acids are too polar to be effectively isolated in low concentrations with the existing fiber coatings. It is believed that derivatization of the short-chain FFAs to less polar esters prior to the analysis is an ideal solution to overcome these problems. The development of new techniques coupling derivatization to SPME sampling for the analysis of volatile fatty acids will be addressed in Chapter 4.

The extraction of free long-chain FFAs in aqueous solution with direct SPME sampling was successful. However, the GC separation and quantitation were poor due to the low volatility of these acids combined with their incompatibility with GC stationary phase. Therefore, the derivatization of these acids, prior to their GC analysis, is essential. Derivatization can convert fatty acids of less polar esters, thus improving their GC separation and quantitation. Derivatization in combination with SPME sampling for the analysis of long-chain fatty acids will be discussed in Chapter 4.

3.8 References

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CHAPTER 4

DERIVATIZATION/SPME TECHNIQUES FOR THE ANALYSIS OF FATTY ACIDS

4.1 Introduction

The previous studies have indicated that direct and headspace SPME were both feasible for the analysis of free fatty acids (FFAs) containing relatively long carbon chains, which are less polar and less water soluble, such as hexanoic to decanoic acids in water.[1] These techniques produced wide fiber linear ranges, typically between 0.001 or 0.01 and 5 ppm. The detection limits for these acids were in low ppb to low ppt ranges. However, for the analysis of very polar and water soluble short-chain C_2-C_5 fatty acids in water, fiber linearity of this particular group was narrow, between 5 and 50 ppm, and the detection limits were in the high ppb to ppm ranges. If lower detection limits or wider fiber linear ranges are desirable, the above two techniques are no longer suitable. Direct SPME sampling for the analysis of C_2-C_5 acids in air was also previously carried out as discussed in Chapter 3. This study indicated that this technique was applicable for monitoring short-chain acids at industrial exposure levels; however it was not sensitive enough for monitoring these acids at indoor air exposure levels. The limitations outlined above were due to the fact that the fiber coatings available during the previous studies were not polar enough to efficiently extract FFAs at very low concentrations. Therefore, other sensitive methods are required.

One way to overcome these problems is to use more polar fiber coatings so analytes will have a larger affinity towards them and extraction efficiencies can be improved. However, there was no such commercially available fiber coating found during this study. The development of new types of fiber coating was not the focus of this project; therefore other alternatives needed to be investigated. These include modifying the existing fiber coatings to make them more suitable for polar analytes and modifying the polar analytes to less polar compounds, so that they can be released from the sample matrix more readily.

The combination of SPME and derivatization for the analysis of short-chain fatty acids was then developed. Derivatization has the advantages of reducing the polarity of polar analytes and increasing their coating/water or coating/gas partition coefficients, thus increasing the SPME extraction efficiency and sensitivity of the method. Another advantage of derivatization is that compounds with low volatilities can be altered into volatile derivatives, hence improving their

chromatographic separation. The previous study using direct SPME for the determination of long-chain C_{16} and C_{18} acids showed poor GC separation and quantitation due to their incompatibilities with GC stationary phases. The derivatization/SPME technique also extends to these compounds to improve their GC behaviour.

The detailed classification of strategies using derivatization coupled with SPME is shown in Figure 4-1: a) derivatization in sample matrix, b) derivatization in SPME fiber coating and c) derivatization in GC injector port.

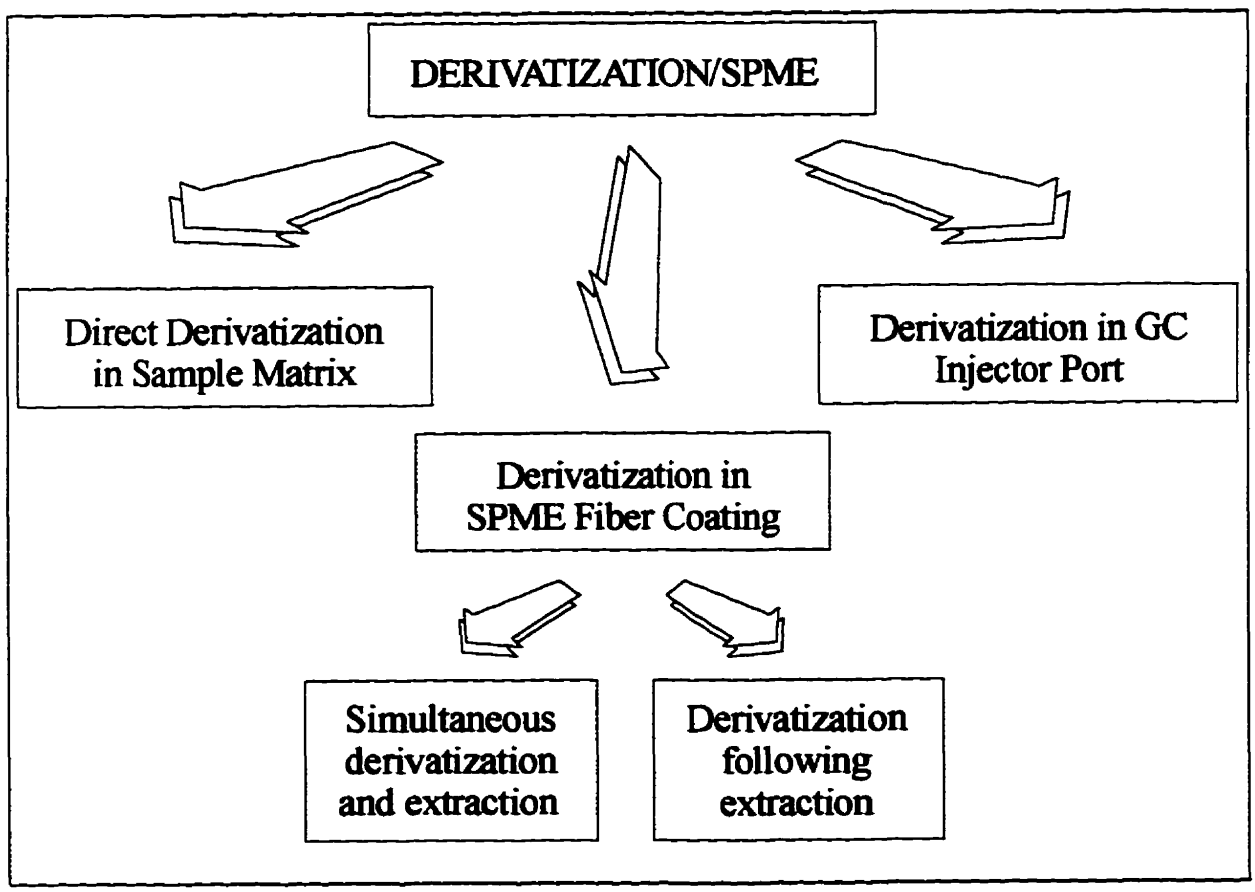

Figure 4-1. Detailed classification of derivatization/SPME techniques.

Derivatization of polar analytes in the sample matrix followed by the isolation of the derivatives formed by using SPME is the simplest way to improve SPME performance and it has been successful for the analysis of phenols in water.[2] Phenols have been converted to phenol acetates in water with acetic anhydride. This technique requires the derivatizing reagents to be relatively stable and reactive towards the target analytes in sample matrices (fluid, air, or sludge) and to produce stable reaction products. Derivatization of FFAs directly in water is quite challenging, since most of derivatizing reagents require the presence of organic media, or otherwise, they are hydrolyzed when used directly in aqueous solution. However, this technique is still possible for the analysis of FFAs in water when a proper derivatizing reagent is used.

Derivatization of analytes directly in a polymeric coating of the SPME device is a newly developed concept to implement with the SPME technique. It provides a new avenue to analyze polar analytes using SPME. It can be done in two ways. One way is to perform derivatization and extraction simultaneously by exposing a fiber containing the derivatizing reagent to the sample. The fiber coating can be thought of as liquid polymer and thus can act as an organic medium. During the partitioning, polar analytes are simultaneously extracted and derivatized to less polar derivatives that are amenable to GC analysis, thus improving the sensitivity of the method. This technique requires that the derivatizing reagent and the reaction derivatives are relatively less volatile, in order to enable the reaction and isolation of derivatives to take place in the fiber stationary phase rather than in the sample matrix. In addition to improving sensitivity, this method can also be used as an integrating sampler when the derivatization reaction is slow and quantitative. This approach eliminates the need for pre-extraction and pre-concentration prior to derivatization, thus simplifying the sample preparation procedure. The alternative way is to perform derivatization following SPME extraction of target analytes, such as long-chain fatty acids, from the sample matrices. Since long-chain acids are less volatile, derivatization will take place mainly in the SPME fiber coating.

The derivatization of FFAs in the GC injector port with an ion-pair reagent is an established technique to improve the chromatographic behaviour of polar and/or relatively non-volatile analytes. However, the combination of this derivatization technique coupled with SPME has not been explored. In this project, FFAs along with the ion-pair reagents were readily

isolated by the SPME fiber and transferred into the hot GC injector port. The pyrolysis of the ion-pair salts in the hot injector port of GC produces ester derivatives of fatty acids.

The aim of this work is to develop simple, time efficient, cost effective, selective, sensitive and solvent-free methods for the analysis of both short-chain and long-chain fatty acids in aqueous and/or gaseous matrices. This chapter presents the development of new strategies combining derivatization techniques coupled with SPME for the sensitive determination of short-chain FFAs, primarily C_2-C_5 acids, in air and water, and long-chain C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20} and C_{22} acids in aqueous solutions.

4.2 Choice of Derivatizing Reagents for the Derivatization/SPME Techniques

Fatty acids are most commonly analyzed by GC either as free acids or as derivatives. In the underivatized form, the sensitivity and reproducibility of analyses are poor.[3] For short-chain fatty acids this is because they are very polar, well soluble in water, and possibly adsorbed in the GC column, especially at the low concentration range (< 1 mmol l^{-1}).[4] For long-chain acids, this is due to their relatively low volatilities and incompatibilities with GC stationary phases that do not allow adequate GC separation. The most widely used procedure to overcome these problems is to prepare relatively volatile, thermally stable derivatives of fatty acids. In this respect, methyl esters of fatty acids are the most frequently prepared derivatives.[5] Methylation is suitable for relatively long-chain acids; however, it is not applicable for short-chain acids since significant losses may occur during sample preparation processes, due to the high volatility of short-chain fatty acid methyl esters.[6] To overcome this problem, higher molecular weight derivatives are desirable. It was reported that even official methods do not necessarily produce accurate results, especially when the samples contain low-molecular weight acids.[7,8]

As presented in Chapter 1, the most popular derivatization techniques used are acid-catalyzed or base-catalyzed esterification.[9] However, the majority of these derivatization reactions require high temperatures, the presence of organic media, and/or the presence of a catalyst which is not suitable for our proposed derivatization techniques.

There are a few characteristics that need to be taken into account when searching for suitable derivatizing reagents for this research: the reagents chosen have to be reactive towards fatty acids in the polymeric, aqueous and/or gaseous phases; they must produce reaction derivatives that are stable in the fiber stationary phase and sample matrices including fluid, air and sludge; they should provide higher molecular weight esters (as compared to methyl esters) for short-chain FFAs and volatile derivatives for long-chain FFAs. With these in mind, the following derivatizing reagents were chosen for the derivatization of short-chain fatty acid: pyrenyldiazomethane (PDAM), pentafluorobenzyl bromide (PFBBr), pentafluorophenyl-diazoethane (PFPDE), and trimethylsilyldiazomethane (TMS-diazomethane). For long-chain fatty acids, diazomethane, tetramethylammonium hydroxide (TMAOH), and tetramethylammonium hydrogen sulphate ($TMAHSO_4$) were selected.

The newly synthesized PDAM[10] was chosen first to perform in-fiber derivatization of volatile fatty acids for GC analysis because it is commercially available, relatively stable under ambient and/or humid conditions, requires mild reaction conditions, produces stable and high yields of reaction products, and has low volatility. This last aspect is necessary to keep the reagent in the fiber coating during the sample extraction and derivatization processes. The other advantage of using this reagent is that its derivatives have relatively high boiling points, which can prevent the interference from volatile compounds during the analysis, thus providing sensitive determination for short-chain fatty acids. In addition, it is always desirable to apply a universal derivatization method which can be used by GC as well as by high performance liquid chromatography (HPLC) in combination with the most frequently used detection methods. PDAM is a reagent that possesses these characteristics, as its derivatives can both be analyzed by GC and HPLC (PDAM is a fluorescent reagent). This reagent has been successfully used to derivatize short-chain dicarboxylic acids such as methylmalonic and ethylmalonic acids for GC and HPLC analysis.[11]

The introduction of electrophore groups into the fatty acid molecules increases their selectivity and sensitivity for electron capture and negative-ion MS detection. Pentafluorophenyl or pentafluorobenzyl derivatives of various functional groups are widely used in GC analysis,[9,12] owing to their excellent GC properties and electron-capturing capabilities. Short-chain C_2-C_5

acids are readily derivatized to their corresponding pentafluorobenzyl ester or pentafluorophenyl ester derivatives by use of PFBBr or PFPDE, respectively. These two reagents effectively derivatize fatty acids in the presence of water which is a distinct advantage for aqueous samples.[13] Furthermore, they provide higher molecular-weight esters than methyl esters for short-chain acids, which prevents possible losses of volatile analytes during the sampling process. The use of pentafluorobenzyl or pentafluorophenyl derivatives of carboxylic acids have been reported in the analysis of a wide range of fatty acids including short-chain acids,[14,15,16] aromatic acids,[15,17] long-chain acids,[14,15,12] and dicarboxylic acids.[15]

TMS-diazomethane is a well-known reagent that can react with carboxylic acids to produce esters. It is suggested that this reagent is a safe substitute for hazardous diazomethane.[18] The advantage of this reagent is that it can provide both methyl esters in the presence of methanol in the reaction solution, or higher molecular weight esters when higher molecular weight alcohols are used to catalyze reactions. Therefore, this reagent was selected for this study.

Diazomethane is the most popular reagent used to derivatize fatty acids. It was chosen for the derivatization of long-chain FFAs because of its high reactivity and ability to provide the most volatile ester derivatives for these acids.

TMAOH and $TMAHSO_4$ were employed for ion-pairing with long-chain fatty acids, since they are the most popular reagents used. They also provide methyl esters of fatty acids via pyrolysis of the ion-pair salt in the hot GC injector port.[19]

4.3 Experimental Section

Materials. The following acids were obtained from Aldrich Chemical Co.: acetic, propionic, butyric, valeric, and ^{13}C labelled acetic and propionic (all > 99%). ^{13}C labelled butyric acid (sodium salt) was purchased from Sigma Chemical Co. (St. Louis, MO). The C_{10} to C_{22} acids were received from UFZ Centre for Environmental Research, Leipzig-Halle, Germany. The derivatizing reagent: 1-pyrenyldiazomethane (PDAM) was purchased from Molecular Probes, Inc. (Eugene, OR) and used without further purification. 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) and trimethylsilyldiazomethane (TMS-diazomethane) were obtained from Aldrich

Chemical Co. 1-pentafluorophenyldiazoethane (PFPDE) was synthesized according to Meese's method.[20] The starting materials 2,3,4,5,6-pentafluoroacetophenone and *p*-toluenesulfonic hydrazide for the synthesis of PFPDE were obtained from Aldrich Chemical Co. Tetramethylammonium hydroxide (TMAOH, 10% in methanol) were received from UFZ Centre for Environmental Research. Tetramethylammonium hydrogen sulphate ($TMAHSO_4$) was purchased from Aldrich. The precursor, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), which releases diazomethane after reaction under alkaline conditions, was purchased from Aldrich Chemical Co. This compound is considered carcinogenic; therefore, it should be handled with caution. Diazomethane is well known as extremely toxic, highly irritating, and explosive. All reactions involving diazomethane should be carried out in an efficient fume hood and behind a sturdy safety shield. PDAM, PFPDE and TMS-diazomethane should be regarded as potentially hazardous, and skin or eye contact should be avoided. PFBBr is a lachrymator and as such a well ventilated facility should be used while dealing with this reagent. All solvents used in this study were of analytical-reagent grade. All aqueous samples were prepared with deionized water (NANOpure).

Two types of fibers were used for this work: the poly(dimethylsiloxane) (PDMS) and the poly(acrylate) (PA) with coating thicknesses of 100 and 85 μm, respectively. The details on how these fibers were conditioned were described in the experimental section of Chapter 3.

The stock standard containing 1000 μg/mL each of acetic, propionic, butyric and valeric acids for the preparation of air and aqueous samples, was prepared in the same manner as described in the experimental section of Chapter 3. The standard solution of 1000 ppm each of C_{10}-C_{22} fatty acids for the aqueous sample analysis, was prepared in methanol and diluted to the desired concentration levels with deionized water to prepare working aqueous solutions.

For derivatization of air samples and sampling with SPME, a 40 mL amber vial, acid washed and silanized prior to the sampling, was used along with a 2.5 cm × 0.8 cm magnetic stir bar (Bel-Art products) to agitate the sample during extraction and derivatization. Two microliters of the standard acid stock solution in methylene chloride were injected into the sample vial. Prior to the sampling, the sample vial was heated in a drying oven at 100°C to help vaporize the acids,

and then cooled to room temperature. A 4.6 mL sample vial with a 10 mm × 4 mm spinbar (Bel-Art products) was employed for headspace analysis of short-chain fatty acids in aqueous solutions. Two milliliters of the aqueous sample were added to the vial. A 40 mL or 4.6 mL vial containing 35 mL or 4 mL aqueous sample was used for long-chain fatty acids analysis in water. 2.5 cm × 0.8 cm or 10 mm × 4 mm stir bar (Bel-Art products) were utilized to agitate the sample during extraction and derivatization.

Synthesis of PFPDE. The synthetic route to PFPDE is presented in Figure 4-2. It mainly consisted of three steps.

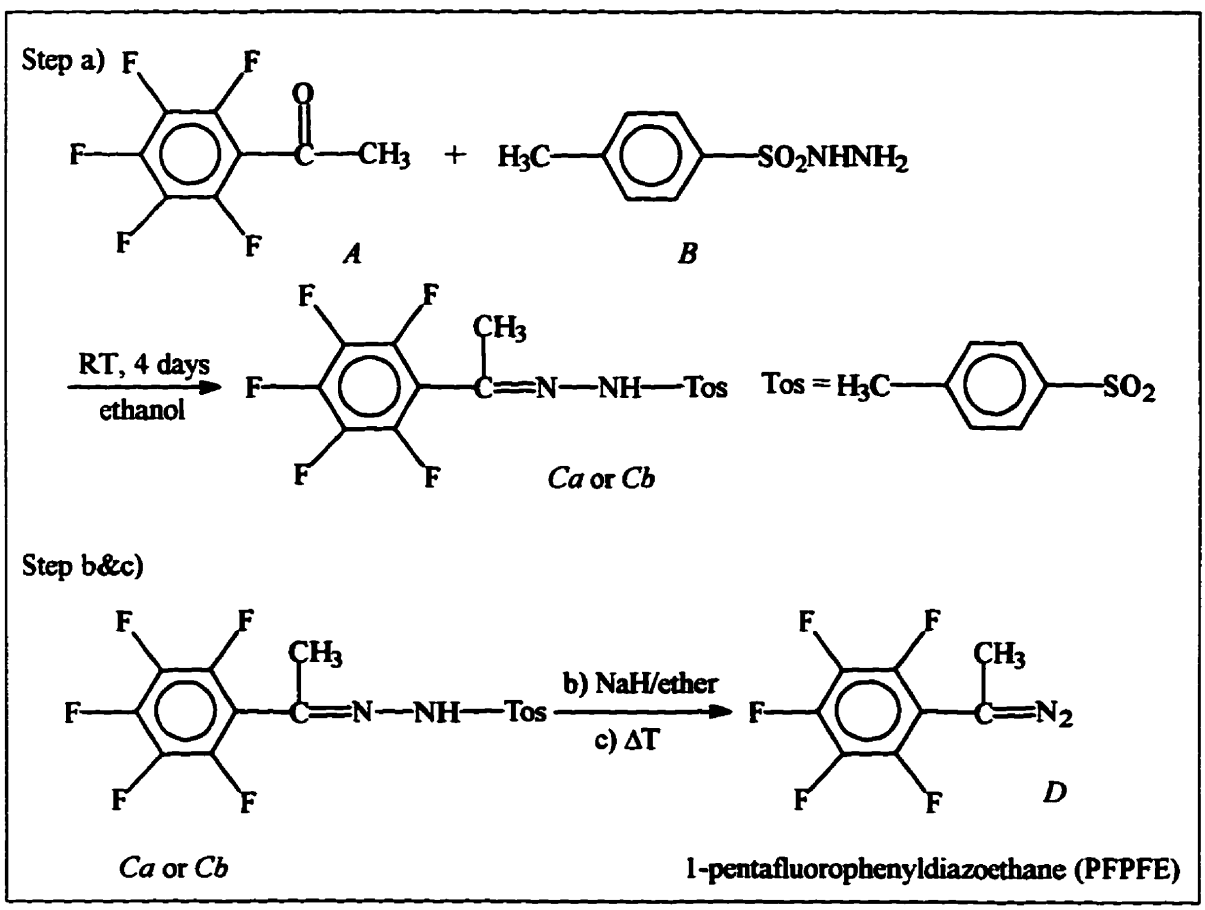

Figure 4-2. The synthetic route to 1-pentafluorophenyldiazoethane (PFPDE).

Step a). The starting materials pentafluoroacetophenone (9 g, 43 mmol) *A* and *p*-toluenesulfonic hydrazide (7.9 g, 42 mmol) *B* were added into a 400 mL round-bottom flask, along with 200 mL of ethanol and a magnetic stir bar. The white suspension was stirred at room temperature for 4 days. The solvent was removed with a vacuum rotary evaporator and a white powder/crystal residue was obtained. This powder/crystal residue was dissolved in 100 mL of boiling ethyl acetate and filtered while the solution was still hot to get rid of undissolved impurities. The mother liquor was then cooled to room temperature and yielded white crystals (isomer *Ca*) after standing at room temperature for 12 hours. These crystals were washed 3 times with 5 mL of ethyl acetate. They were then re-dissolved using hot ethyl acetate (as little as possible) and recrystallized at room temperature. After recrystallization, the mother liquor was transferred into another flask and the white crystals (isomer *Ca*) were dried under vacuum. The mother liquor was concentrated, stored at 4°C for 12 hours, and yielded off-white crystals (isomer *Cb*). The off-white crystals were re-dissolved in hot ethyl acetate and recrystallized at 4°C. After recrystallization, the mother liquor was removed and the crystals (isomer *Cb*) were dried under vacuum. Isomer *Cb* can be changed to isomer *Ca* if the raw material is heated in hot ethyl acetate for a longer period of time. Both isomers *Ca* and *Cb*, can be used for the next step reaction. The total weight of isomer *Ca* and *Cb* was 12.3 g (77 %), close to the reported yield of 80%.[20]

Isomers *Ca* and *Cb* were characterized by ^{1}H-NMR ($CDCl_3$) spectroscopy. The data for isomer *Ca* is shown in Figure 4-3. The reported ^{1}H-NMR ($CDCl_3$) data for isomers *Ca* were δ = 2.12, 2.45, 7.33, 7.86, 8.07.[20] As it can be seen from this figure, the ^{1}H-NMR ($CDCl_3$) data from this experiment are the same as the reported one.

Step b). The mixture of NaH (60% dispersion in mineral oil, 622.6 mg, 8.94 mmol) and isomers *Ca* or *Cb* (3.4735 g, 8.94 mmol) in 120 mL of dry diethyl ether was stirred under Ar at room temperature for 24 hours. Very fine solid material was formed and was dried under vacuum. The solid was washed with 20 mL of dry diethyl ether and the procedure was repeated 5 times. The solid was then dried again under vacuum to yield 3.5189 g (98%) of an almost colourless sodium salt . The reported yield for this reaction was 80%.[20]

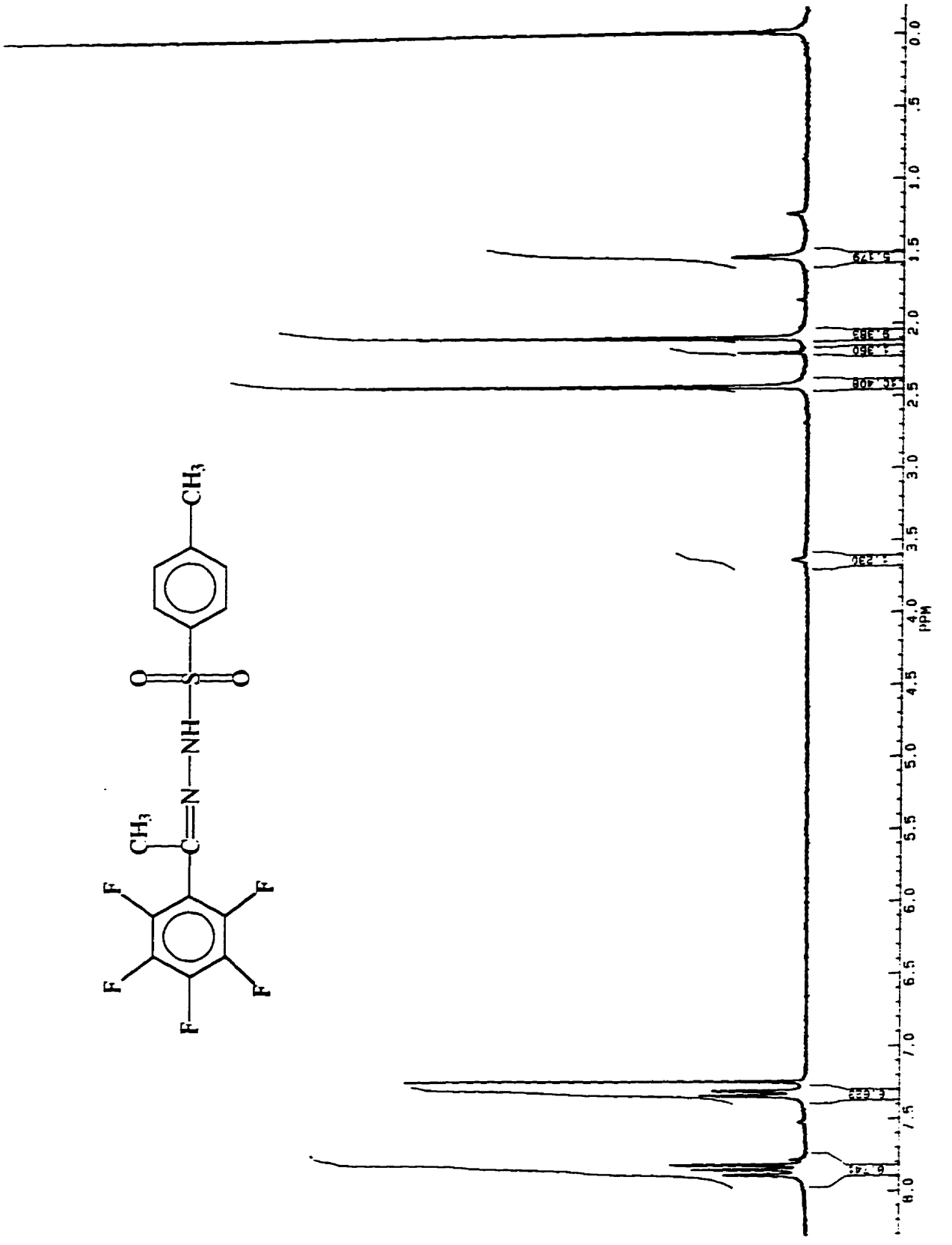

Figure 4-3. ^{1}H-NMR ($CDCl_3$) spectrum for isomer *Ca*.

Step c). Vacuum pyrolysis of the above sodium salt yielded 1-pentafluorophenyl-diazoethane (PFPDE) *D*. The above sodium salt (1.9784 g, 4.94 mmol) was added into a 25 mL round bottom flask along with a magnetic stir bar. A stick filter was connected to the flask and it was used to avoid the blow up of the sodium salt powder during the vacuum pyrolysis. The other end of the stick filter was connected with a short path distillation apparatus which had a Vigreux column in the head, a thermometer on top, and a closely coupled condenser and vacuum connection. The Vigreux indentation can increase surface area within the vapour path. This promotes better refluxing prior to condensation and yields a better separation. The condenser was connected with a rotating distillation receiver that had three outlets connected with three 5 mL flasks. These three small flasks were used to collect different fractions during high vacuum distillation. The whole system was under vacuum during pyrolysis and was wrapped with aluminium foil to protect against light, since PFPDE is light sensitive. It was not necessary to run cooling water through the condenser during the pyrolysis. The three flasks connected with the receiver adapter were cooled to -78°C with dry ice/acetone during the vacuum distillation. The flask containing the sodium salt was heated in an oil bath. The initial oil bath temperature was 80°C and it was gradually increased to 120°C within 60 minutes. At 100°C, an orange coloured gas (PFPDE) was seen and condensed as orange crystals in the receiver flask at -78°C. After pyrolysis, the condensed raw material was warmed up to room temperature (at room temperature, PFPDE is an orange coloured oil). If necessary, the above procedure can be repeated to purify the raw material. The yield was between 45-60%. PFPDE is stable at -80°C for 5 weeks and 2-3 weeks at -20°C. PFPDE was characterized by ^{1}H-NMR ($CDCl_3$) and the data are shown in Figure 4-4. It can be seen that the results obtained in this experiment are the same as the literature data which was characterized by $\delta = 2.22$ in $CDCl_3$.[20]

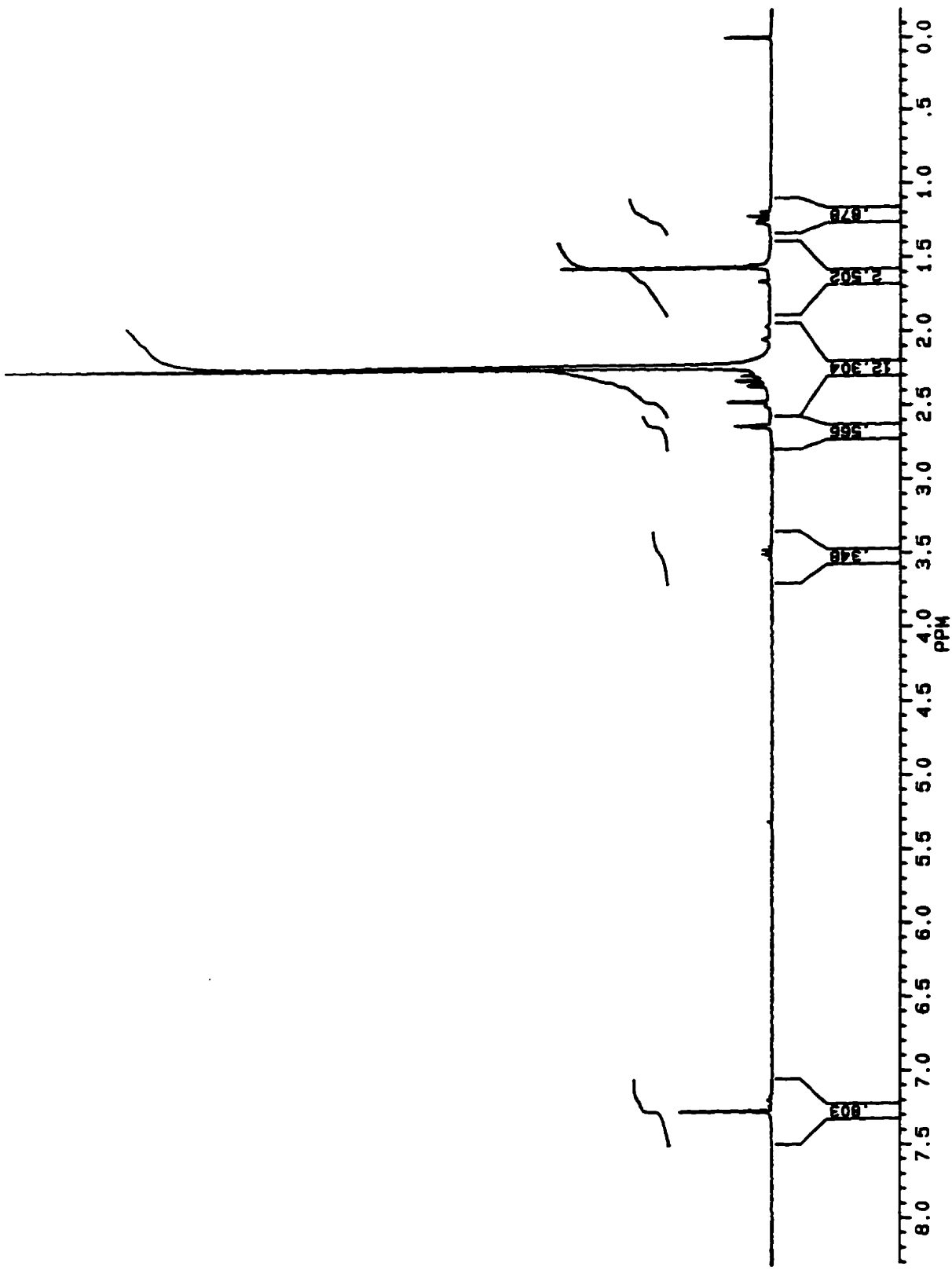

Figure 4-4. The ^{1}H-NMR ($CDCl_3$) spectrum of PFPDE.

Instruments and apparatus.

Gas chromatography/flame ionization detection (GC/FID): The same Varian 3400 gas chromatograph equipped with a flame ionization detector (FID) as described in Chapter 3, was used (Varian Canada). The column used was a 30 m, 0.25 mm ID SPB-5 with a 1 μm stationary phase (Supelco Canada). The GC conditions for the analysis of fatty acid/PDAM esters were as follows: the initial oven temperature was 40°C for 6 minutes, then programmed to 265°C at a rate of 30°C/min and from 265°C to 300°C at a rate of 25°C/min with a final hold time of 20 minutes; the FID was held at 300°C; the injector temperature was programmed from 40 to 300°C at a rate of 250°C/min for solvent injections and it was held at 300°C for SPME fiber injections; the fiber desorption time was 5 minutes. The GC conditions for the analysis of long-chain fatty acid methyl esters were as follows: the column temperature was programmed from 60 to 280°C at a rate of 20°C/min and held at 280°C for 11 minutes; the injector temperature was programmed from 60 to 275°C at a rate of 250°C/min for solvent injections and it was held at 300°C for fiber injection, the desorption time was 5 minutes; the detector was held at 300°C.

Gas chromatography/electron capture detection (GC/ECD): GC/ECD analysis was carried out using the same GC mentioned above that was also equipped with a ^{63}Ni electron capture detector (Varian Canada). The column used was the same as for the GC/FID analysis. The GC conditions were as follows: the oven temperature was programmed from 40 to 180°C at a rate of 10°C/min and then from 180 to 280°C at a rate of 20°C/min; the ECD was held at 300°C; the injector temperature was programmed from 40 to 260°C at a rate of 250°C/min for solvent injections and it was held at 250°C for SPME fiber injections, and the fiber desorption time was 4 minutes.

Gas chromatography/ion trap mass spectrometry (GC/ITMS): Gas chromatography/mass spectrometry (GC/MS) analysis was carried out on a Varian Saturn II system which included a Varian 3400 gas chromatograph coupled to an ion trap detector (Varian Canada). The column used was a 30 m, 0.25 mm ID SPB-5 with a 0.25 μm stationary phase (Supelco Canada) for the analysis of PDAM derivatives and methyl esters of long-chain fatty acids, or a 30 m, 0.25 mm ID Omegawax with the film thickness of 0.25 μm (Supelco Canada) for the analysis of the PFPDE

and PFBBr derivatives. The same GC conditions as for the GC/FID analysis were used to carry out the quantitative analysis of the PDAM derivatives, whereas the same conditions as for the GC/ECD analysis were employed to complete the quantitative analysis of the PFPDE and PFBBr derivatives. The transfer line temperature was set at 280°C. For the analysis of methyl esters of long-chain fatty acids, the initial column temperature was 50°C for 3 minutes and ramped to 270°C at a rate of 7°C/min. The injector and transfer line were held at 300 and 250°C, respectively, for fiber injections. The fiber desorption time was 4 minutes. A mass range of 45-400 amu was scanned for all GC/ITMS analyses.

Derivatization Procedure.

Simultaneous in-fiber derivatization/SPME: The simultaneous in-fiber derivatization/SPME of C_3-C_4 acids in air and water was carried out with PDAM. The set-up of this technique is shown in Figure 4-6.

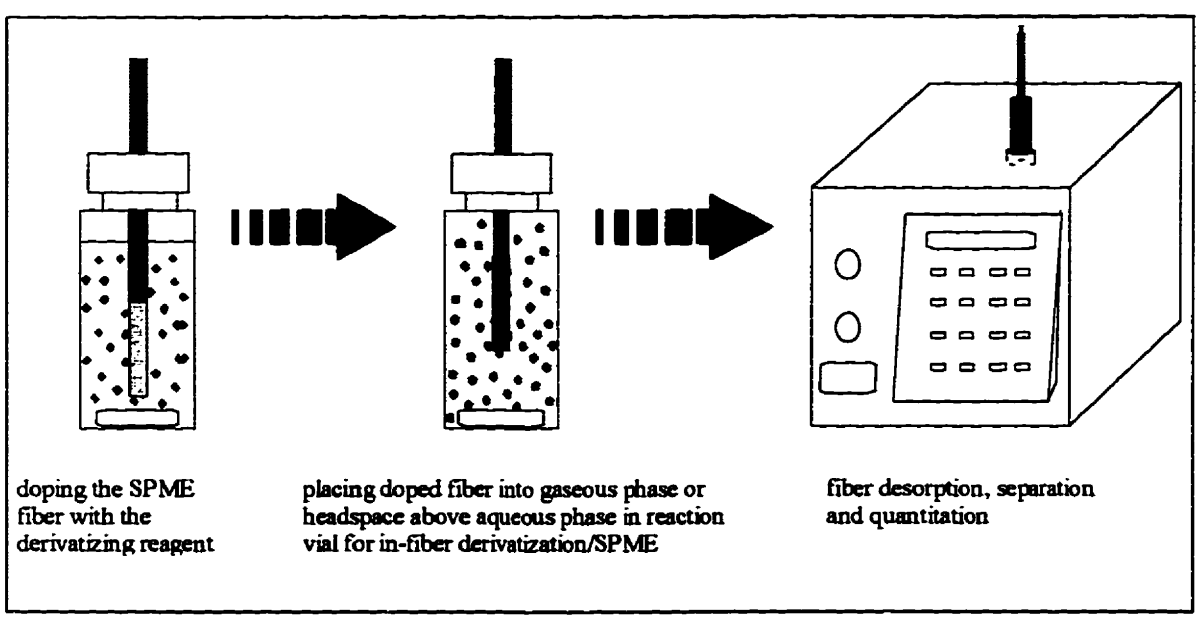

Figure 4-6. The experimental set-up for simultaneous in-fiber derivatization/SPME technique.

Prior to derivatization, a PA coated fiber was placed in a 5 mg/mL PDAM/hexane solution for 30 minutes to coat PDAM into the fiber stationary phase. A similar procedure was also used for the PDMS coated fiber. Since the PDMS fiber has a lower absorption capacity towards PDAM, 30 mg/mL derivatizing reagent was used in order to have the same amount of PDAM in the PDMS fiber coating as in the PA fiber. For the derivatization of air samples, the doped fiber was inserted into the sample vial which contained fatty acids in the gaseous phase. The analytes were allowed to partition into the fiber coating and react with the derivatizing reagent at 25°C and/or 60°C for a specific time period. Similarly, for the derivatization of aqueous samples, the doped fiber was introduced into the headspace above the aqueous solution. The sample matrix was modified to saturated NaCl salt and pH 1.5 conditions. Reactions were tested at both 25°C and 60°C. For sewage sample analysis, 10 mL of deionized water was added to 10 mL of sewage sludge in a 40 mL vial along with a magnetic stir bar. Saturated salt and pH 1.5 conditions were used to modify the sample matrix. Internal standards of ^{13}C-labelled C_2-C_4 acids were added to the same sample solutions. Since PDAM is sensitive to light, all vials containing PDAM were wrapped with aluminium foil to keep them in the dark at all times.

Reactions of C_2-C_5 fatty acids with PDAM in hexane were carried out for syringe calibration. A 500 μL of 5 mg/mL PDAM/hexane solution was added into a 500 μL hexane solution containing fatty acids with the concentration ranging from 2-500 ppm. The solution was mixed with a magnetic stir bar at room temperature for 24 hours, at which time the GC area counts of target peaks would no longer increase and remained constant. The solution (0.2 μL) was injected onto the GC column. GC area counts collected from each injection versus the amount of the acids in nanogram (*ng*) that reacted with PDAM were used to plot the calibration curve for external calibration of the in fiber derivatization. The correlation coefficients of the syringe calibration curves ranged from 0.99581 for C_2/PDAM ester to better than 0.99794 for C_3-C_5/PDAM esters when GC/FID was used. The correlation coefficients were better than 0.99893 for all of C_2-C_5/PDAM esters while GC/ITMS was employed. The masses of the solutes extracted by the fiber coatings were all within the calibration ranges to ensure accuracy of the technique.

***Post-derivatization following SPME extraction*:** The post-derivatization following SPME extraction of the long-chain C_{10}-C_{22} acids from aqueous solutions was carried out with diazomethane. The set-up for this technique is illustrated in Figure 4-7. Prior to derivatization, a PA coated fiber was placed in a 40 mL sample vial to isolate long-chain fatty acids. After extraction equilibrium was reached, the fiber was transferred into the headspace of another vial which contained diazomethane/ether to carry out the reaction. The reaction was completed within 20 minutes.

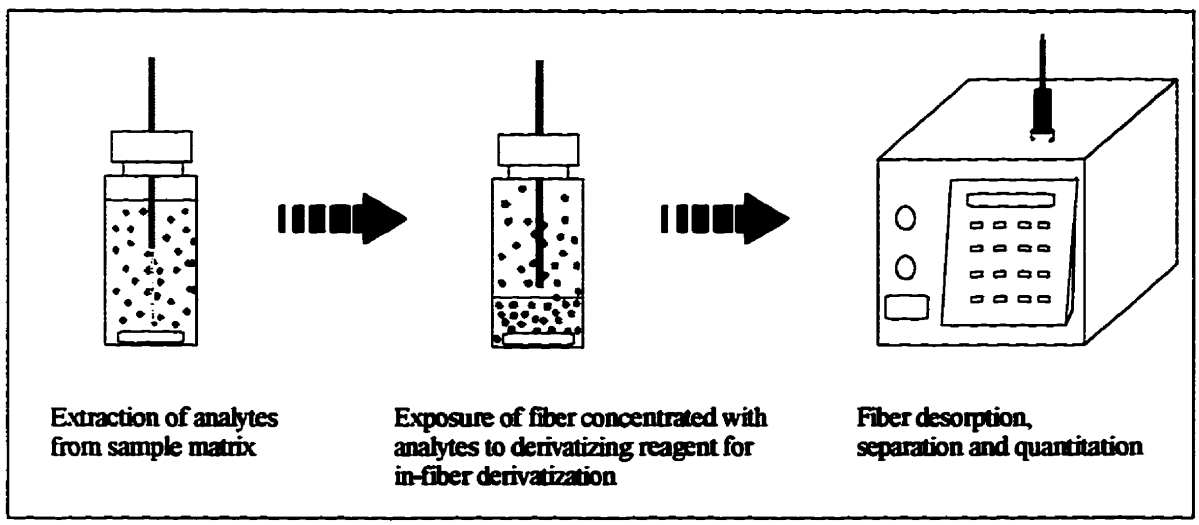

Figure 4-7. The experimental set-up for post-derivatization following SPME technique.

Similarly, reactions of the long-chain FFAs with excess amounts of diazomethane in ether were used to test the syringe calibration curve. Samples contained long-chain FFAs ranging from 50 to 1000 ppm. Reactions were monitored at room temperature and completed within 2 hours. The correlation coefficients were between 0.99157 and 0.99439 for all the methyl esters of long-chain fatty acids when GC/FID was used. Masses extracted by fiber coatings were also within the mass ranges of the calibration curves.

In-matrix derivatization/SPME: The set-up of this technique for derivatization of both gaseous and aqueous samples is demonstrated in Figure 4-8.

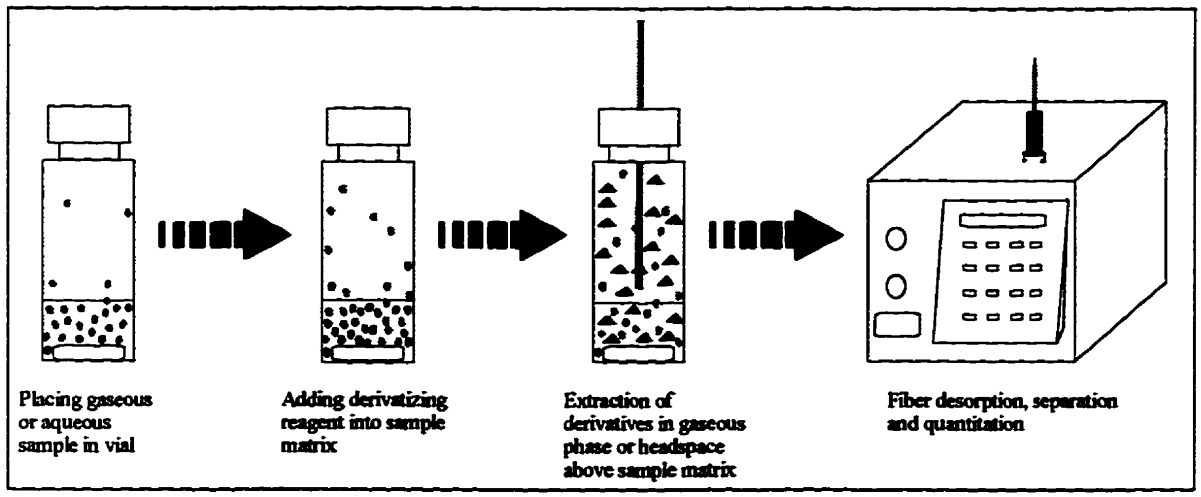

Figure 4-8. The experimental set-up for in-matrix derivatization/SPME technique.

In-matrix derivatization/SPME for the analysis of C_2-C_5 acids in air was carried out with PFPDE. Microliter amounts of the standard fatty acid mixture were spiked into 40 mL silanized amber vials. After FFAs were fully vaporized, 1 μL of 6.2 mM PFPDE/toluene solution were spiked into the vial and the reaction took place at room temperature. A fiber was then placed in the reaction vial to extract reaction derivatives. Reactions of PFPDE with C_2-C_5 acids in the concentration ranging from 5 to 500 ppb in ether were used to carry out the syringe calibration and the external calibration for the fiber extraction and analysis by GC/ECD. The correlation coefficients were between 0.99583 and 0.99829 for all the short-chain acid/PFPDE esters. Masses extracted by fiber coatings were also within the mass ranges of the calibration curves.

The in-matrix derivatization of C_2-C_5 acids in aqueous solutions was examined with both PFPDE and PFBBr.

In-solution derivatization with PFPDE: The short-chain FFAs were dissolved in water. Two milliliters of this solution with concentrations ranging from 1 to 500 ppb were transferred into a 4.6 mL silanized vial. Five microliters of 4.7 mM PFPDE/toluene solution was added. Then a PA coated fiber was placed in the headspace of the sample vial to extract the derivatives.

In-solution derivatization with PFBBr: The short-chain FFAs, along with one molar equivalent of potassium carbonate and excess amount of PFBBr, were dissolved in water in a sealed reaction vial. The reaction vial was then placed in a water bath at 55°C for 3 hours. After the reaction, the vial was taken out of the water bath and cooled to room temperature. A 2 μL aliquot was transferred into a 4.6 mL sample vial. A PA coated fiber was introduced into the headspace above the aqueous solution to extract reaction derivatives. Reactions of 1-500 ppb C_4-C_5 acids and one equivalent of potassium carbonate with PFBBr in acetone at 55°C, for 3 hours, were used to carry out calibration by GC/ECD. The correlation coefficients were 0.99255 and 0.99625 for C_4 and C_5 acid/PFBBr esters, respectively. Masses extracted by fiber coatings were also within the mass ranges of the calibration curves.

The derivatization of waste water samples with both PFPDE and PFBBr was done using the same procedure as described above.

Injector port derivatization/SPME: The set-up of this technique was similar to that for in-matrix derivatization/SPME. This technique was investigated using long-chain fatty acids and ion-pair reagents TMAOH and $TMAHSO_4$. Fatty acids and ion-pair reagents were dissolved in 4 mL water in a 4.6 mL vial. After a magnetic stir bar was added into the solution, the PA coated fiber was placed in the aqueous phase to isolate the ion-pair salts. After the desired extraction time was reached, the PA coated fiber was transferred into the hot GC injector port for desorption, derivatization, separation and quantitation.

The syringe injections of fatty acids with excess amount of ion-pair reagents showed very poor quantitation (all the free long-chain fatty acids were found in the GC/ITMS chromatograms). Therefore, the calibration curve was determined by injecting methyl esters produced from reactions of fatty acids ranging from 50 to 1000 ppm with CH_2N_2 in ether. The correlation coefficients were between 0.99463 and 0.99991 for all the methyl esters of long-chain fatty acids analyzed using GC/ITMS. Masses extracted by fiber coatings were within the mass ranges of the calibration curves.

4.4 SPME/Derivatization of Short-Chain Fatty Acids in Air

4.4.1 In-Fiber Derivatization with PDAM

The derivatization/SPME technique was first examined with PDAM for the derivatization of C_2-C_4 acids in air. Since PDAM is a relatively non-volatile reagent, in-fiber derivatization by introducing the SPME fiber doped with PDAM reagent into reaction vial was carried out. Both the PA and PDMS coated fibers with film thicknesses of 85 and 100 μm, respectively, were used.

According to the literature,[11] ethyl acetate was the best solvent to carry out reactions of acids with PDAM; therefore ethyl acetate was first used to find out the retention times of the target peaks. These tests indicated that a C_2 acid/PDAM ester peak was always seen in all the solvent injections for the reactions of C_3 or C_4 acid with PDAM without the presence of C_2 acid in the reaction solutions. These were likely due to the ester-exchange reactions between the solvent ethyl acetate and C_3 or C_4 acid/PDAM ester during the prolonged reaction times. Therefore, other solvents had to be considered. Hexane was the second choice in the reference and it was employed in the subsequent experiments. After hexane was used, no C_2 acid/PDAM ester was found in reactions of other acids with PDAM.

Next, absorption of PDAM with both the PA and PDMS coated fibers was examined. Fibers were placed in a 2 mL vial containing 1.5 mL of 5, 20 and 30 mg/mL PDAM/hexane solution. The PDAM/hexane solution was stirred with a magnetic stir bar during the fiber absorption process. An exposure time profile was used to monitor the time at which both fibers absorb enough amounts of reagent for derivatization. The solvent calibration of PDAM/hexane was used to calibrate the fiber injection. It was found that 3.4 μmol of PDAM, a 45 times excess for derivatization of 5 ng/mL of C_3 and C_4 acids (200 ng each in a 40 mL vial) in air, was absorbed by the PA coating after it was soaked in 5 mg/mL PDAM/hexane for 30 minutes. However, in order to have the similar amount of PDAM in the PDMS coating, the PDMS fiber needed to be doped with 30 mg/mL PDAM/hexane for 30 minutes. This indicated that the PDMS coating had lower absorption capabilities than the PA coating towards PDAM.

It was also noticed that PDAM was suspended in the hexane solution after its concentration was higher than 1 mg/mL. This however, did not seem to affect the consistency and reproducibility for absorption by either of the two fiber coatings. The precisions (%RSD for 10 replicates) for the absorption of PDAM by the above two coatings were below 10%.

Since the PDMS coating swelled in hexane, the surface of this coating scratched against the syringe needle when the fiber was withdrawn into it after SPME sampling. To avoid this, the cap of the vial containing PDAM/hexane was loosened after sampling and the PDMS fiber was directly taken out of the PDAM/hexane solution without withdrawing it into the syringe needle. The hexane was allowed to evaporate in air for a few seconds. The PDMS fiber was then able to be drawn back to the syringe needle without being scratched and subsequent desorption and analysis were performed.

The previous experiments indicated that it was necessary to acid wash the sample vials and silanize them with 10% of dimethyldichlorosilane/toluene solution prior to introducing air samples into them. This was done to prevent possible adsorption of the volatile fatty acids onto the glassware during the SPME sampling.[1] Therefore, all the vials used to carry out the derivatization of the volatile FFAs in air throughout this chapter were acid washed and silanized prior to use.

The SPME fiber desorption temperatures and times were determined. It was found that less than 2% of carryover was reached when the PA and PDMS coated fibers with target analytes were desorbed in the GC injector at 300°C for 5 or 4 minutes, respectively.

Figure 4-9 shows the reaction time profiles for the derivatization of 0.5 ng/mL each of propionic and butyric acids in the doped PA and PDMS coated fibers. Reaction and extraction took place in the fiber stationary phase at the same time. This figure illustrates that reactions of short-chain FFAs with PDAM in both of the above fiber coatings were very slow. It took 3-4 hours for the reaction to be complete in both fibers. It can be seen that it was an exhaustive reaction in which 96-99% of the acids were converted into their PDAM/esters in the SPME fiber stationary phase after derivatization and extraction. Because of its non-volatile characteristics, PDAM is ideal as an integrating reactor to monitor acids in air.

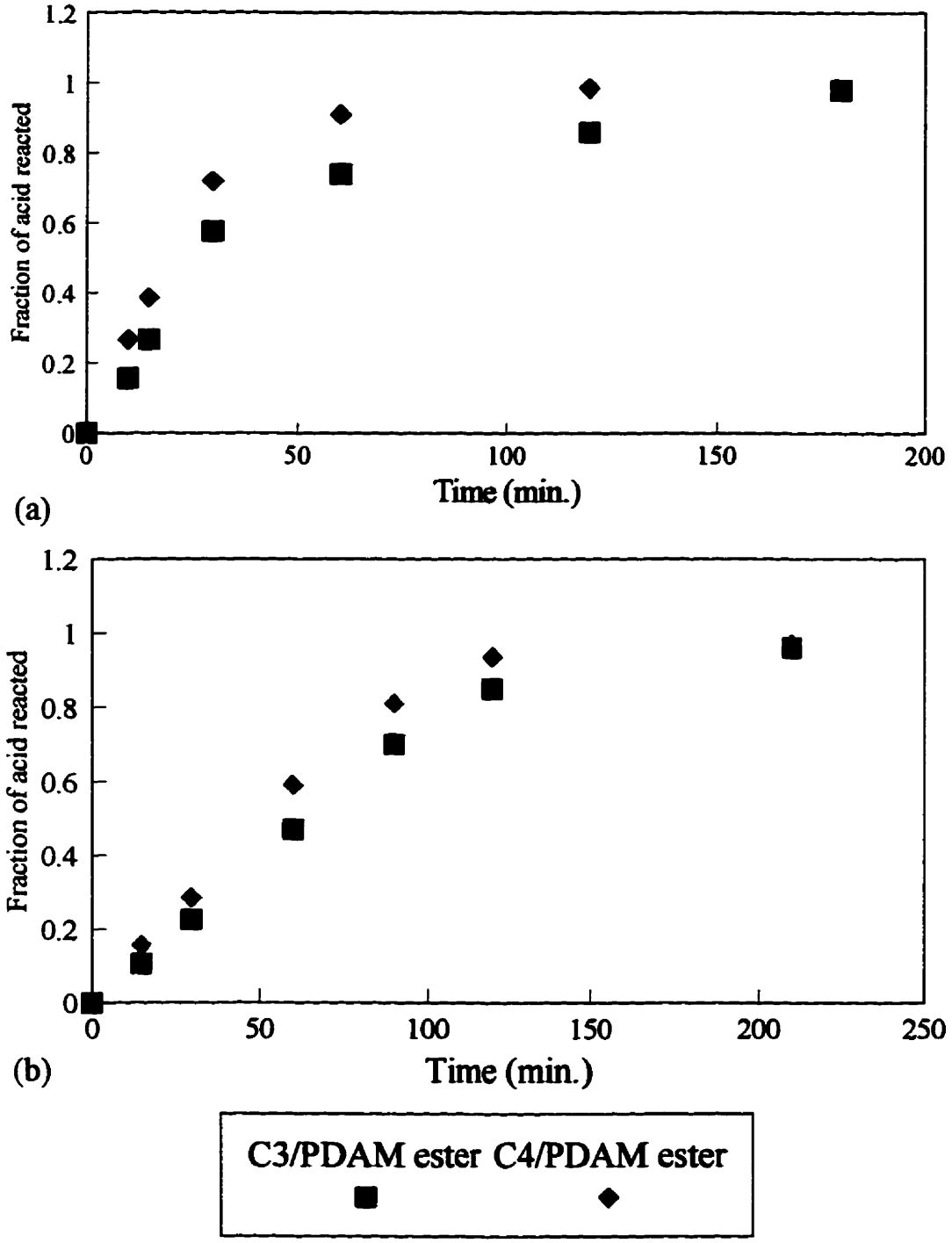

Figure 4-9. Reaction time profiles for the derivatization of 0.5 ng/mL each of gaseous C_3 and C_4 acids with PDAM in the doped (a) PDMS and (b) PA coated fibers at room temperature.

Since in-fiber derivatization of C_3 and C_4 acids with PDAM at room temperature was slow, derivatization at a higher temperature of 60°C was examined. Higher temperatures usually increase the reaction rate and shorten the reaction time. The 40 mL sample vial was immersed in an oil bath heated to 60°C. The doped fiber was then placed in the sample vial to carry out derivatization. The comparison of reaction time profiles for the derivatization of propionic and butyric acids with PDAM in the doped PA and PDMS coatings at 25°C and 60°C is illustrated in Figure 4-10.

It can be seen that heating did not increase the reaction rate, but rather decreased it slightly. This occurs because absorption of the analytes into the fiber is an exothermic process and heating the sample and its surroundings decreases the partition coefficients of the analytes in the fiber coating.[21] Zhang *et al.* also found that heating the BTEX sample during headspace SPME sampling decreased partition coefficients for those compounds.[21] These decreased partition coefficients resulted in a reduced mass loadings of the analytes in the fiber coating at higher temperature. This led to a lower reaction rate compared to reactions that were performed at room temperature. From Figure 4-10 it can also be seen that the yields of reaction at 60°C are slightly lower (between 92-94%) than those at 25°C (96-99%) for both the PA and PDMS coated fibers. This indicates that decomposition of reaction derivatives or desorption of PDAM esters from the fiber coating may also be possible when derivatization is performed at higher temperatures.

4.4.2 Reaction Kinetics for the In-Fiber Derivatization with PDAM

To better understand the in-fiber derivatization technique, the reaction kinetics for the derivatization of gaseous propionic and butyric acids with PDAM in both the PA and PDMS coated fibers was examined. The magnitude of the reaction rate constant, k, for different acids with the different polymeric coatings must be addressed because it is the main factor that controls the derivatization reaction.

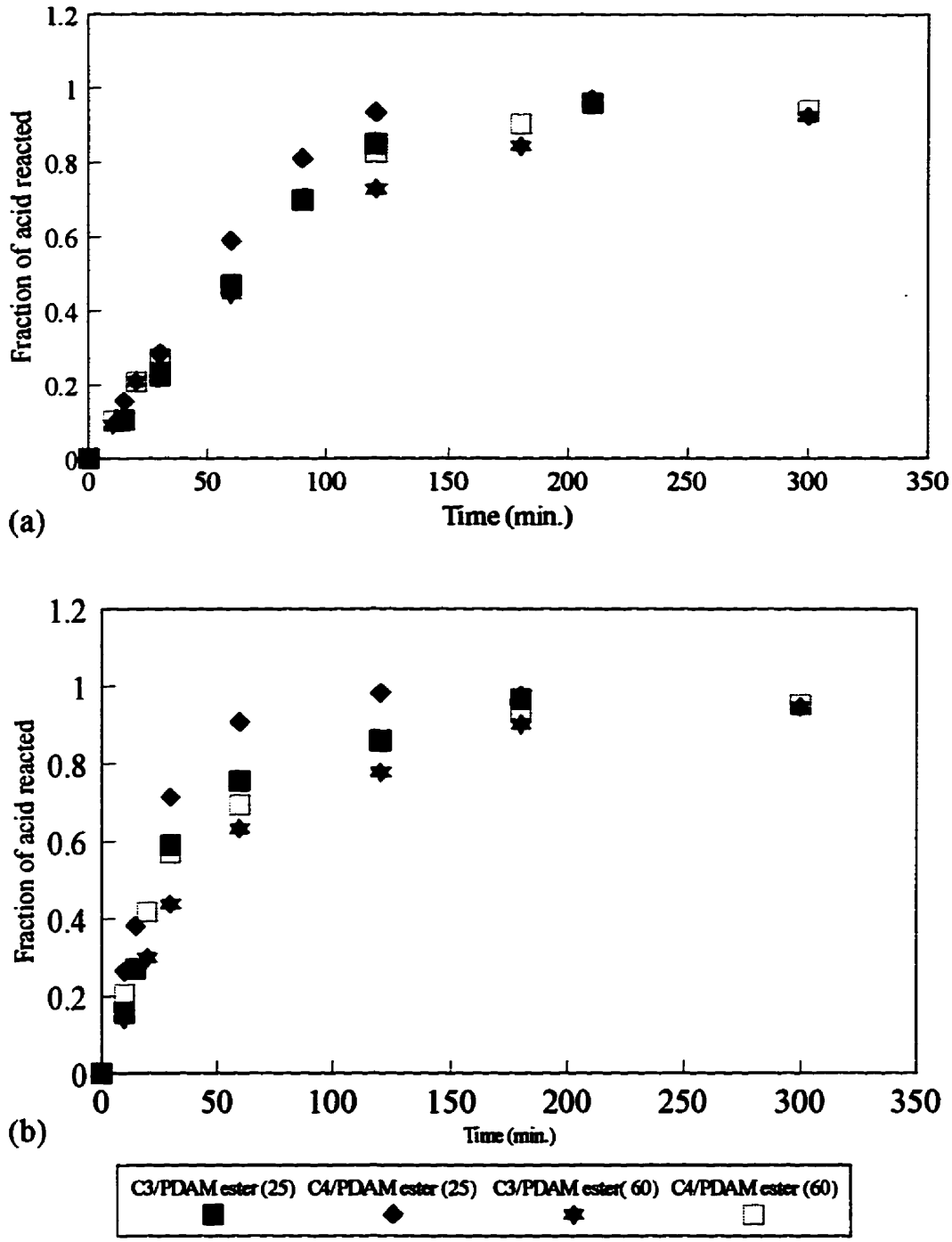

Figure 4-10. Comparison of reaction time profiles for the derivatization of 0.5 ng/mL each of gaseous C_3 and C_4 acids with PDAM in the doped (a) PA and (b) PDMS coated fibers at 25°C and 60°C.

As mentioned before, this in-fiber derivatization is an exhaustive reaction. To test if any PDAM or reaction product were left over in the sample vial after derivatization was complete, a freshly conditioned new fiber without derivatizing reagent was inserted into the same sample vial for 3 hours, then analyzed by GC/FID. The chromatogram showed that no derivatizing reagent or reaction derivatives were detected. Since PDAM has a negligible volatility at room temperature, with the above experimental evidence, it is assumed that the reaction of the acids with PDAM is most likely taking place only in one phase, the polymeric coating of the fiber, during the SPME/derivatization process. The amount of PDAM in the fiber coating was in excess compared to the total amount of the acids in the sample vial during the reaction. The chromatograms confirmed that the concentration of PDAM in the coating remained constant before and after the reactions, within the limits of experimental error. Therefore, the rate of reaction depended only on the acid concentration in the fiber coating. These results imply that the derivatization is a pseudo-first order reaction. The reaction scheme is shown in Figure 4-11. It is a protonation of CHN_2 moiety of pyrenyldiazomethane with fatty acids RCOOH followed by nucleophilic substitution of N_2 with $RCOO^-$ to form ester derivatives.[22]

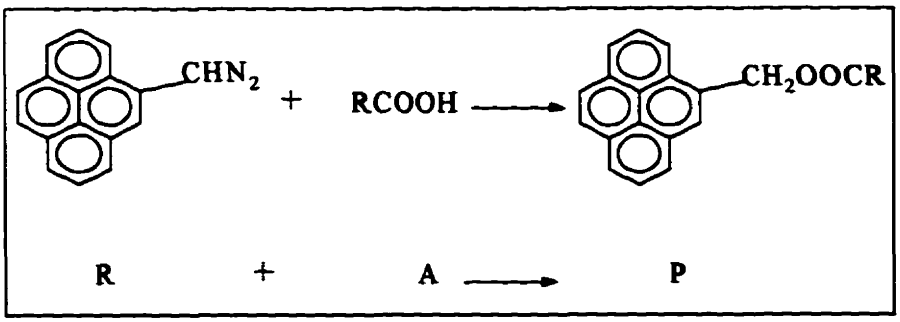

Figure 4-11. Reaction scheme for the derivatization of fatty acids with PDAM. Symbols R, A and P represent PDAM (Reagent), fatty acids (Acid) and acid/PDAM esters (Product), respectively.

According to the rate law, the rate of the reaction in the coating anywhere and at anytime can be described as:[23]

$$\frac{d[P]}{dt} = k'[A_f] \tag{4-1}$$

where $[P]$ and $[A_f]$ are the concentration of the reaction product (acid/PDAMester) and the acid in the fiber coating, respectively, at different reaction times. $k' = k[R]$, where k is the reaction rate constant, and $[R]$ is the concentration of the derivatizing reagent in the fiber coating.

Although the derivatization takes place only in the fiber coating, the overall kinetics have to be considered by examining the two phase system consisting of a polymeric coating and a gaseous phase. This is because the amounts of the analytes derivatized in the polymeric coating are related to the overall partitioning of the acids between the two phases.

A mathematical model for this derivatization system was derived.[1] The following assumptions were made: the coating contains excess amount of reagent so that analytes react everywhere and at all times in the coating at a rate $k[A](r,t)$, where $[A](r,t)$ represents analyte concentration at radius r and time t; diffusion is the only mass transport process in the coating with no interactions with the fiber's inner core or with vial surfaces, and the analytes are perfectly mixed in the gaseous phase.

The equations describing analyte diffusion in the coating are

$$\frac{\partial^2[A]}{\partial r^2} + \frac{1}{r}\frac{\partial[A]}{\partial r} = \frac{1}{D}\frac{\partial[A]}{\partial t} + \frac{k[A]}{D} \qquad \text{where } a < r < b,\ t > 0, \qquad (4\text{-}2)$$

$$\frac{\partial[A]}{\partial r} = 0 \qquad \text{where at } r = a,\ t > 0 \qquad (4\text{-}3)$$

and

$$\frac{K}{V_g}\left(n_0 - 2\pi bLD\int_0^t \frac{\partial[A]}{\partial r}dt\right) = [A] \qquad \text{where at } r = b,\ t > 0, \qquad (4\text{-}4)$$

Symbols a and b represent the inner and outer radii of the fiber coating; L is the length of the fiber coating; D is the diffusion coefficient of the analyte in the fiber coating; K is the partition coefficient between fiber coating and air and $K = [A]_f / [A]_g$, where $[A]_f$ is the concentration of the

analyte in the fiber coating and $[A]_g$ is the concentration of the analyte in the gaseous phase; n_0 is the initial amount of the analyte in the sample vial; and V_f and V_g are the volumes of the fiber coating and the gaseous phase, respectively.

Applying the Laplace transform[24] to the above equations, we have

$$2\pi Lk\int_0^t\int_a^b [A]rdrdt = 4\pi LDn_0k\sum_{\alpha j}\frac{[e^{(D\alpha^2-k)t}-1][b\{K_1(\alpha a)I_1(\alpha b)-I_1(\alpha a)K_1(\alpha b)\}]}{-(D\alpha^2-k)\partial/\partial\alpha\Delta(\alpha)\rfloor_{\alpha j}} \quad (4\text{-}5)$$

whereas K_1 and I_1 are the Bassel functions.[25] The detailed derivation and explanation of the mathematical model is given in the Appendix B. A computer program written by Marc Adams was used to calculate the theoretical estimates of reaction rate constant with Equation (4-5).

Comparison of the actual reaction time profiles with the theoretical model for the reaction of 0.5 ng/mL each of propionic and butyric acids with PDAM in the doped PA coated fiber is shown in Figure 4-12. Similar profiles were also observed with the PDMS coated fiber. In the first 60 minutes the formation of the pyrenylmethyl esters was linear with respect to the reaction time for the PA coated fiber, then levelled off afterwards. After 4 hours, all the acids (> 96%) had reacted with PDAM. The levelling off is caused by the dramatic decrease of the acid concentration in the gaseous phase as well as in the fiber coating. This figure shows qualitative agreement between the theoretical model and the experimental data.

The experimental data was used to estimate the reaction rate constant (k') with Equation (4-5) by minimizing the sum of squared differences between data points and estimates. The calculated reaction rate constants (k') for propionic and butyric acids using both the PDMS and PA coated fibers are listed in Table 4-1. Although the PDMS coating is non-polar, the reaction in this fiber coating is faster than in the polar PA coated fiber. The different rate constants might be due to the different polarity of the fiber coatings. The nature of the fiber coating might also have a large impact on the rate of reaction.

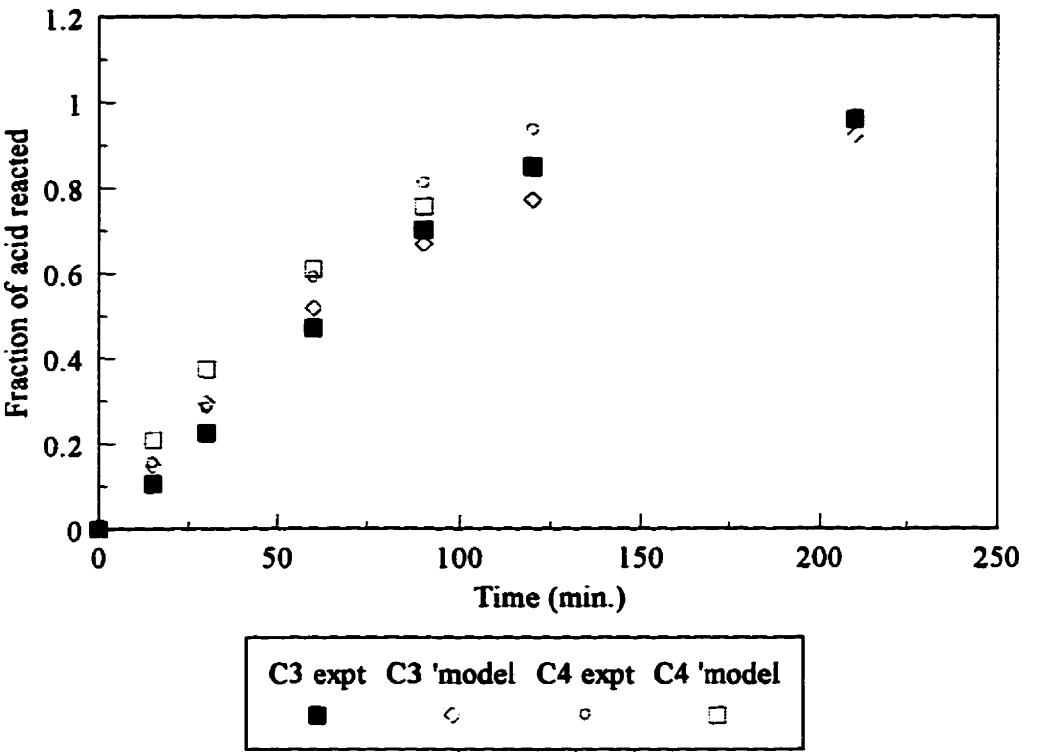

Figure 4-12. Comparison of the actual reaction time profiles with the theoretical model for the derivatization of 0.5 ng/mL each of gaseous C_3 and C_4 acids with PDAM in the doped PA coated fiber.

Table 4-1. The calculated reaction rate constants (k', sec^{-1}) for the reactions of gaseous propionic and butyric acids with PDAM in the PDMS and PA coated fibers.

Compound	PDMS (k', sec^{-1})	PA (k', sec^{-1})
Propionic acid	8.9×10^{-3}	3.8×10^{-4}
Butyric acid	7×10^{-3}	4.2×10^{-4}

4.4.3 In-Matrix Derivatization with PFPDE

Since PFPDE is a relatively volatile reagent, the derivatization with it was performed by spiking excess amounts of PFPDE into the reaction vial containing target acids. As PFPDE contains pentafluorobenzyl moiety which has a large electron capture cross section, it is sensitive towards ECD detection. Therefore, GC/ECD was used for the analysis of the PFPDE derivatives. Only the PA coated fiber was tested for this technique. The PA coated fiber was placed in the gaseous phase of the reaction vial immediately after PFPDE was spiked. Derivatization mainly took place in the gaseous phase. The reaction and extraction started at the same time. The SPME fiber desorption temperature and time were determined. It was found that less than 0.5% carryover was reached when the PA coated fibers were desorbed in the GC injector at 250°C for 4 minutes.

The reaction and extraction time profiles for the derivatization of 0.5 ng/mL of C_2-C_5 acids with PFPDE are presented in Figure 4-13. Since the reaction derivatives of PFPDE are relatively volatile, they mainly remain in the gaseous phase during reaction and extraction. Fiber absorption is an equilibrium process. The reaction and extraction time profiles indicate that both the completion of reaction and reaching the extraction equilibrium were fast, within 10 minutes. Since the GC run time is about 15 minutes, a 20 minutes reaction and extraction time was used to be compatible with the GC analysis.

The yields of reaction with PFPDE were investigated using reagent concentration of 39, 78, 117 and 156 mM, being 1.5, 3, 4.5 and 6 molar equivalent to the total molarity of 20 ng each of C_2-C_5 acids in a 40 mL sample vial. The PA fiber extraction time was 20 minutes. Figure 4-14 indicates that with reagent concentration of 117 and 156 mM, the yields of the reaction products were the highest. Experiments also indicate that the concentration of reagent higher than 156 mM did not produce more derivatives. Thus, PFPDE concentration of 156 mM was used for subsequent experiments.

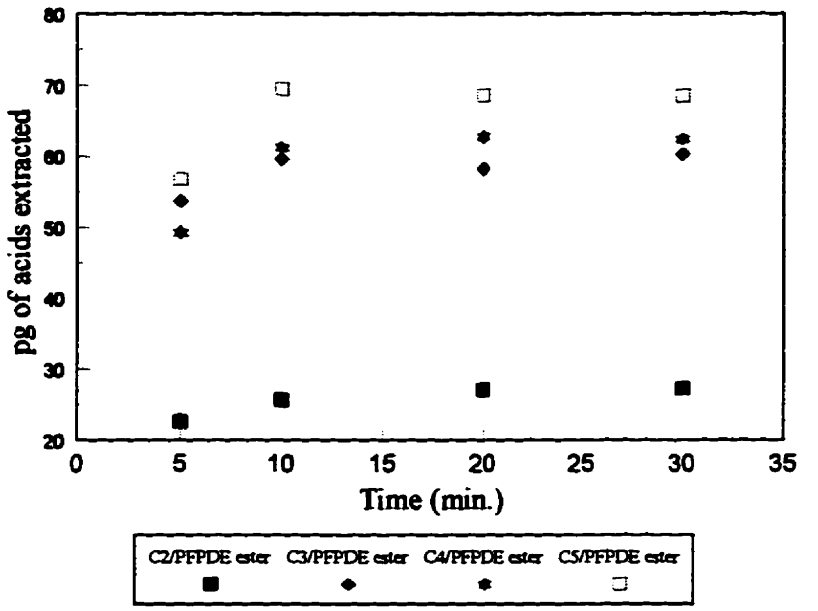

Figure 4-13. Reaction time profiles for the derivatization of 0.5 ng/mL each of C_2-C_5 acids with PFPDE in air using the PA coated fiber to isolate acid/PFPDE esters.

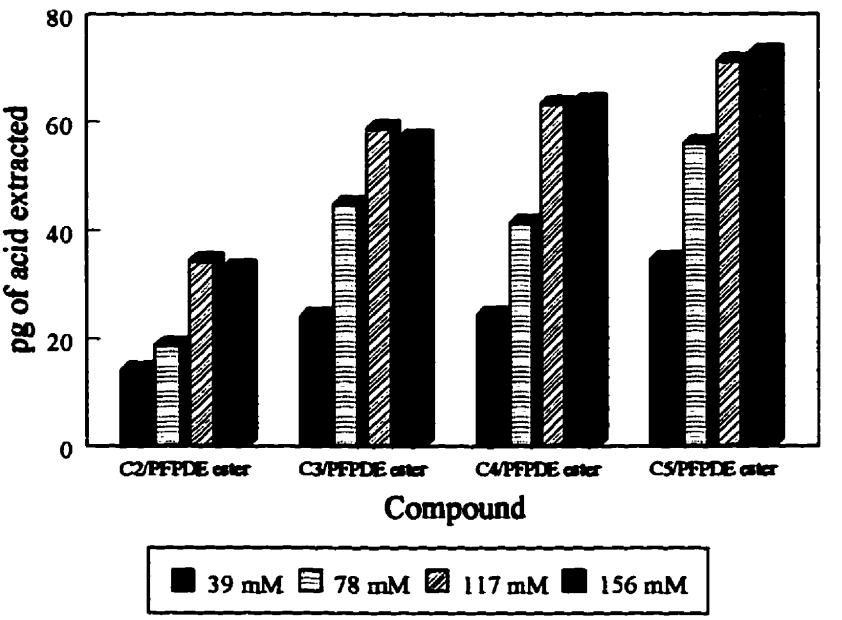

Figure 4-14. Effect of the PFPDE concentrations on the yields of the derivatization of C_2-C_5 acids in air (with the PA coated fiber to isolate acid/PDPFE esters).

Figure 4-15 illustrates the humidity effect on the derivatization of 0.5 ng/mL each of C_2-C_5 acids with PFPDE in air. Humidity of less than 10% (condition 1) represents the relative humidity (R.H.) conditions in a drying oven at 100°C. The sample vial was heated in the drying oven for longer than 60 minutes without the lid (the lid was also heated in the same oven for the same period of time). The sample vial was sealed with a hole cap and a Teflon faced silicon septum right after it was taken out from the drying oven. When it was still hot, 0.5 ng/mL each of C_2-C_5 acids was added with a syringe, to help these acids fully vaporize. After the vial was cooled to room temperature, PFPDE/toluene was added into the sample vial with a syringe. A PA coated fiber was placed in the vial immediately. The sampling time was 20 minutes. During the SPME sampling, the mixture was stirred vigorously with a magnetic stir bar.

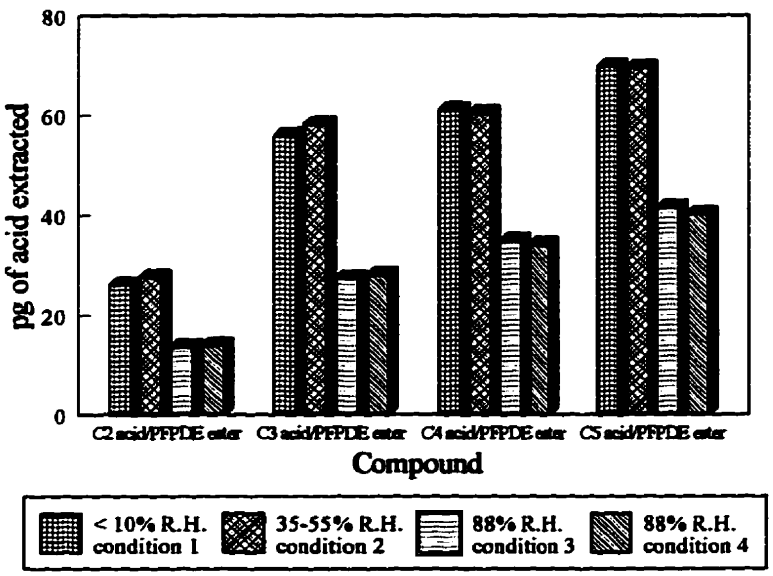

Figure 4-15. Humidity effect on the extraction of 0.5 ng/mL each of C_2-C_5 fatty acids as their PFPDE esters from air using the PA coated fiber.

The humidity of 35-50% (condition 2) was found to be the R.H. conditions at room temperature (25°C) using the unheated sample vial, prior to the addition of FFAs. After the sample was added into the sample vial, the vial was sealed and put in the heated oven for 5 minutes to help the FFAs fully vaporize. The sample vial was then taken out of the oven, cooled

to room temperature, and spiked with PFPDE. A PA coated fiber was next placed in the vial for sampling.

More than 88% humidity (condition 3) represents the R.H. conditions reached by adding 0.5 μL H_2O into the unheated sample vial after volatile fatty acids were added to it (R.H. in the sample vial was 35-55% without the addition of 0.5 μL H_2O and when 0.5 μL H_2O was added the R.H. increased by 53% in the sample vial; the detailed calculation is shown in the Appendix C). The same heating procedure as described for Condition 2 was used. Once the vial was cooled to room temperature, a PA coated fiber was introduced into the gaseous phase to isolate reaction derivatives. It can be seen from Figure 4-15 that when the humidity in the system was less than 50%, the amount of the analytes eluted was not affected. When the humidity was increased to more than 85%, the amount eluted decreased almost by half for all the acids tested. Chai *et al.* found that increasing the humidity in the sample container (air bulb) for the analysis of VOCs in air decreased the amount of VOCs extracted by SPME sampling.[26]

In this study, the humidity can affect either derivatization or extraction or both. Therefore, another experiment was performed (condition 4) in which 0.5 μL H_2O was added in the sample vial after the derivatization of 0.5 ng/mL each of gaseous C_2-C_5 acids with PFPDE in an unheated vial (vial containing 35-55% R.H.). After water was added to the sample vial, the air sample was stirred at room temperature for 30 minutes to help vaporize the water and the analytes. The same PA coated fiber was then placed in the sample vial for 20 minutes to isolate acid/PFPDE esters. In this situation, the derivatization was complete before the R.H. was increased to higher than 85% in the vial. The derivatization therefore, was not affected by the higher humidity conditions. As it can be seen from Figure 4-16, the amount of acids eluted at condition 4 is the same as in condition 3. This indicates that the higher humidity affected the extraction process rather than the derivatization process. It seemed that when R.H. increased, trace amount of water was condensed to the stainless steel tubing attached to the fiber. During the desorption step, water and the analytes were desorbed off the fiber coating and the stainless steel tubing and were vaporized in the inlet of GC injector. Since the volume of the hot vapour exceeded the volume of the inlet, it resulted in a back-flush from the injector. As a consequence, some of the analytes were lost during fiber injection which caused the amount of the analytes

detected to decrease. Therefore, the humidity in the system has to be controlled to less than 55% for air sampling so that the SPME extraction process could not be affected.

4.4.4 Comparison of Derivatization/SPME Sampling with Direct SPME Sampling

The summary of derivatization/SPME techniques and direct SPME technique for the analysis of short-chain FFAs in air is presented in Table 4-2. Reaction of short-chain FFAs with PDAM was quite long, approximately 3-4 hours, whereas reaction of these acids with PFPDE was relatively short, within 10 minutes. Extraction of short-chain FFAs as PDAM derivatives was an exhaustive process, whereas extraction of these acids as PFPDE derivatives and the extraction of FFAs with direct SPME were equilibrium processes. The desorption temperature for the PDAM derivatives was high at 300°C, whereas desorption for the PFPDE derivatives could be done at 250°C.

Table 4-2. Summary of SPME techniques for the analysis of short-chain fatty acids in air.

Summary	Reaction with PDAM	Reaction with PFPDE	Extraction of free acids
Reaction time	3-4 hrs.	10 min	N/A
Extraction Type	exhaustive	equilibrium	equilibrium
Desorption Temp.	300°C	250°C	250°C
Desorption Time	4-5 minutes	4 minutes	3 minutes
Fiber used	PA and PDMS	PA	PA and PDMS
Detector used	FID or ITMS	ECD	FID

The linear range is an important characteristic for quantitative SPME analysis. Table 4-3 presents the summary of fiber linear ranges for the extraction of free C_2-C_5 acids and their PDAM and PFPDE derivatives from air.

Table 4-3. Summary of fiber linear ranges for the analysis of C_2-C_5 acids in air using derivatization/SPME and direct SPME techniques.

	Fiber linear range (ng/mL)			
	Acetic acid	Propionic acid	Butyric acid	Valeric acid
PDAM (PA, GC/FID)	NT	0.025-2.5	0.025-2.5	NT
r^2	-	0.99911	0.99882	-
PDAM (PDMS, GC/FID)	-	0.05-5	0.05-5	-
r^2	-	0.99971	0.99925	-
PDAM (PA, GC/ITMS)	0.005-5	0.005-5	0.005-5	0.005-5
r^2	0.99974	0.99998	0.99999	0.99999
PFPDE (PA, GC/ECD)	0.05-1	0.05-1	0.025-1	0.025-1
r^2	0.99459	0.99858	0.99281	0.99584
Direct SPME (PA, GC/FID)	0.25-10	0.25-10	0.05-10	0.05-10
r^2	0.99758	0.99589	0.99886	0.99635

NT = not tested

Since reactions of short-chain FFAs with PDAM were very slow (3-4 hours), it was not economical to use such a long time to perform quantitation. The reaction kinetic studies indicated that the derivatization is a pseudo-first order reaction and the amounts of acids reacted versus reaction time were linear within the first one and a half hours. As demonstrated before, the amounts of analyte extracted with SPME sampling also have a linear relationship with the initial sample concentration. Quantitation can then be done by sampling for less than the equilibrium time, as long as the sensitivity remains satisfactory. Therefore, a 1 hour extraction and reaction time was used to carry out the fiber linear range experiments for the in-fiber derivatization with PDAM. For PFPDE, a 20 minute extraction and reaction time was used to test fiber linear

ranges. Table 4-3 demonstrates that when the derivatization techniques were used the lower ends of the fiber linear ranges were all smaller than those for the direct SPME technique. The widest fiber linear range (0.005-5 ng/mL) was obtained when the derivatization was performed with PDAM in the PA coated fiber and analysis by GC/ITMS. The lower end of this fiber linearity was almost 2 orders of magnitude smaller than that of the direct SPME technique. According to the NIOSH and OSHA guide-lines, the exposure limits to these volatile acids in indoor air are between 0.025-0.25 ng/mL. It can be seen that derivatization with PDAM in the PA fiber coating and analysis by both GC/FID (fiber linearity between 0.025-2.5 ng/mL) and GC/ITMS (0.005-5 ng/mL) can be used to examine the indoor air quality. Other methods were not feasible for the determination of indoor air quality of volatile fatty acids, but they could be used to monitor the presence of these volatile acids in industrial atmosphere. It can be seen that the PA fiber coating had a narrower linearity when derivatization was done with PFPDE. This was because that the higher end of this linear range was limited by the linear response of the ECD. This is understandable since the ECD usually has a linear response spanning only 2 to 4 orders of magnitude. The linear range was not tested for concentrations higher than 5 ng/mL when PDAM was used since those higher concentrations were already covered by the direct SPME technique.

The limits of detection (LODs) for the derivatization/SPME techniques and direct SPME technique for the analysis of short-chain fatty acids in air are summarized in Table 4-4. The LODs for the derivatization techniques were determined by the reagent blank, not by the detection ability of the detector. For the GC/FID analysis, the area counts which were 3 times higher than the area counts in the reagent blank at the target regions were used to calculate the detection limits. For the GC/ITMS analysis, the signal to noise ratio which was 3 times higher than that of the reagent blank at the target regions, was used to estimate the detection limits. The data shown in Table 4-4 indicates that simultaneous in-fiber derivatization with PDAM using the PA coated fiber and analysis by GC/ITMS provided the lowest detection limits for all the selected analytes. Compared to direct SPME sampling of FFAs, the derivatization techniques can lower the detection limits by 1-4 orders of magnitude. The results demonstrate that derivatization/SPME techniques with both PFPDE and PDAM reagents using the PA coated fiber can be validated to monitor industrial and indoor air quality.

Table 4-4. Summary of LODs for the analysis of C_2-C_5 acids in air using derivatization/SPME and direct SPME techniques.

	LOD (pg/mL)			
	Acetic acid	Propionic acid	Butyric acid	Valeric acid
PDAM (PA, FID)	NT	4	2	NT
PDAM (PDMS, FID)	NT	16	8	NT
PDAM (PA, ITMS)	0.06	0.2	0.5	0.9
PFPDE (PA, ECD)	1	10	7	6
Direct SPME (PA, FID)	170	60	33	18

Table 4-5 summarizes the percent relative standard deviation (%RSD) for the analysis of volatile fatty acids in air using both the derivatization/SPME and the direct SPME techniques. The RSD (%) values were between 1 and 8.5% for all the techniques presented, which was considered to be satisfactory.

Table 4-5. Summary of RSDs (%) for the analysis of C_2-C_5 acids in air using derivatization/SPME and direct SPME techniques.

	RSD (%)			
	Acetic acid	Propionic acid	Butyric acid	Valeric acid
PDAM (PA, FID, n=3, 0.1 ng/mL)	NT	2.7	4.2	NT
PDAM (PDMS, FID, n=3, 0.1 ng/mL)	NT	2.8	2.3	NT
PDAM (PA, ITMS, n=3, 0.05 ng/mL)	5.2	2.9	2.2	1.4
PFPDE (PA, ECD, n=3, 0.1 ng/mL)	2.7	3.4	3.3	4.1
Direct SPME (PA, FID, n=7, 0.5 ng/mL)	4.1	4.7	5.3	6.2

NT = not tested

4.5 Derivatization/SPME of Short-Chain Fatty Acids in Water

4.5.1 In-Fiber Derivatization with PDAM

As demonstrated previously, direct SPME and headspace SPME sampling techniques were not sensitive enough for the analysis of short-chain FFAs in water. One reason for this is that the currently used fibers are not polar enough compared to both the volatile FFAs and the aqueous matrix. The free acids have larger affinities towards water than the fiber coatings, thus fiber extraction of FFAs is poor. One way to improve the SPME technique is by modifying the existing fiber coatings to make them more suitable for the extraction of polar analytes. Specifically, this can be done by doping relatively non-volatile derivatizing reagents into the fiber coating to derivatize FFAs in the fiber stationary phase. Once the FFAs are absorbed by the fiber coating containing the derivatizing reagent, they are converted into less polar and less volatile derivatives which remain in the fiber coating rather than going into the sample matrix. The FFAs are continuously consumed by the SPME fiber coating as a result of the derivatization process. This allows more FFAs to be absorbed into the fiber coating, which, in turn, leads to an increase in the analyte's ability to partition into the coating. Consequently, more fatty acids can be released from the aqueous phase and SPME extraction efficiency can be improved. When the derivatizing reagent in the fiber coating is in excess with respect to the total amount of fatty acids in the sample, this process will continue until all the fatty acids are extracted.

To develop a new SPME method, the choice of sampling mode is the primary step. It is important to determine if direct SPME or headspace SPME is a more appropriate sampling technique for the in-fiber derivatization. The following parameters were considered: the volatility of the target analytes, the stability of the derivatizing reagent when it is in direct contact with the sample matrix, and the suitability of the sampling mode for real sample analysis. As a consequence, the in-fiber derivatization in water was carried out with PDAM using headspace SPME sampling. These conditions were chosen since short-chain FFAs were volatile and the reagent was believed not to be very stable when it comes in direct contact with water for prolonged periods of time. Also, real samples tend to contain particles or materials that could

contaminate or damage a fiber coating if it is directly exposed to the matrix, which would interfere with the extraction. Headspace SPME could avoid these adverse matrix effects.

As mentioned before, headspace analysis involves three phases: coating, gas and liquid. The amount of analyte absorbed into the coating once equilibrium has been reached can be written as:[27]

$$n = \frac{C_0 V_f V_s K_1 K_2}{K_1 K_2 V_f + K_2 V_h + V_s} \tag{4-6}$$

where V_f, V_h and V_s are the volumes of the fiber coating, headspace and liquid, respectively; K_1 and K_2 are the coating/gas and gas/water partition coefficients, respectively. Derivatization can increase both K_1 and K_2, which results in an increase of the overall partition coefficient, K, where $K = K_1K_2$. K_2 for polar analytes is usually very small due to their hydrophilicity (for example, for butyric acid, K_1=115000 (Chapter 3) and K=1.3[1] in saturated salt at pH 1.5 conditions using the PA coated fiber, therefore, K_2=1.17×10^{-5}). When V_h and V_s are of the same order of magnitude, $K_2V_h << V_s$. V_f is often 4-5 orders of magnitude smaller than V_s. K_1K_2=K is also very small for polar analytes (see above example for butyric acid). Thus, $K_1K_2V_f < V_s$. From Equation 4-6 it can be seen that, when the overall K increases, the increasing factor in the numerator will be larger than in the denominator which leads to an increase in the amount of the analyte extracted by the fiber coating, n. Therefore, the sensitivity can be improved.

Matrix effect was examined by analyzing samples containing 18% salt, saturated salt (36% salt) and saturated salt at pH 1.5, as a means of increasing the amounts of acids extracted into the PA coated fiber. The comparison of the above three conditions is displayed in Figure 4-16. The samples contained 100 ppb each of C_3 and C_4 acids and the sampling time was 5 hours. As demonstrated before, for polar fatty acids, adding salt into aqueous matrix can decrease their solubility in water, thus increase the mass loadings of fatty acids in the fiber. It was observed that the area counts of acids extracted under the 18% salt conditions were only half of those under the saturated salt conditions (containing 36% salt). Therefore, the addition of saturated salt was necessary to have the maximum "salting out" effect on these acids. It is well known that

decreasing pH of aqueous solution can further decrease the polarity of acids in the aqueous phase. Thus, by combining the above two factors, more acids were driven into the headspace (and eventually to the fiber coating) from the aqueous phase and greater amounts of acids were reacted with PDAM in the fiber coating. It can be seen that under the conditions of saturated salt and pH 1.5, the highest amount of reaction derivatives was reached. Therefore, these optimized conditions were used for subsequent experiments.

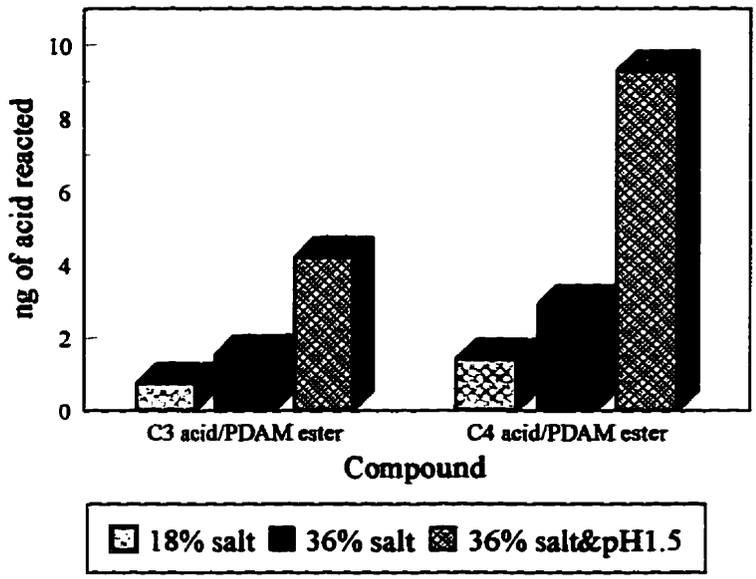

Figure 4-16. Matrix effects on the in-fiber derivatization of 100 ppb each of C_3 and C_4 acids with PDAM in the PA coated fiber using headspace sampling.

Figure 4-17 (a) illustrates the reaction time profiles for the formation of the pyrenylmethyl esters of propionic and butyric acids in the doped PA coated fiber when the aqueous layer of samples was held at room temperature. The acid concentration was 100 ppb each. The yields of reaction products increased linearly at the beginning and tapered off after 4 hours. Only 5% and 12% of propionic and butyric acids respectively, reacted with PDAM in the PA fiber coating after the reaction took place for 35 hours. The tapering off might be due to the combined effects of insufficient acid concentration and the loss of derivatizing reagent in the fiber coating after the prolonged reaction time. Since the acids could not be effectively extracted and reacted in the fiber

coating when sample solutions were maintained at room temperature, heating the aqueous layer of samples to elevated temperatures was investigated. Higher temperatures should facilitate the mass transfer of volatile acids from the aqueous phase to the headspace.

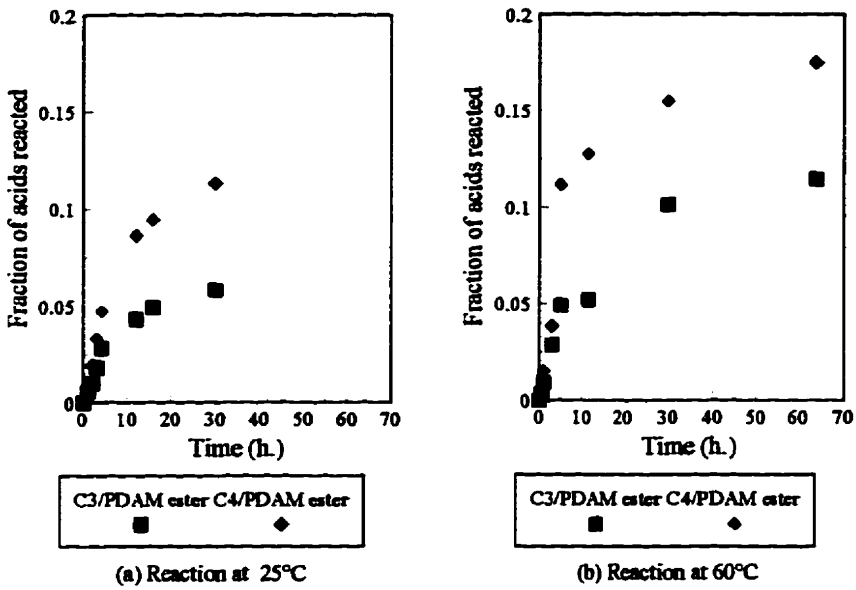

Figure 4-17. Reactions of 100 ppb each of C_3 and C_4 acids with PDAM in the doped PA coating when sample solution was at (a) 25°C and (b) 60°C.

Figure 4-17 (b) illustrates the reaction time profiles for the derivatization of 100 ppb each of C_3 and C_4 acids with PDAM in the doped PA coating when the sample solution was heated to 60°C. During the experiment, the portion of the vial which contained the aqueous solution was heated in a water bath, while the headspace of the vial was retained above the heated water. The fiber was placed in the headspace of the vial which was not directly heated, yet it was expected that the temperature of the fiber was lower than 60°C but slightly higher compared to room temperature. It can be seen that the reaction was linear for the first 5 hours and then tapered off. The yields of the fatty acid/PDAM esters almost doubled when compared to the reaction at room temperature in the first 5 hours. The previous studies for the reaction of PDAM with the gaseous C_3 and C_4 acids in the PA coated fibers in air samples at 60°C revealed that higher temperature did not increase the reaction rate, but decreased it slightly. The experiment indicates that the main effect of the higher temperature in this case is the improvement of mass transfer of the analytes

into the headspace from the aqueous phase. This driving force is powerful enough to compensate for the decrease of the amount of the acids partitioning into the fiber due to the higher temperature, thus accelerating the derivatization reaction. However, the overall reaction still did not run into completion in 65 hours after the temperature was increased.

From the reaction of gaseous C_3 and C_4 acids with PDAM in the doped PA coating (in air samples), it can be seen that the levelling off for the derivatization started after more than 60% of the acids reacted with PDAM, due to a significant decrease of the acid concentration in the air. However, the tapering off in this case started when less than 15% each of C_3 and C_4 acids in the aqueous solution were reacted. If by then the amount of PDAM was still in excess compared to the total amount of acids in the aqueous solution, the derivatization would still increase linearly, like the reaction in the air samples, then levelling off until most of the acids would have been reacted with PDAM.

During derivatization of aqueous samples, it was noticed that there were two peaks present near the retention time of the reagent peak when water samples were analyzed, compared to one reagent peak for gaseous sample analysis. In order to investigate these problems, the examination of the reagent blank with the PA coated fiber for the analysis of aqueous samples was carried out. A PA coated fiber doped with PDAM was placed in the headspace of a 40 mL vial containing only deionized water without the target acids inside. The vial was then heated to 60°C in a water bath. After a certain period of time, the fiber was taken out of the vial and introduced into the GC injector for analysis. It was found that the reagent blank contained two large peaks instead of one compared to the reagent blank that have been done in the gaseous phase. One exactly fell in the retention time which was confirmed as 1-methylpyrene with GC/ITMS, the same peak that was seen in the reagent blank of gaseous sample analysis. The second peak which eluted slightly behind the 1-methyl pyrene peak was confirmed as pyrenylmethanol with GC/ITMS. The second peak was not seen in the reagent blank for gaseous sample analysis. Therefore, this peak is considered to be the hydrolysis product of PDAM during analysis of aqueous samples. It was also noticed that the second peak was getting larger when the reaction time was getting longer. This phenomenon confirmed that the second peak was the hydrolysis product of the reagent. Although PDAM was not directly in contact with the aqueous solution,

even in the headspace above water, the system was still very humid. Therefore, the incompletion of the above derivatization for aqueous sample analysis is likely due to the decomposition of PDAM after long reaction times and at high reaction temperatures combined with the humid conditions. The decomposition of PDAM esters in the fiber coating is also possible. This situation may be improved by designing a more appropriate reagent in the future.

A PDMS coated fiber was also studied for headspace extraction/derivatization. However, it did not perform very well. The experimental results indicated that a doped PDMS fiber produced detection limits that were at the part per million level. This suggests that fatty acids have less tendency to partition into a non-polar PDMS coating compared to the polar PA coating in the coating/gas/water system. Among the three phases, water is the most favoured phase for acids since it is the most polar. In order to compete with the aqueous phase, a polar coating is needed.

4.5.2 Direct Derivatization in the Aqueous Phase with PFBBr, PFPDE, PDAM and TMS-diazomethane

For effective analysis of analytes in water using SPME techniques, the target analytes must first be released from the aqueous matrix. They can then be efficiently extracted by the selective SPME fiber coatings. Several techniques can be used to reduce their interactions with water, such as adding salt and lowering the pH of the sample solution to decrease the polarity and solubility of the polar analytes in water. However, the previous studies using direct SPME and headspace SPME indicated that these were not effective enough to release very polar short-chain FFAs from water.

Another method used to improve the release of analytes from water is to heat the water samples to an elevated temperature, as was demonstrated in section 4.5.1. The higher sampling temperature provides the energy for analyte molecules to overcome energy barriers which tie them to the matrix,[28] enhances the mass transfer, and increases the vapour pressure of the analytes. Zhang *et al.* also presented that heating soil and clay samples containing BTEX to elevated temperatures improved their release from sample matrices.[27] Since heating the sample

and its surrounding area decreased the partitioning of the analytes into the SPME fiber coating, the fiber stationary phase had to be cooled by liquid CO_2 during SPME sampling.[27] Although this technique was effective, the procedure was quite complicated and the CO_2 flow rate was difficult to control. Therefore, other techniques which are easier to handle are needed to be developed.

For short-chain fatty acids, a simpler method can be employed to decrease their interactions with water. This is accomplished by converting the polar analytes to less polar derivatives via in-matrix derivatization. It is well known that as the polarity of a compound decreases, its interaction with water also decreases; therefore, it is released from water more effectively, which leads to an improvement in the extraction efficiency.

As indicated before in the literature, the majority of the current derivatization techniques still use acid- or base-catalyzed alkylation reagents for the analysis of fatty acids in aqueous matrices. Since these reactions require the presence of organic media, some extraction techniques had to be applied in order to isolate FFAs from water into the organic solution prior to derivatization. These techniques are usually time consuming and expensive. In addition, the esters of the fatty acids undergo hydrolysis in acidic or basic aqueous solutions; hence, these reagents are not suitable for direct derivatization of free acids in water. PFBBr and PFPDE were used for the current study because they are selective and sensitive. The use of both reagents to derivatize fatty acids directly in water has not been found in the literature. The reaction schemes for both reagents with the acids are presented in Figure 4-18.

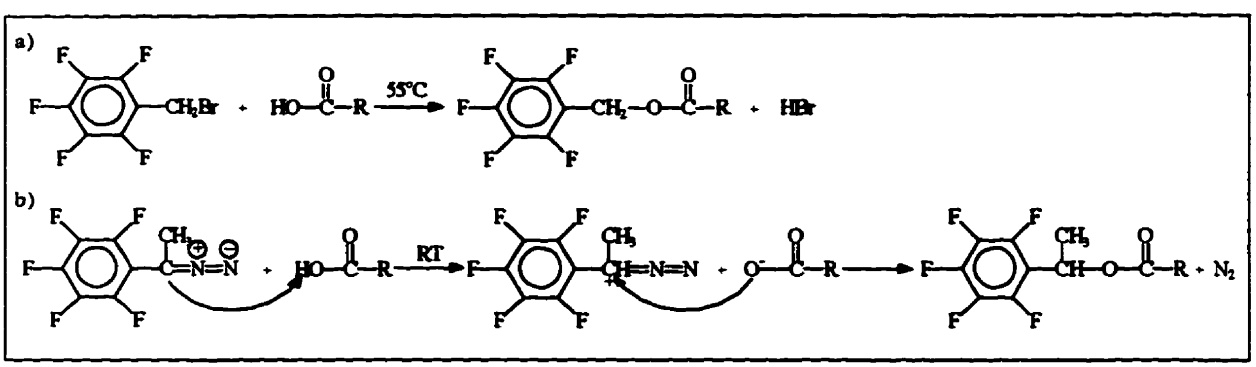

Figure 4-18. The reaction scheme for the derivatization of fatty acids with (a) PFBBr and (b) PFPDE reagents.

PFBBr can only react with strong nucleophiles such as fatty acids and phenols and PFPDE is an excellent alkylating reagent for fatty acids; thus they are selective when dealing with samples containing other groups of compounds besides fatty acids. Both reagents provide the reaction derivatives containing the pentafluorobenzyl moiety which has a large electron capturing cross section. Hence, these reagents are very sensitive for the analysis of fatty acids when ECD is used. PFBBr was only used to derivatize acids in water because the reaction requires high temperatures. Experiments indicated that the reaction derivatives with both reagents were stable in water for a few days.

(a) With PFBBr: Reactions of C_2-C_5 acids with PFBBr in the presence of potassium bicarbonate were carried out at 55°C. The reaction time profiles were monitored for 1 to 4 hours and are shown in Figure 4-19.

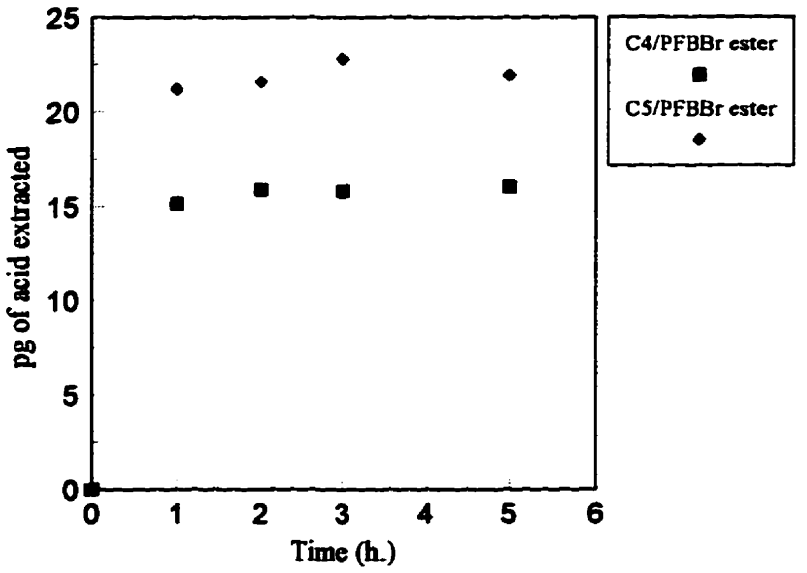

Figure 4-19. Reaction time profiles for the extraction of C_4 and C_5 acids as PFBBr esters from the headspace above the water using the PA coated fiber.

After each time point was reached, the reaction vial was taken out from the water bath and cooled to room temperature. A 2 μL aliquot along with a 10 mm × 4 mm stir bar were

transferred into a 4.6 mL vial. A PA coated fiber was used to extract the reaction derivatives from the headspace above the aqueous solution. A 30 minute extraction time was used. Since interfering peaks covered the target peaks of the derivatives of acetic and propionic acids when ECD was used as the detector, the data for C_2 and C_3 acids could not be reported. Figure 4-19 indicates that a nearly constant level of reacted acid was reached for all the selected compounds after 2-hours. To ensure the completion of the reactions, a 3 hour reaction time was used for the subsequent experiments.

The exposure time profiles for the extraction of 50 ppb each of C_4-C_5 acid (as their PFBBr esters) from the headspace above aqueous solution were also tested with a PA coated fiber. The solution was stirred vigorously during SPME sampling. Experiments showed that equilibrium was reached within 30 minutes. Therefore, a 30 minute extraction time was employed for further experiments. During this investigation, it was also found that the speed of the stirring of aqueous solution had a significant effect on the time to reach equilibrium. When the vortex reached the bottom of the sample vial, the fastest equilibrium could be reached. Górecki *et al.*[29] also found that the stirring speed had a significant impact on headspace SPME sampling of tetraethyllead formed by the derivatization of Pb (II) with sodium tetraethylborate in aqueous samples.

The amount of reagent used is one of the key factors affecting the yields of derivatization. Figure 4-20 shows the yields of derivatives for the reactions of 50 ppb each of C_4-C_5 acids with PFBBr at pH 5.5 with the concentrations of the reagent at 12.8, 25.7, 38.5, 64.2, 128.3 and 192.5 μM. The results indicate that the concentrations of 128.3 and 192.5 μM gave similar yields of the derivatives. However, increasing the concentration of the reagent from 128.3 to 192.5 μM resulted in a significant increase in the background, due to a large tailing reagent peak. The other drawback of using high reagent concentrations was that it increased the formation of by-products which affected the sensitivity of the method as well as shortened the life time of the column used. Thus the reagent concentration of 128.3 μM was selected for all further experiments.

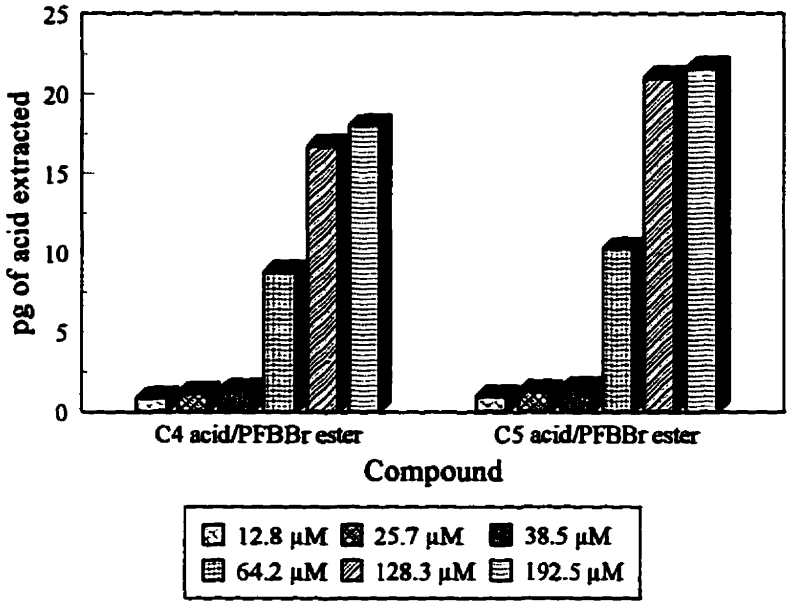

Figure 4-20. The effect of the amount of PFBBr used on the yields of derivatization of 50 ppb each of C_4 and C_5 acids in water.

The pH is also an important factor that strongly affects the derivatization of short-chain FFAs in water. pH values of 4, 5.5, 7, 8 and 10 were examined and the comparison of the amounts of acids extracted under such conditions is shown in Figure 4-21. It demonstrates that the highest amounts of acids were extracted at pH 5.5. The reaction mechanism for the derivatization of fatty acids with PFBBr indicates that it is a nucleophilic displacement of the Br^- from PFBBr with the $RCOO^-$ group. In water, OH^- ions are also present; therefore a competition reaction takes place at the same time. At alkaline conditions such as pH 8 and 10, the concentration of the hydroxyl ions was much higher than that of $RCOO^-$ ions. As a result, the main reaction was the hydrolysis of PFBBr rather than esterification of fatty acids. Under basic conditions, the hydrolysis of esters is also possible. The amount of derivatives extracted at pH 8 and 10 was therefore very low. Under acidic conditions, the magnitude of pH controlled the amount of $RCOO^-$ dissociated from the neutral form. The lower the pH, the smaller the amount of $RCOO^-$ generated. Therefore, the acidic pH conditions should be optimized. The pH value of 5.5 was used for further experiments.

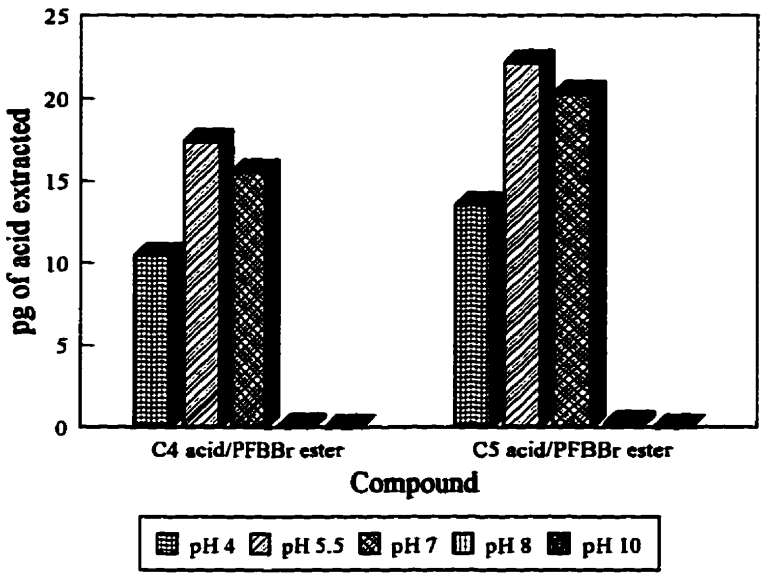

Figure 4-21. The effect of pH on the derivatization of 50 ppb each of C_4 and C_5 acids with PFBBr in water using the PA coated fiber to absorb PFBBr esters in the headspace.

Figure 4-22 shows the stability of the fatty acid/PFBBr esters in water at room temperature. It can be seen that these PFBBr esters are stable in water at room temperature for 2-3 days. Since the experiments were done over a few days, the area counts obtained in each experiment were adjusted according to the detector response factor calculated by using a quality control (QC) injection. The QC injection was performed everyday to ensure the GC detector was working properly.

Figure 4-23 shows the effect of solvent on the amount of acids extracted for the derivatization of C_4 and C_5 acids with PFBBr in water. It can be seen that the extraction was not affected when 10% of polar solvents such as acetonitrile or acetone were present in the aqueous solution. However, when non-polar solvents such as pentane and hexane were present in the system, the amounts of acid extracted were reduced proportionally to the amounts of solvent added. The more non-polar solvent present into the system, the less PFBBr derivatives were extracted.

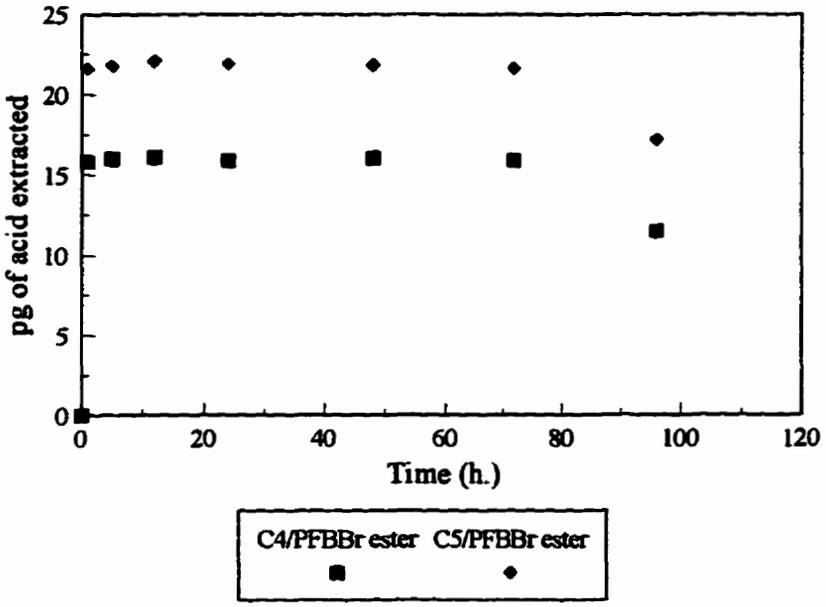

Figure 4-22. The stability of fatty acid/PFBBr esters at room temperature in aqueous solutions.

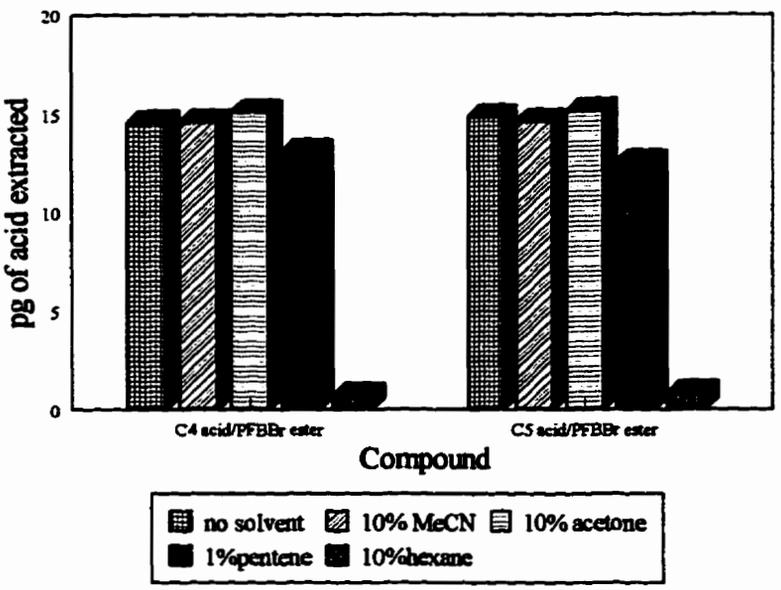

Figure 4-23. The effect of solvent on the amounts of acids extracted for the derivatization of 50 ppb each of C_4 and C_5 acids with PFBBr in water (with the PA coated fiber).

(b) With PFPDE: Since PFBBr was not suitable for the derivatization of acetic and propionic acids in water when ECD was used, the PFPDE reagent was further investigated. The only difference between the reaction derivatives of the two reagents is that the PFPDE esters have an extra CH_2 compared to the PFBBr esters of short-chain fatty acids.

The effect of reagent concentration on the completion of derivatization was first examined. No significant differences between the area counts of the target peaks were observed when 77.8 μM and 118 μM PFPDE were used. To ensure the completion of derivatization, 118 μM of PFPDE concentration was used in all further experiments.

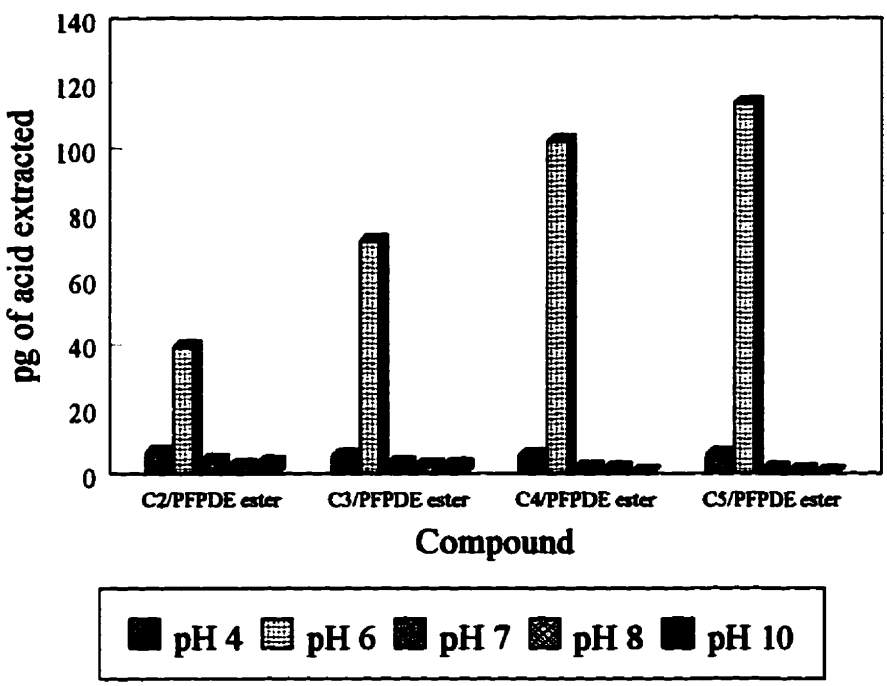

Figure 4-24. Effect of pH on the derivatization of 100 ppb each of C_2-C_5 acids with PFPDE in water using the PA coated fiber to extract reaction derivatives from the headspace.

The reaction time profiles for the derivatization of 100 ppb each of C_2-C_5 acids with PFPDE in water were also monitored for up to 15 hours. The PA coated fiber was used to isolate PFPDE derivatives. The experiments indicated that a nearly constant level of acid reacted was reached for all the selected compounds after a 12 hour period.

The stability of the fatty acid/PFPDE esters at room temperature in the aqueous solution was also examined. They were found to be stable for at least 2 days when they were stored at room temperature in water.

The pH effect on the reaction of 100 ppb each of C_2-C_5 acids with PFPDE in a water solution was also investigated. Figure 4-24 indicates that a pH of 6 is the optimum condition that yields the highest amount of reaction derivatives. At pH 4, 7, 8 and 10 the amount of the derivatives produced was significantly reduced. When examining the reaction mechanism for the derivatization of PFPDE with FFAs one can see that this is a proton catalyzed reaction in which the initial protonation of diazoethane of PFPDE with FFAs generates pentafluorobenzyl derivatives with an excellent leaving group, molecular nitrogen, N_2. At neutral and alkaline conditions, there are no protons or not enough protons present to trigger the reactions; therefore the amounts of acids derivatized were very small. When the solution is too acidic, the diazoethane is destroyed by stronger acid rather than reacting with FFAs; therefore the amounts of FFAs reacted would be also small. In order to have maximum amounts of PFPDE esters generated, matrix conditions of pH 6 should be used.

(d) With PDAM: The derivatization of short-chain FFAs with PDAM directly in aqueous solutions was first tested for this in-matrix derivatization technique since it was the first reagent that was available for this project. As it was believed that the reagent would decompose under acidic conditions, the experiments were carried out under neutral conditions. 60 µL of 30 mg/mL PDAM/hexane was added into a 40 mL vial containing a 30 mL aqueous sample. A series of acid concentrations were then examined. The experiments showed that only when the acid concentration was higher than 5 ppm, the C_3 and C_4 acid/PDAM esters were found when the extracts were analyzed with GC/FID. This suggested that the derivatization of fatty acids with PDAM directly in the aqueous solution was not effective and could not be used for trace analysis

of short-chain FFAs in water. Therefore, no further investigation with PDAM was performed for the in-matrix derivatization technique.

(e) With TMS-diazomethane: TMS-diazomethane was chosen to derivatize short-chain FFAs because it can provide different kinds of esters when its reactions are catalyzed by different kinds of alcohols. The derivatization was carried out directly in water solution since the reagent was too volatile to be used for in-fiber derivatization technique. Both methanol and butanol were employed as catalysts to produce methyl esters and butyl esters for these short-chain fatty acids. However, TMS-diazomethane was found not pure enough to be suitable for trace analysis. The extracts were analyzed with GC/FID. The reagent blank, even in organic solution, showed numerous peaks indicating the presence of impurities. The derivatization of short-chain FFAs with this reagent in water was successful when the acid concentration was in the tens of ppm level analyzed with FID. It was observed that the working concentrations of the target acids could be decreased to the high ppb levels when GC/ITMS was used. Even with GC/ITMS, the signal to noise ratios for the target analytes were very small. Therefore, no further investigation was done.

4.5.3 Analysis of Fatty Acids in Real Samples with PDAM, PFBBr and PFPDE

The ultimate test for any sample preparation technique is how it performs in the analysis of real samples. Three kinds of samples were selected: wastewater, sludge and milk. Wastewater samples usually contain fatty acids in low concentrations and they have relatively clean matrices. They were therefore examined with the in-matrix derivatization coupled with headspace SPME sampling. Both PFBBr and PFPDE were tested. Sludge and milk samples often have very complex matrices; thereby the in-fiber derivatization with PDAM coupled with headspace SPME techniques was used to examine these samples.

(a) Wastewater Samples

Both PFBBr and PFPDE reagents were utilized to analyze wastewater samples (obtained from Waterloo Wastewater Treatment Plant, Waterloo, Ont.). Standard addition was employed for quantitation. When the derivatization was carried out with PFBBr, 128.3 μM of PFBBr was used for each of the wastewater samples tested. The pH of the wastewater samples was adjusted

to 5.5 so that the maximum derivatization yields could be reached. The real samples without the addition of the standard fatty acids and the ones spiked with 20, 50 and 100 ppb each of C_4-C_5 fatty acids showed linear response with the correlation coefficients of 0.99349 and 0.99287 for butyric and valeric acids, respectively (acetic and propionic acids could not be determined due to the interfering peaks). These experiments indicated the presence of 19.9 ppb (average of three replicates) of butyric acid in the wastewater samples. The concentration calculated for valeric acid was negligible and no valeric acid was found during the analysis of wastewater samples without the addition of standard fatty acids. The results were confirmed by GC/ITMS and the reconstructed GC/ITMS chromatogram for wastewater sample derivatized with PFBBr is shown in Figure 4-25. The mass spectrum of C_4/PFBBr ester showed a molecular ion at *m/z* 267 and a fragmented ion at *m/z* 181. The latter fragment may result from the cleavage of the ester bond between the pentafluorophenyl group and the carboxylate.

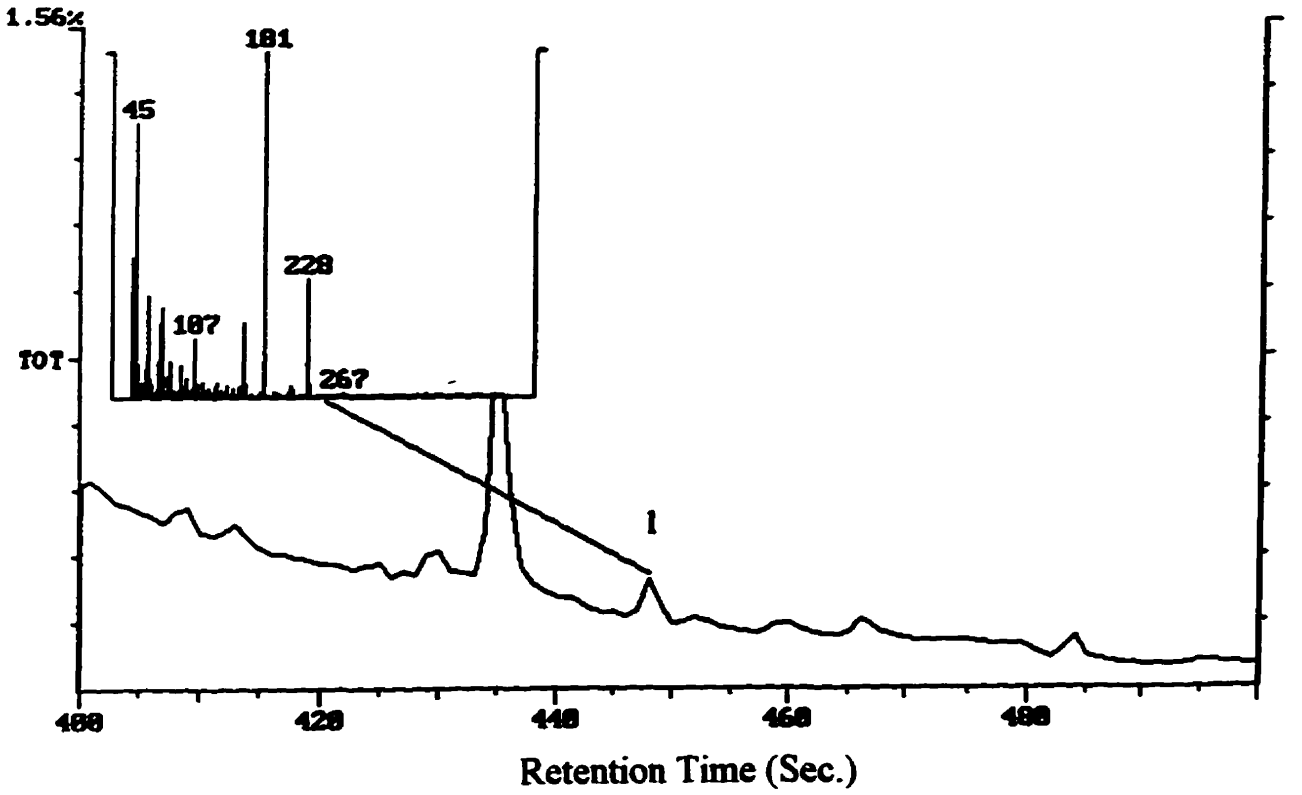

Figure 4-25. Reconstructed GC/ITMS chromatogram for the derivatization of short-chain fatty acids in a wastewater sample with PFBBr, indicating the presence of butyric acid.

The same wastewater samples were also examined with PFPDE and PFPDE concentration of 118 μM was used for the derivatization of real samples. The pH of the wastewater solution was adjusted to 6. The real samples without the addition of standard fatty acids and the ones with the addition of 20, 50 and 100 ppb each of C_2-C_5 acids showed linear responses for C_2-C_5 acids with the correlation coefficients of 0.99074, 0.99256, 0.99381 and 0.99485, respectively. The above analyses indicated the presence of 18.8, 7.3 and 22.6 ppb of acetic, propionic and butyric acids (average of three replicates), respectively, in the wastewater. The GC/ECD chromatogram for the analysis of a wastewater sample with PFPDE is presented in Figure 4-26. The concentrations of butyric acid obtained with the two reagents, PFBBr and PFPDE, were very close, indicating the good accuracy of the method. No valeric acid was found in the wastewater samples with both reagents.

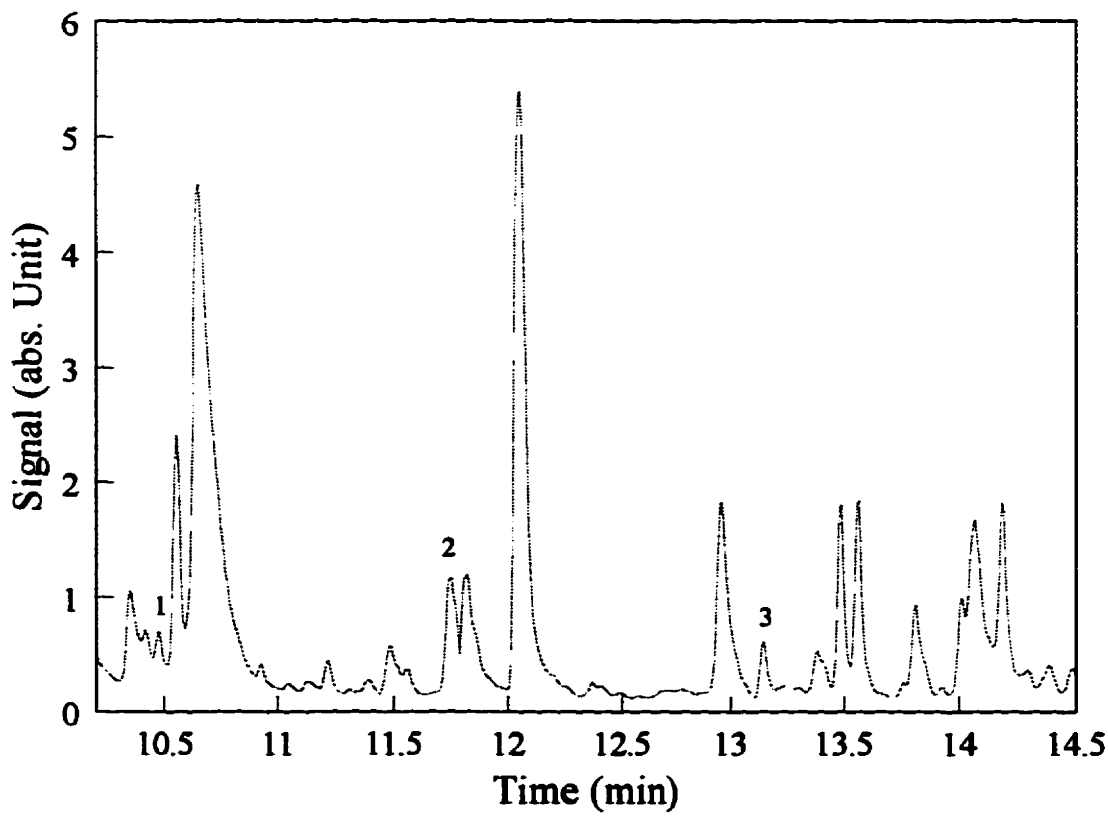

Figure 4-26. GC/ECD chromatogram of short-chain fatty acids as their PFPDE esters after derivatization of a wastewater sample: (1) acetic; (2) propionic; (3) butyric acid.

(b) Sludge Sample

As presented before, the in-fiber derivatization of aqueous samples using PDAM cannot be completed in a reasonable time, which indicates that it is not feasible to use an exhaustive reaction to perform quantitation. However, quantitation is still possible. In this study, quantitation was performed by spiking isotopically labelled (^{13}C) analogues to the sample solution. The addition of isotopically labelled analogues of the target analytes as internal standards can be used to directly determine the concentration of the native target analytes without the need to obtain 100% extraction with the fiber. The advantage of using isotopically labelled compounds is that they have the same physical and chemical properties and chemical structures as their native analogues, and, as such, they behave in a similar manner.

PA fiber calibration curves of C_2-C_4 acids, with the addition of 1 ppm each of the ^{13}C labelled analogues as internal standards, were examined. The calibration curves were linear over the concentration ranges from 250 ppb to 25 ppm for C_2-C_4 acids. The correlation coefficients were all above 0.99433. A 3 hour reaction and extraction time was used for sampling.

During the real sample analysis, 10 mL of deionized water was added to 10 mL sewage samples in a 40 mL amber vial. The sample matrix was then modified to saturated salt at pH 1.5 conditions. The unknown amount of fatty acids was calculated by comparing the area counts of isotopically labelled acids versus the area counts of the native acids using GC/ITMS. Figure 4-27 shows a reconstructed GC/MS chromatogram of a sewage sample obtained from the Wastewater Technology Centre, Burlington, ON. The sample was found to contain 6.6, 2.6 and 13.8 ppm of acetic, propionic and butyric acids, respectively, using the PA coated fiber. Isobutyric, valeric, isovaleric, pivalic and hexanoic acids were also found in the sewage sample.

The GC/ITMS analysis of each fatty acid/PDAM ester peak showed a molecular ion and a fragmented ion at *m/z* 215 (see Figure 4-28 for an example of the propionic acid/PDAM ester). The latter fragment may result from the cleavage of the ester bond between the pyrenylmethyl group and the carboxylate. This very same fragment was also seen in the GC/MS chromatogram of methylmalonic acid methyl monoester.[17]

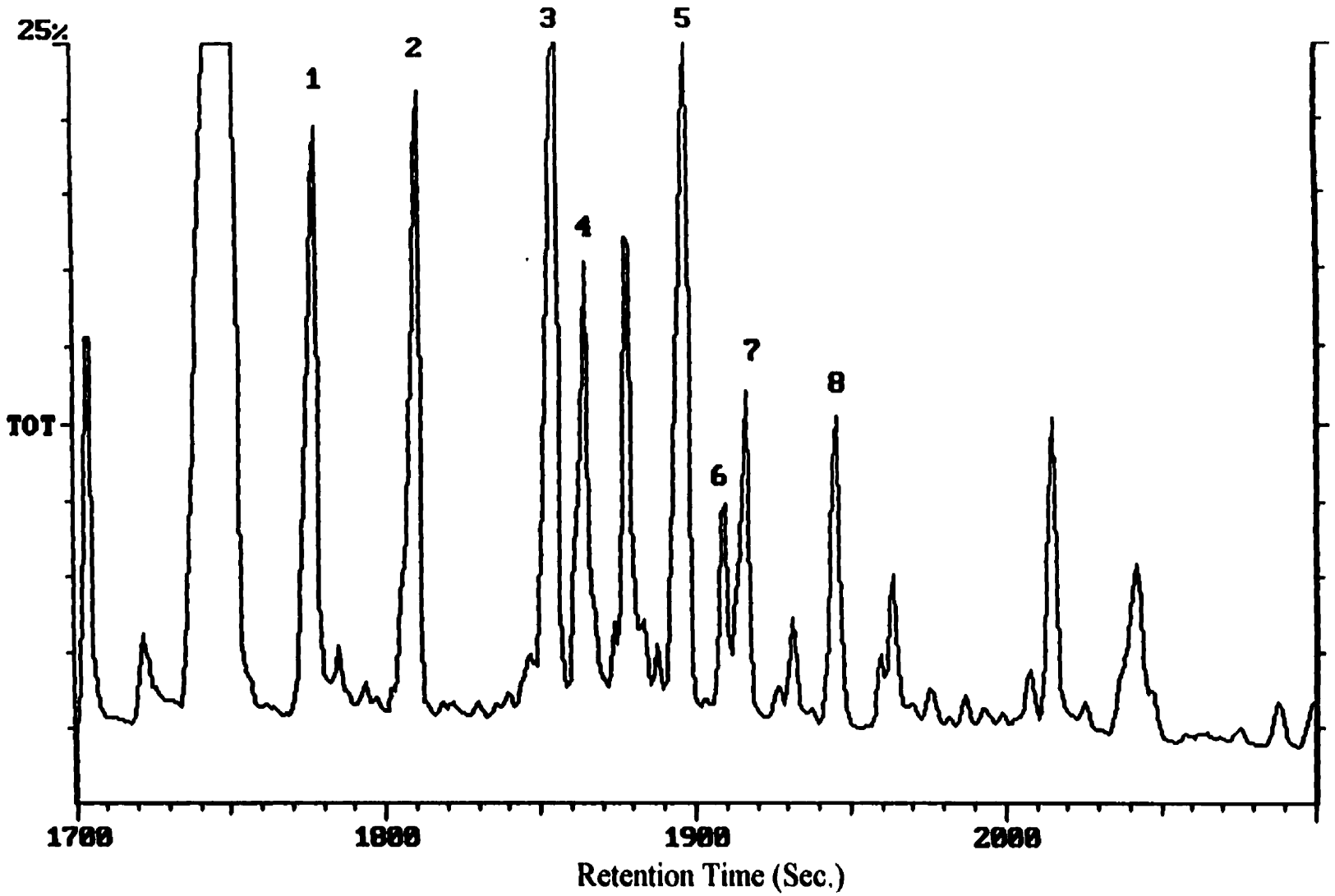

Figure 4-27. Reconstructed GC/ITMS chromatogram indicating short-chain fatty acids in a real sewage sample obtained with the PA coated fiber. The sample was found to contain the following acids: 1, acetic; 2, propionic; 3, isobutyric; 4, butyric; 5, pivalic; 6, isovaleric; 7, valeric; and 8, hexanoic. The actual peaks shown are pyrenylmethyl esters of these acids.

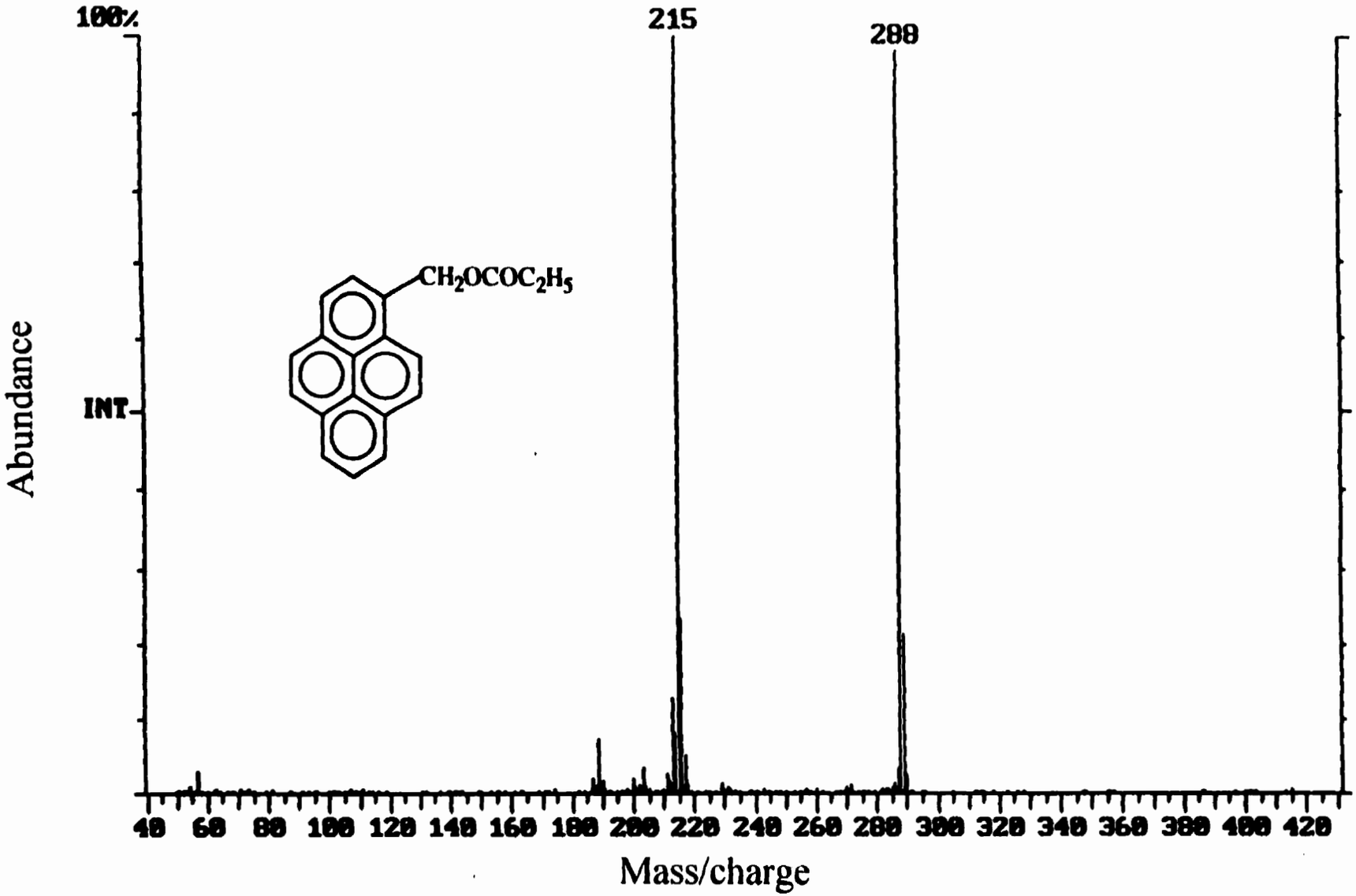

Figure 4-28. An example of mass fragmentogram and the structure of propionic acid/PDAM ester isolated from the real sample.

For the GC/ITMS analysis of the sewage sample, all the target analytes were scanned in extracted-ion chromatogram mode and quantitation was performed with the molecular ion. Table 4-6 shows the elution order of acid/PDAM esters, their corresponding FFAs, their molecular ion masses and quantitation masses. It can be seen that the elution order follows the order of chain length; iso-acids precede their straight-chain isomers. For the extracted-ion plot where the base ion is *m/z* 316, two peaks at different retention times were found which were only 29 second apart. To confirm that these were isomer peaks rather than one peak split in two, a known amount of valeric and isovaleric acids in the ratio of 1:2 ppm was spiked into water samples. The matrix was modified with saturated salt and adjusted to a pH of 1.5. The PA coated fiber soaked with PDAM was placed into the headspace for 3 hours, then subjected to GC/ITMS analysis (Figure 4-29). Two peaks, 29 seconds apart, in a ratio of 2:1 were found, supporting the assumption of the presence of isomers in sewage samples. Similarly, two peaks with molecular ion *m/z* 302 were confirmed as the butyric acid isomers.

(c) Milk Samples

In-fiber derivatization technique can be used both quantitatively (as above) and qualitatively for the analysis of fatty acid in real samples. In this section, qualitative analysis of fatty acids in milk samples was performed. These experiments indicated that simultaneous in-fiber derivatization with PDAM not only can be used to derivatize volatile C_2-C_6 fatty acids, it can also be used for the analysis of higher molecular weight fatty acids e.g. C_7-C_{10}. Figure 4-30 shows the GC/ITMS analysis of 2% milk using the in-fiber derivatization with PDAM. The PA coated fiber, doped with PDAM, was placed into the headspace above the sample matrix. The sample matrix was also modified to saturated salt conditions and adjusted to a pH of 1.5. The sampling time was 3 hours and the derivatization was carried out at room temperature. It was observed that fatty acids containing even carbon chain numbers were in much higher concentration than their odd number counter parts in the 2% milk samples. This is understandable since most of fatty acids occurring in nature are the ones with even carbon chain number. During these experiments, fatty acids larger than C_{10} acid were not detected. This is likely due to their low volatilities that could not provide sufficient quantity in the headspace during the shorter sampling times.

Table 4-6. Summary of elution order of fatty acid/PDAM esters, their corresponding fatty acids, their molecular ion masses and quantitation masses.

Elution order	Corr. fatty acid	Formula	Molecular ion	Quan. mass
1	Acetic acid	$C_9H_5O_2$	274	274
1	^{13}C-Acetic acid	$*C_9H_5O_2$	275	275
2	Propionic acid	$C_{10}H_7O_2$	288	288
2	^{13}C-Propionic acid	$*C_{10}H_7O_2$	289	289
3	iso-Butyric acid	$C_{11}H_9O_2$	302	302
4	Butyric acid	$C_{11}H_9O_2$	302	302
4	^{13}C-Butyric acid	$*C_{11}H_9O_2$	303	303
5	Pivalic acid	$C_{12}H_{11}O_2$	316	316
6	iso-Valeric acid	$C_{12}H_{11}O_2$	316	316
7	Valeric acid	$C_{13}H_{13}O_2$	316	316
8	Hexanoic acid	$C_{13}H_{13}O_2$	330	330

* = ^{13}C labelled analogue

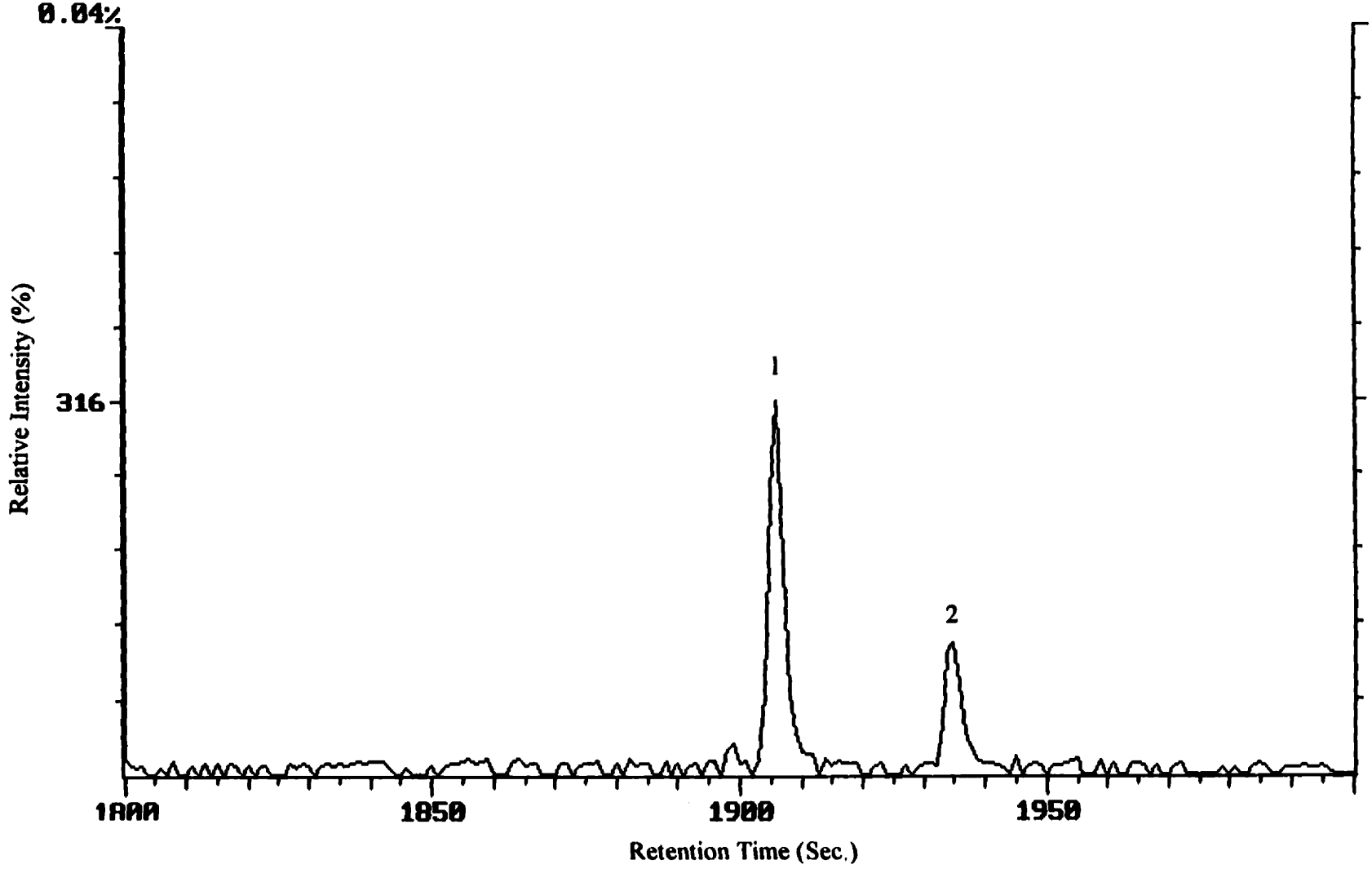

Figure 4-29. GC/ITMS chromatogram showing 1. isovaleric and 2. valeric acids (concentration of valeric/isovaleric acid = 1:2) as their PDAM esters, 29 sec. apart, confirming the presence of isomeric compounds in the sewage samples.

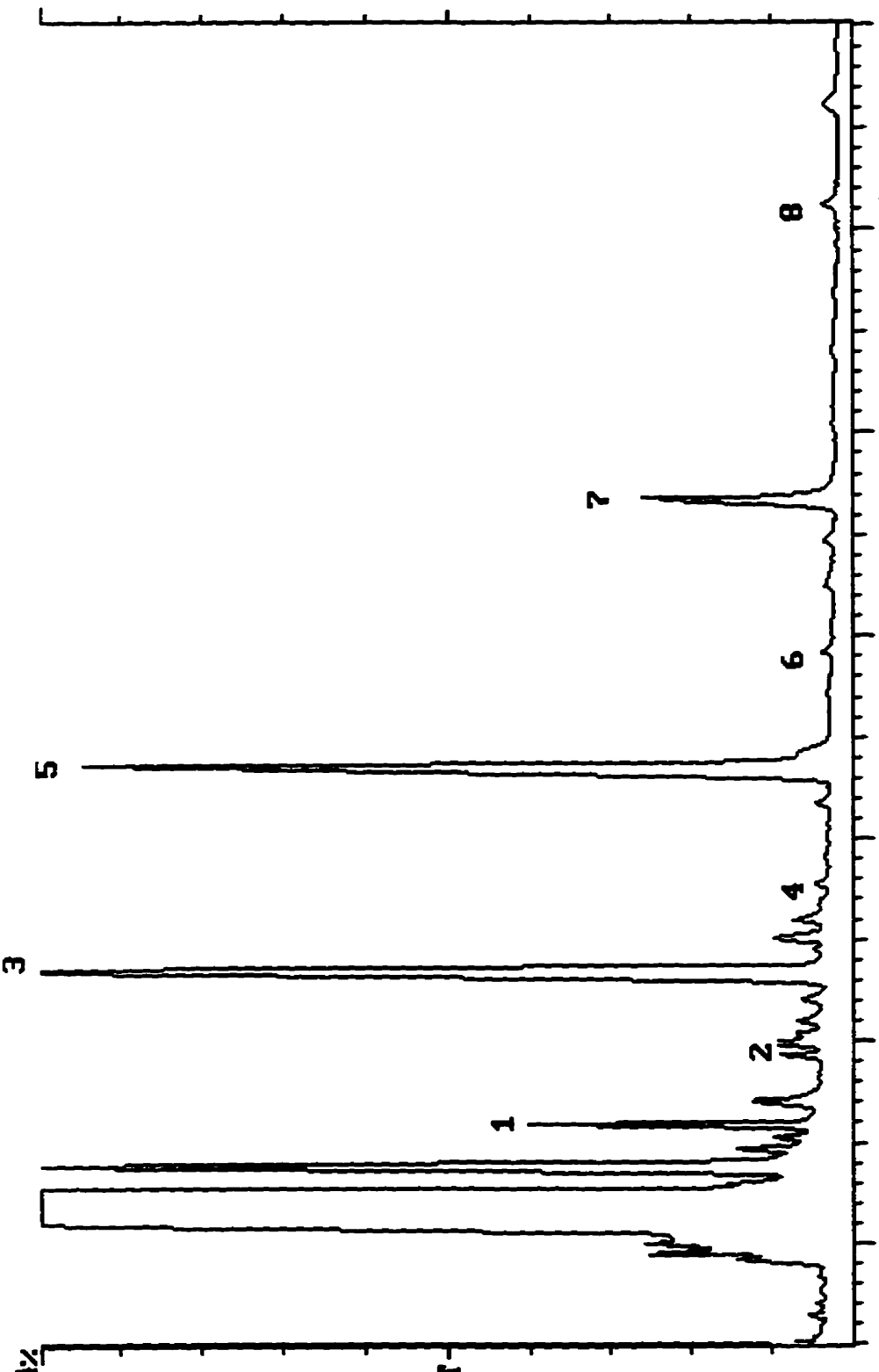

Figure 4-30. Reconstructed GC/TMS chromatogram indicating fatty acids in a 2% milk sample obtained with the PA coated fiber. The sample was found to contain the following acids: 1, acetic; 2, propionic; 3, butyric; 4, valeric; 5, hexanoic; 6, heptanoic; 7, octanoic and 8, decanoic. The actual peaks shown are pyrenylmethyl esters of these acids.

4.5.4 Comparison of Derivatization/SPME Sampling with Direct SPME Sampling

A summary of derivatization/SPME and direct SPME techniques for the analysis of short-chain FFAs in water is presented in Table 4-7. It was found that reactions of these acids with PDAM were not complete even after 35 hours. Derivatizations with PFPDE were complete within 12 hours, whereas derivatizations with PFBBr were complete within 2-3 hours. Extraction of short-chain fatty acids as PDAM derivatives should be an exhaustive extraction, however, the reactions never ran to completion. Extraction of short-chain fatty acids as PFBBr and PFPDE derivatives and extraction of free acids with direct SPME were equilibrium extractions. The desorption temperature for PDAM derivatives was high, 300°C, whereas for PFBBr and PFPDE derivatives, desorption was done at 250°C. For the in-fiber derivatization of fatty acids with PDAM and extraction of FFAs, sample matrices were modified to saturated salt at pH 1.5 conditions so that maximum amounts of fatty acids could be isolated by the fiber coating. For the in-matrix derivatization of fatty acids with PFBBr and PFPDE, the aqueous matrices were adjusted to pH of 5.5 and 6, respectively, to maximize the yields of derivatization.

Table 4-7. Summary of the derivatization/SPME techniques with PDAM, PFBBr and PFPDE and direct SPME sampling for the analysis of short-chain fatty acids from the aqueous phase.

Summary	PDAM	PFBBr	PFPDE	Extr. of free acids
Reaction time	> 65 hrs.	2 hrs.	12 hrs.	N/A
Reaction Temp.	25°C	55°C	25°C	N/A
Extraction type	exhaustive	equilibrium	equilibrium	equilibrium
Desorption Temp.	300°C	250°C	250°C	250°C
Desorption Time	5 min.	4 min.	4 min.	3 min.
Fiber used	PA	PA	PA	PA and PDMS
Detector used	FID or ITMS	ECD	ECD	FID
Matrix	36% salt & pH 1.5	pH 5.5	pH 6	36% salt & pH 1.5

Table 4-8 presents the summary of fiber linear ranges for the extraction of free C_2-C_5 acids and their PDAM, PFBBr and PFPDE derivatives from water. For PDAM analysis, a 3-hour sampling time was used. For PFBBr analysis, a 30-minute sampling time was employed, whereas for PFPDE a 2-hour extraction and reaction time was used to test the fiber linear range. As indicated in this table, the fiber linear ranges with PFBBr and PFPDE were relatively narrow compared to those with PDAM. This is because the lower end of the linear range for both PFBBr and PFPDE was limited by the background of the reagent blanks. The higher end was limited by the ECD linear range. PDAM, on the other hand, gave a wider linear range, but the sensitivity was not as good as those with the two fluorine tagging reagents.

Since PFBBr could not provide information for C_2-C_3 acids due to the interfering peaks when the analysis was done with GC/ECD, the fiber linear range was tested with GC/ITMS. This time, the data for all the short-chain fatty acids were obtained. Although the GC/ITMS analysis was less sensitive than the GC/ECD analysis, the fiber linear ranges were 2 orders of magnitude wider than for the ECD analysis. Together the three reagents covered a linear range from 1 to 5000 ng/mL. They can be used to analyze trace amounts of short-chain acids at any level in this range with the selection of a suitable reagent.

The LODs of derivatization/SPME techniques and direct SPME technique for the analysis of short-chain fatty acids in aqueous solutions are summarized in Table 4-9. Again, the LODs for the derivatization were determined by the reagent blank, not by the detectability of the detector. The data shown in Table 4-9 indicate that the in-matrix derivatization with PFPDE using the PA coated fiber and analysis with ECD gave the lowest detection limits for C_2-C_3 acids, whereas PFBBr provided the lowest LODs for C_4 and C_5 acids. Compared to direct SPME sampling of FFAs, the derivatization/SPME technique can lower the detection limits by 1 to 3 orders of magnitude.

Table 4-10 summarizes RSDs (%) for the analysis of volatile fatty acids in the aqueous solution using both the derivatization/SPME and direct SPME techniques. The RSDs were between 2.9 and 10% for all the techniques presented, which is acceptable.

Table 4-8. Summary of fiber linear ranges for the analysis of C_2-C_5 acids in the headspace above aqueous solution using derivatization/SPME and direct SPME techniques.

	Fiber linear range (ng/mL)			
	Acetic acid	Propionic acid	Butyric acid	Valeric acid
PDAM (PA, FID)	NT	10-5000	10-5000	NT
r^2	NT	0.99216	0.99643	NT
PFBBr (PA, ECD)	ND	ND	1-50	1-50
r^2	ND	ND	0.99193	0.99323
PFBBr (PA, ITMS)	100-10000	100-1000	50-1000	50-1000
r^2	0.98983	0.98954	0.99343	0.99241
PFPDE (PA, ECD)	2-200	1-100	1-100	1-100
r^2	0.99235	0.98951	0.98939	0.99054
Direct SPME (PA, FID)	1000-50000	1000-50000	1000-50000	50-5000
r^2	0.99485	0.99714	0.99928	0.99999

NT = not tested, ND = not detected

Table 4-9. Summary of LODs for the analysis of C_2-C_5 acids in aqueous solutions using derivatization/SPME and direct SPME techniques.

	LOD (ng/mL)			
	Acetic acid	Propionic acid	Butyric acid	Valeric acid
PDAM (PA, FID)	NT	2.5	1.4	NT
PFBBr (PDMS, ECD)	ND	ND	0.5	0.1
PFBBr (PA, ITMS)	2	0.8	0.6	0.5
PFPDE (PA, ECD)	0.8	0.6	0.5	0.4
Direct SPME (PA, FID)	760	290	122	3.1

Table 4-10. Summary of RSDs (%) for the analysis of C_2-C_5 acids in the aqueous phase using derivatization/SPME and direct SPME techniques.

	RSD (%)			
	Acetic acid	Propionic acid	Butyric acid	Valeric acid
PDAM (PA, FID), n=3, 50 ng/mL	NT	4.4	3.3	NT
PFBBr (PDMS, ECD), n=3, 10 ng/mL	ND	ND	6.6	5.2
PFBBr (PA, ITMS), n=3, 100 ng/mL	8.5	7.9	7.3	5.1
PFPDE (PA, ECD), n=3, 25 ng/mL	10	3.2	4.4	7.2
Direct SPME (PA, FID), n=10, 5 ng/mL	5	4	3.1	2.9

NT = not tested, ND = not detected

4.6 Derivatization/SPME of Long-Chain Fatty Acids in Water

4.6.1 In-fiber Derivatization with Diazomethane

Although HPLC is now becoming competitive in the separation of fatty acids, the method of choice for the characterization of fatty acids is still capillary gas chromatography because it provides better separation efficiency.[6] Since free long-chain fatty acids do not chromatograph well by GC, derivatization of these acids into derivatives with better GC characteristics is often needed to improve their gas chromatographic behaviour.

Conventional methods often require the use of organic solvents to isolate long-chain FFAs into organic media prior to derivatization and/or analysis, which is time-consuming and expensive. In the previous chapter, studies have demonstrated that direct SPME can be used to effectively isolate long-chain C_{16} and C_{18} fatty acids from water; however, the GC separation of these fatty acids was poor. In this chapter, a new technique combining post-derivatization with SPME extraction for the GC analysis of long-chain fatty acids is presented. The initial experiments were performed with palmitic and stearic acids. Since the previous experiments shown in Chapter 3 demonstrated that the PDMS coated fiber was the best one to isolate free palmitic and stearic acids from water, the 100 μm PDMS coated fiber was used to extract the selected long-chain fatty acids from aqueous solution prior to derivatization.

After a sufficient extraction time, the PDMS fiber was placed in the headspace of another vial containing diazomethane/ether to carry out derivatization. The derivatization mainly took place in the stationary phase of the fiber coating, since these acids have low volatilities. The reaction time profiles indicated that the derivatization could be finished at room temperature within 20 minutes.

The comparison of the GC analysis of non-derivatized palmitic and stearic acids with the methylated palmitic and stearic acids is shown in Figure 4-31. It can be seen that before derivatization, quantitation of free C_{16} and C_{18} acids was difficult owing to their incompatibilities with GC stationary phases which resulted in poor GC separation (peaks were flat and broad).

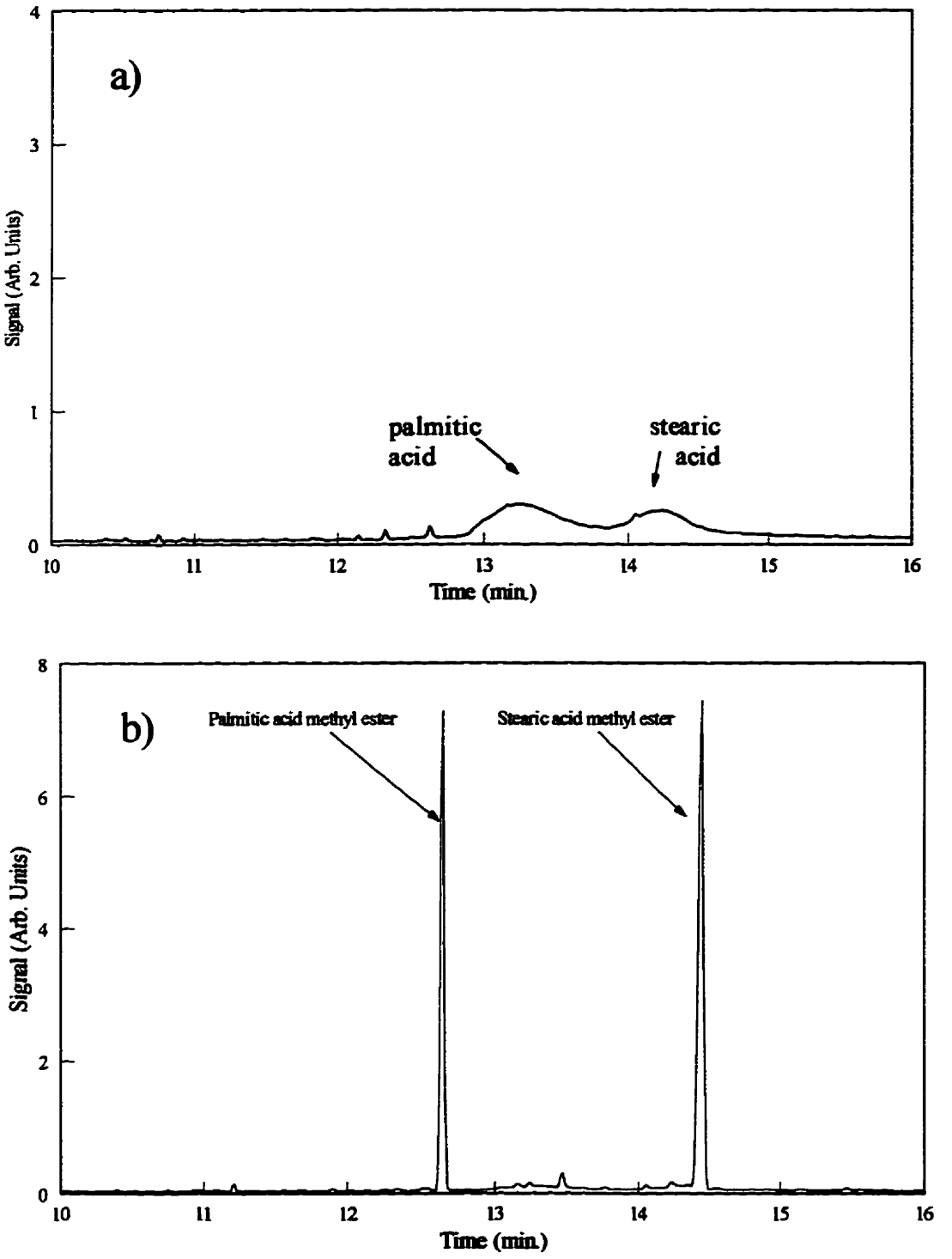

Figure 4-31. GC/FID analysis of (a) underivatized palmitic and stearic acids using direct SPME and (b) methylated palmitic and stearic acids using in-fiber derivatization. Note: the scale in (a) is half of that in (b).

After the derivatization technique was applied, the methylated peaks were very sharp, which significantly improved quantitation. Since the presence of the free acids was not detected, it was assumed that quantitative derivatization was achieved. This technique simplified sample concentration and derivatization steps thus it was simpler and more time efficient than conventional techniques.

As the in-fiber post-derivatization of C_{16} and C_{18} acids was successful, this technique was extended to the analysis of all the C_{10}-C_{22} fatty acids. Reaction time for the completion of post-derivatization of C_{10}-C_{22} fatty acids with CH_2N_2 was also examined. After a 2 hour extraction of 250 ng/mL each of C_{10}-C_{22} acids in aqueous solution, the PDMS fiber with concentrated acids was transferred into the headspace of a 4.6 mL vial which contained 3 mL of 0.03 M CH_2N_2 in ether. The ether solution was stirred vigorously during the reaction. The reaction time profiles are displayed in Figure 4-32. As shown in this figure, the yields of methyl esters peaked at 20 minutes for most of the long-chain acids examined. After 20 minutes, the amount of methyl esters started to decrease. For the C_{10} acid, the loss of methyl ester occurred after 15 minutes. This indicates that 20 minutes reaction time should be used in order to eliminate the loss of volatile derivatization products as well as to ensure proper quantitation.

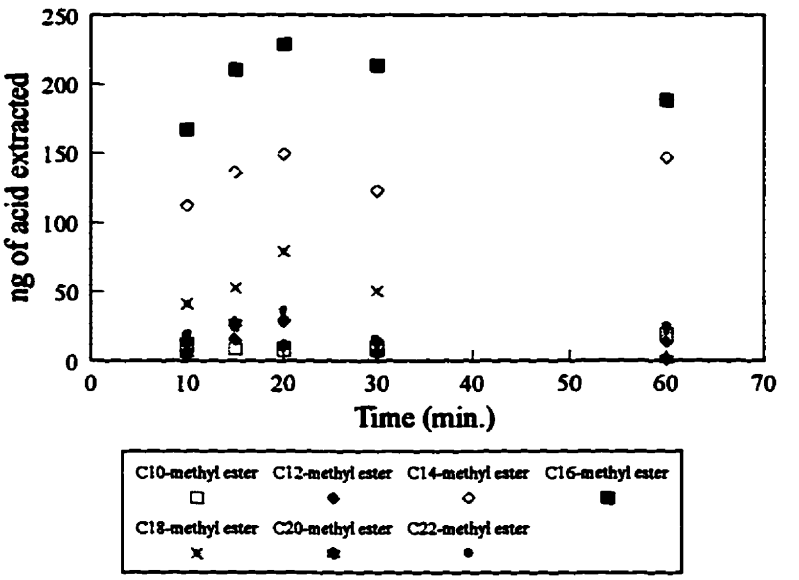

Figure 4-32. Reaction time profiles for the in-fiber derivatization of C_{10}-C_{22} acids with CH_2N_2.

The PDMS fiber linear range for the post-derivatization of C_{10}-C_{22} acids was also tested. All the acids tested showed a linear range between 0.5 and 250 ng/mL. Concentrations higher than 250 ng/mL were not tested since long-chain FFAs are no longer soluble in water at those concentrations.

Since both CH_2N_2 and the methyl esters of long-chain fatty acids have larger affinity towards ether, elimination of this organic solvent during derivatization would increase the reactivity of CH_2N_2 towards fatty acids in the SPME fiber coating. The elimination of the use of ether would also prevent the loss of methyl esters during reaction. Therefore, in-fiber derivatization of long-chain FFAs was examined again. This time, derivatization was performed by directly placing the fiber concentrated with long-chain FFAs in its coating in the headspace of the CH_2N_2 generator. The set-up of this experiment is shown in Figure 4-33. After CH_2N_2 gas was released from the decomposition of 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) with the addition of alkaline solution, it reacted with long-chain FFAs in the PA coated fiber before it was trapped in the ice cold ether solution.

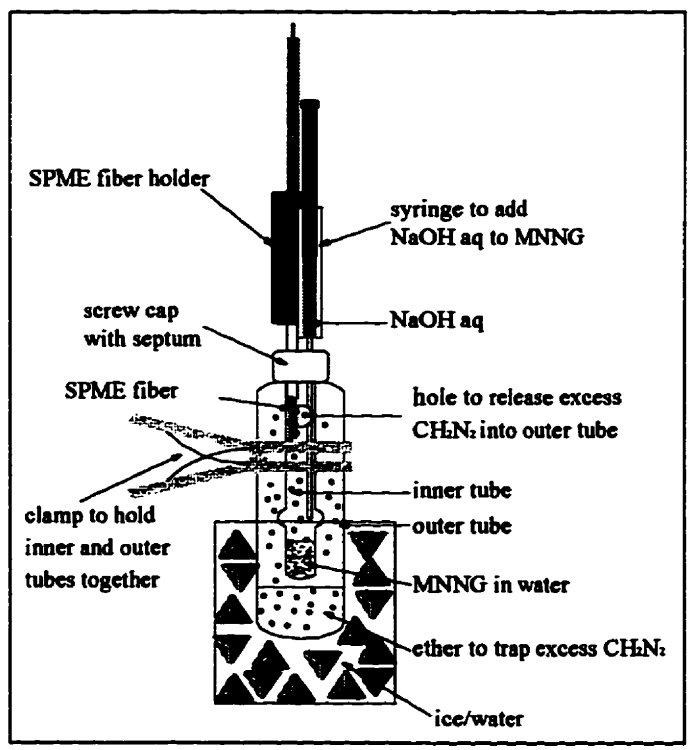

Figure 4-33. The set-up for post in-fiber derivatization of C_{10}-C_{22} acids in the CH_2N_2 generator.

Figure 4-34 compares the amounts of acids extracted by the PDMS coated fiber after the derivatization was performed in the diazomethane gas generator for 5 and 20 minutes, to that performed in the headspace above a diazomethane/ether solution (3 mL solution in a 4.6 mL vial) for 20 minutes. It can be seen that reactions performed in the diazomethane gas generator produced larger amounts of fatty acid/methyl esters even at a 5 minutes reaction time than those performed in the diazomethane/ether solution for 20 minutes. These experiments demonstrated that the elimination of the direct exposure of the fiber concentrated with target analytes to the ether solution during the in-fiber derivatization is essential to reduce the derivatization time. The lower amounts of fatty acid/methyl esters generated with the diazomethane/ether solution were likely due to the combination effect of the desorption of fatty acids as well as their derivatives from the fiber coating into the ether solution during derivatization process. Derivatization performed in the gas generator can eliminate these disadvantages.

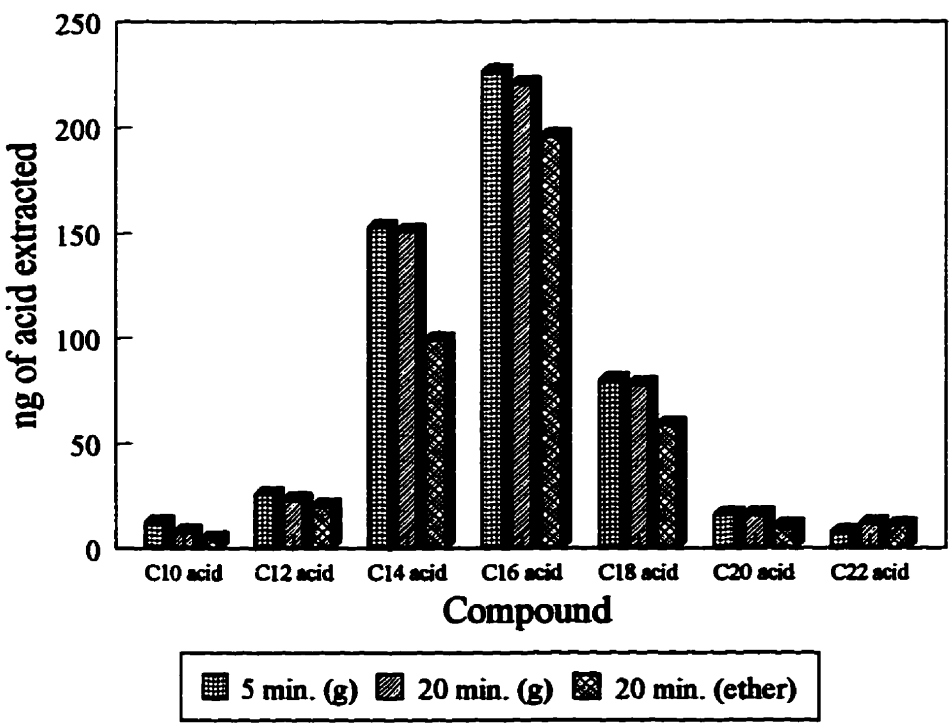

Figure 4-34. Comparison of the amount of C_{10}-C_{22} acids extracted by the PDMS coating after the in-fiber derivatization performed in the diazomethane gas generator for 5 min. and 20 min. with 20 minutes reaction in the diazomethane/ether solution.

When the in-fiber derivatization was performed in the diazomethane gas generator the majority of the fatty acids showed no difference in the amount of methyl esters produced between a reaction time of 5 minutes and 20 minutes. The only exception was C_{22} acid, which had a slightly lower amount of methyl ester obtained in a 5 minute reaction than in 20 minute reaction. This indicates that the longer chain fatty acids have slower reaction rates with diazomethane than the shorter chain fatty acids. When the longer chain fatty acids are the main target analytes during the analysis, a slightly longer reaction time would be appropriate to ensure the completion of derivatization.

4.6.2 In Injector-Port Derivatization of Long-Chain Fatty Acids with Ion-Pair Reagents

Since in-injector port derivatization technique is simple and straightforward, it was also combined with SPME sampling to improve the GC characteristics of long-chain fatty acids. Reactions of long-chain C_{10}-C_{22} fatty acids with ion-pair reagents, TMAOH and $TMAHSO_4$, were investigated using GC/ITMS.

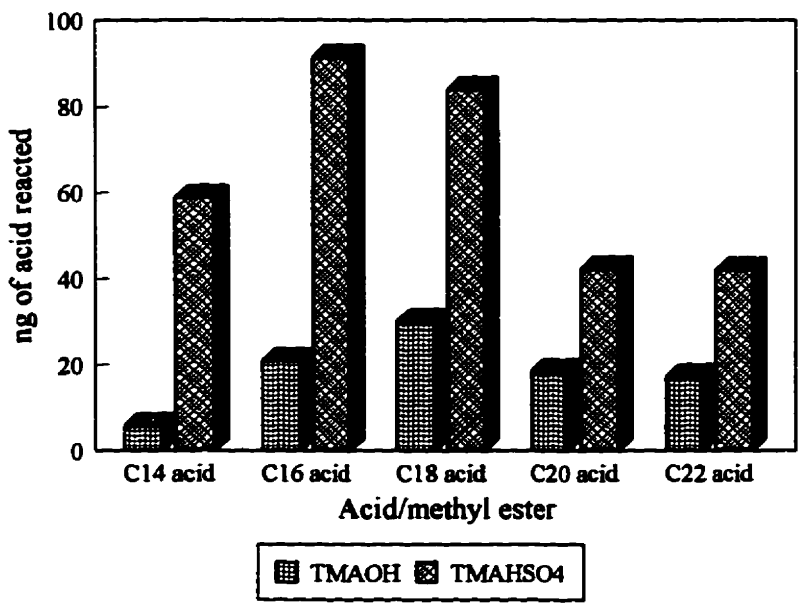

Figure 4-35. The effect of ion-pair reagents used on the yields of derivatization of C_{10}-C_{22} acids.

Figure 4-35 shows the effect of the reagent used on the yields of reaction (with the PA fiber coating). Samples contained 1 ppm each of C_{14}-C_{22} acids. Both reagent concentrations were 1000 times excess than the acid concentration. The pH of each test was controlled at 9. Theoretically, the derivatization is most likely taking place by nucleophilic attack of $RCOO^-$ to the methyl group on TMA^+ ion to form methyl esters under high temperature. Therefore, the amounts of esters generated by above two reagents were expected to be the same. However in this experiment, it was observed that $TMAHSO_4$ gave a higher yield of methyl esters for all the acids tested. The difference between the above two ion-pair reagents is that they have different counter ions. These might have some impacts on the situations encountered above. However, the real reason behind this case is not clear.

In-injector port derivatization technique was not suitable for the derivatization of C_{10} and C_{12} acids, as the methyl esters of these two acids were not found in the GC/ITMS chromatogram. In fact, the experiment for the analysis of C_2-C_8 acids with $TMAHSO_4$ under the same conditions above was also carried out. However, no methyl esters for these acids were found during the GC/ITMS analysis. This revealed that in-injector port derivatization technique was not suitable for the determination of relatively volatile fatty acids. The above problems might be due to the fact that these acids are too polar under the experimental conditions and they could not be extracted efficiently with the PA coated fibers along with the ion-pair reagent. Therefore, the data for C_{10} and C_{12} acids could not be reported.

The effect of reagent concentration on the yield of reaction was also tested. TMAOH was first investigated as the ion-pair reagent and the results are shown in Figure 4-36. As it can be seen from this figure, when the reagent/acid ratio of 20:1 was used, the methyl esters of C_{20} and C_{22} acids could not be seen; therefore, higher reagent concentrations were needed. Changing the reagent/acid ratio from 20:1 to 100:1 did not increase the yields of methyl esters significantly for C_{14}-C_{18} acid. Increasing the ratio from 100:1 to 1000:1 resulted in a significant increase of methyl esters produced for all the long-chain fatty acids tested.

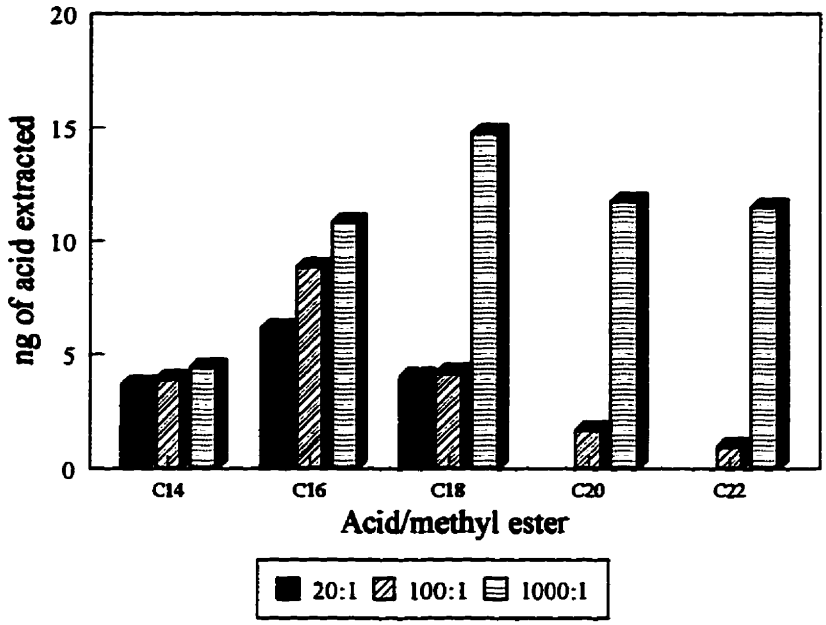

Figure 4-36. Ratio of TMAOH/acid on the yield of derivatization of C_{14}-C_{18} acids.

Since it was found later that $TMAHSO_4$ produced higher yields of the methyl esters than TMAOH, higher concentrations of $TMAHSO_4$ were further investigated. Figure 4-37 indicates that a further increase of the reagent/acid ratio from 1000:1 to 3000:1 resulted in an increase in the yields of the methyl esters for C_{16}-C_{22} acids. A ratio higher than 3000:1 is not recommended since higher concentrations would produce higher background which would affect the sensitivity of the method. When the ion-trap mass spectrometer is used, higher concentrations could contaminate the trap, which would also affect the accuracy of analysis. As well, higher concentrations could also shorten the column lifetime.

The effect of injector temperature on the efficiency of derivatization and fiber desorption was also examined. The fiber desorption time was 4 minutes. As shown in Figure 4-38, increasing the injector temperature from 275°C to 300°C resulted in an increased signal for all the acid/methyl esters. The test for carryover indicated that no carryover was found after the injector temperature was increased to 300°C.

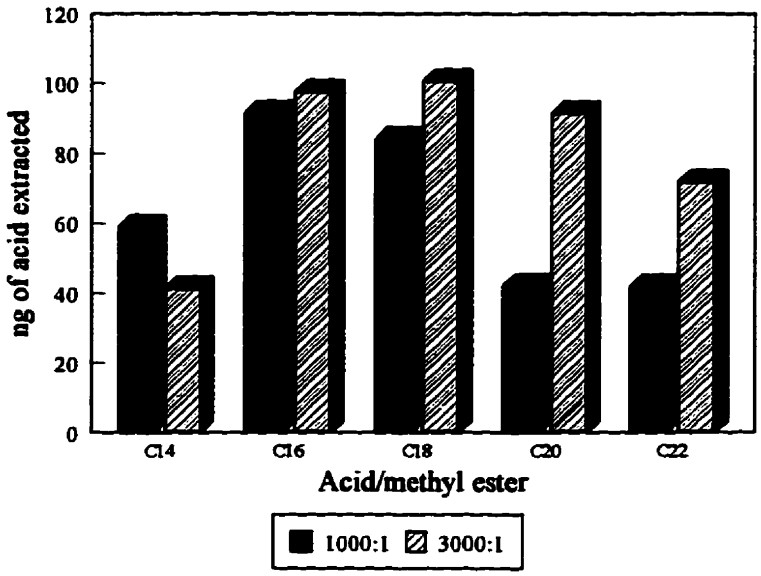

Figure 4-37. Amount of reagent $TMAHSO_4$ used on the yield of derivatization.

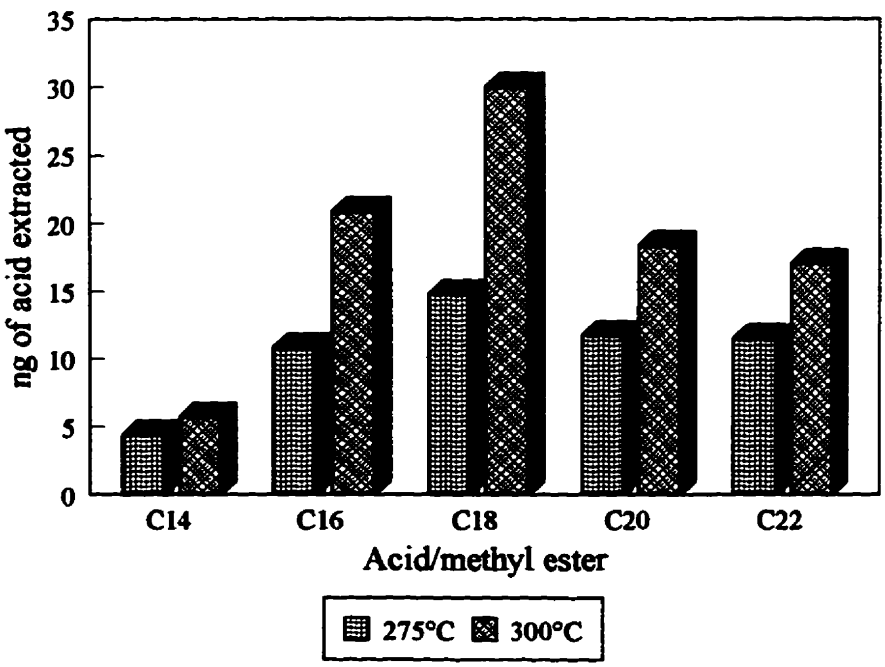

Figure 4-38. The effect of the injector temperature on the efficiency of fiber desorption and derivatization.

During the preliminary experiments, free acids were also observed in the GC/ITMS chromatogram. This might have been due to the fact that the amount of TMA^+ in the PA fiber was not sufficient to derivatize all the fatty acids in the GC injector port. To overcome this problem, an extra amount of reagent was injected by syringe right before fiber injection. No free acids were seen after this technique was applied (see Figure 4-39 for an example). Since the derivatization actually takes place in the hot injector port, the insufficient reaction time would also be a reason for the presence of free fatty acids. Because of the special geometry of the SPI injector, all the analytes injected into it would elute into the column within a few seconds. This is good for the regular injection since it provides alternative way for on-column injection. However, in this experiment, it did not provide sufficient time for the reaction of long-chain FFAs (as $RCOO^-$) and the TMA^+ counter ions to take place in the hot injector before they were deposited onto the cold column. Once they got into the column, the temperature was not high enough to carry out the reaction completely. This problem could be overcome by using a split/splitless injector, as the analytes could remain in this kind of injector about 40 seconds before they are eluted into the GC column. Thus, fatty acids and their counter ions would have longer reaction times in the hot GC injector to complete derivatization.

The effect of pH on the yields of methyl esters was also examined. Two pH values , 6 and 9, were examined in these preliminary experiments. Figure 4-40 indicates that pH 6 resulted in a higher yield for the smaller C_{14} acid, while it decreased the yield of formation of the methyl esters for longer chain acids C_{20}-C_{22} acids. When the pH was higher, the yields increased for the longer chain acids, whereas for the shorter chain C_{14} acid the yield was decreased. For C_{16} and C_{18} acids no significant difference was observed in the yield between the two pH values. The effect of pH might be associated with the pK_a values of the acids tested. Since the majority of analytes have a increased yields at a pH 9, it should be used for subsequent experiments.

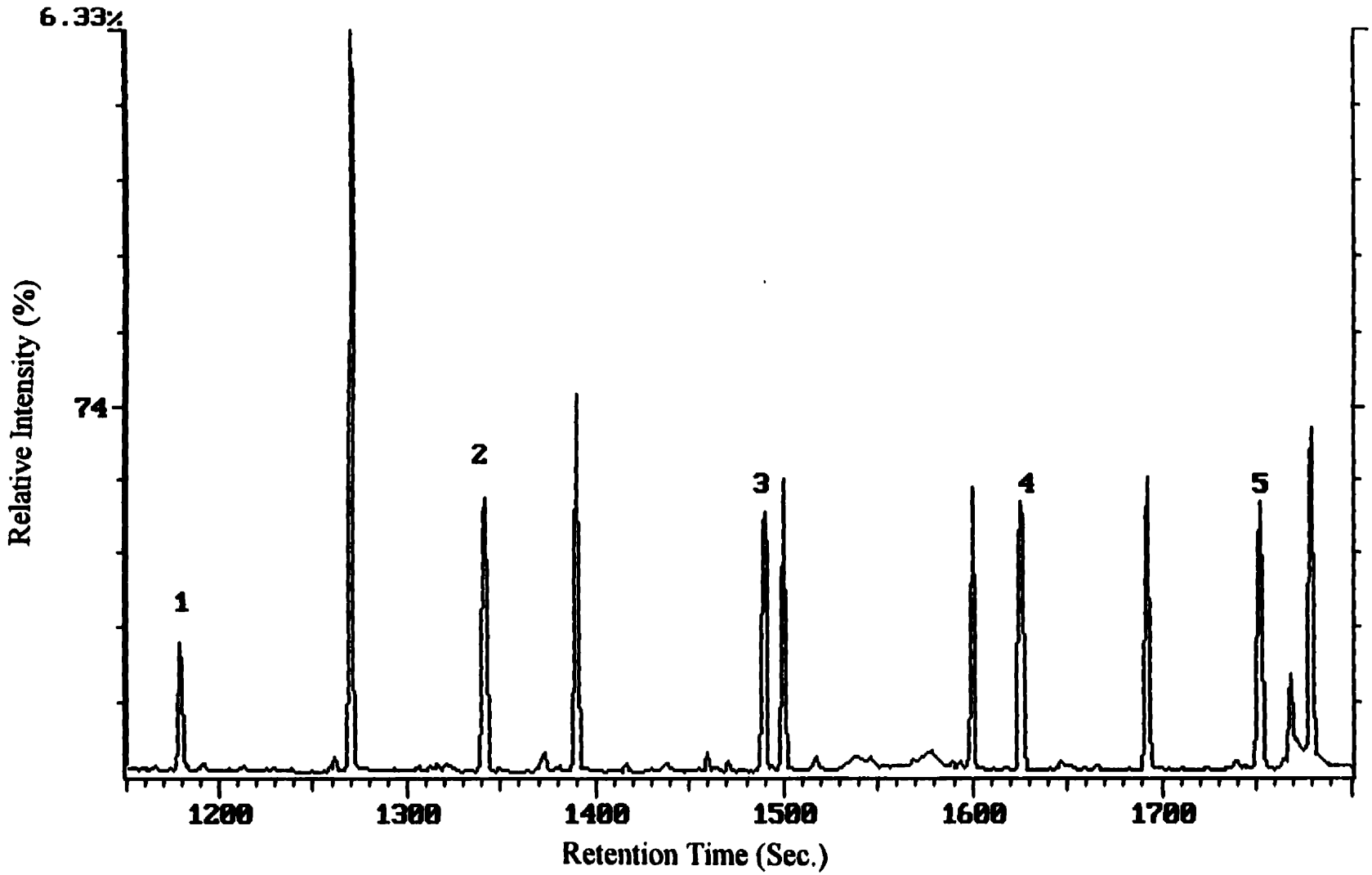

Figure 4-39. Extracted-ion GC/ITMS chromatogram with the base ion of *m/z* 74, for the derivatization of: 1, C_{14}; 2, C_{16}; 3, C_{18}; 4, C_{20} and 5, C_{22} acids with $TMAHSO_4$. The actual peaks shown are methyl esters of these long-chain fatty acids.

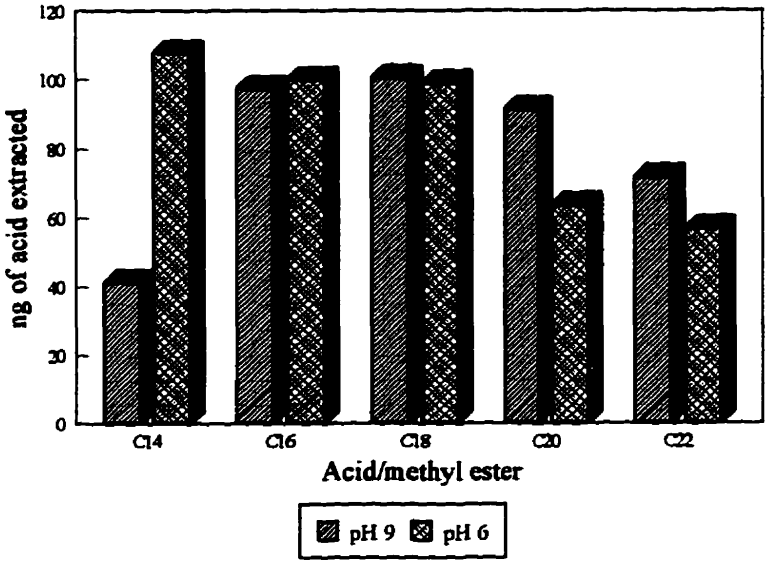

Figure 4-40. The effect of pH on the yields of derivatization of 1 ppm of C_{10}-C_{22} acids with $TMAHSO_4$.

4.7 Summary

This chapter has presented new derivatization/SPME techniques to successfully extract and quantify fatty acids from aqueous solutions and/or the gaseous phase. For air sample analysis, the in-fiber derivatization with PDAM and the in-matrix derivatization with PFPDE significantly improved the LODs compared to direct SPME sampling. The LODs for the analysis of C_2-C_5 acids were in low pg/mL to high fg/mL levels after derivatization/SPME techniques were applied, with the fiber linearity covering 3 orders of magnitude.

For the analysis of C_2-C_5 acids in aqueous solution, three reagents, PDAM, PFBBr and PFPDE, were examined to carry out derivatization/SPME. Derivatization coupled with SPME techniques has sub ppb limits of detection, 2-3 orders of magnitude more sensitive than direct and headspace SPME sampling. The method linear ranges spanned from 1 to 5000 ng/mL, for the combined three reagents tested. They can be used to analyze trace amounts of short-chain fatty acids at any level in this range with the selection of a suitable reagent. Derivatization combined with SPME also significantly improved the GC separation and quantitation for long-chain fatty

acids in aqueous solution. In general, several parameters should be optimized so that the derivatization/SPME could be used effectively and efficiently for the analysis of fatty acids in water: SPME sampling mode (direct SPME versus headspace SPME), the type of reagents, the ratio of the reagent/target analytes, the matrix conditions (pH or salt), the presence of organic solvents and reaction temperatures.

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CHAPTER 5

DETERMINATION OF AMINES IN AIR AND WATER USING SOLID-PHASE MICROEXTRACTION

5.1 Introduction

In the previous two chapters, studies of the determination of fatty acids in aqueous and/or gaseous matrices using direct SPME, headspace SPME and derivatization coupled with SPME were presented. These studies showed that these techniques can be selectively used for the sensitive determination of analytes of interest. In the current chapter, the study of another challenging group of polar compounds, organic amines, in gaseous as well as aqueous matrices using SPME techniques is explored.

5.2 Experimental

Material. The following amines were obtained from Aldrich Chemical Co. and used as received: methyl, ethyl, diethyl, isobutyl, butyl, amyl, and hexyl amines. The derivatizing reagents 1,2-naphthoquinone-4-sulfonate (NQS) and 2,3,4,5,6-pentafluorobenzylaldehyde (PFBAY) were both obtained from Aldrich Chemical Co., whereas 4-nitrophenyl trifluoroacetate (NPTFA) was synthesized by Dr. Chong's group (Department of Chemistry, University of Waterloo, Waterloo, Ont.). All the above three derivatizing reagents were used as received without further purification. NPTFA, NQS, and PFBAY should be regarded as potentially hazardous, and skin or eye contact should be avoided. All solvents used in this study were analytical-reagent grade. Deionized water (NANOpure) was used to prepare aqueous samples.

Three types of fibers obtained from Supelco were used for this work: carbowax divinylbenzene [CAX(DVB)], poly(dimethylsiloxane) (PDMS), and poly(acrylate) (PA) with the film thicknesses of 65, 100 and 85 μm, respectively. The details on how these fibers were conditioned were described in the experimental section of Chapter 3.

The stock standard containing 2000 μg/mL each of methyl, ethyl, diethyl, isobutyl, butyl, amyl, and hexyl amines was prepared by dissolving the pure analytes in acetonitrile (MeCN). This solution was then diluted with either deionized water or MeCN by factors ranging from 100 to 500 to prepare the working sample solutions. A pH 10 buffer solution was prepared according to the reference.[1]

For air sample analysis, 40 mL amber vials were used along with 2.5 cm × 0.8 cm stir bars (Bel-Art products) to agitate the samples during extraction. The vials were sealed with Teflon faced silicone septa and hole caps. Two microliters of the stock solution in MeCN were injected into a sample vial. Prior to the sampling, the sample vial was heated in a drying oven at 100°C to help vaporize the amines and then cooled to room temperature. For aqueous sample analysis, 15 mL sample vials and 1.2 cm × 0.8 cm stir bars were used. Ten mililiters of an aqueous sample solution were added to the sample vial.

Instruments and apparatus. The same Varian 3400 GC equipped with FID (Varian Canada) as described in Chapter 3, was used for the analysis of both free amines and their derivatives. The column used was a 30 m, 0.25 mm ID SPB-5 with a 1 μm stationary phase (Supelco Canada). For the analysis of free amines and their NPTFA and PFBAY derivatives, the GC conditions were as follows: the initial oven temperature was 40°C for 1.5 minutes, then programmed to 135°C at a rate of 15°C/min and from 135°C to 280°C at a rate of 20°C/min with a final hold time of 2 minutes; the FID was held at 300°C; the injector temperature was programmed from 40 to 275°C at a rate of 250°C/min for solvent injections and it was held at 275°C for SPME fiber injections. The fiber desorption time was 5 minutes.

The FID response for the analysis of free amines including methyl, ethyl, diethyl, isobutyl, butyl, amyl, and hexyl was linear between 5 and 400 ng (10-2000 ppm). The correlation coefficients of the syringe calibration curve were between 0.99241 and 0.99615 for all the amines tested.

Derivatization Procedure.

With NPTFA: Reactions of amines with NPTFA in methylene chloride (CH_2Cl_2) were carried out for syringe calibration. Five hundred microliters of 10 mg/mL NPTFA/CH_2Cl_2 solution was added to 500 μL CH_2Cl_2 solution containing amines with the concentrations ranging from 20-5000 ppm. The solution was mixed with a magnetic stir bar at room temperature for 30 minutes, at which time the GC/FID area counts of target peaks no longer increased and remained constant. The calibration curve showed a linear response for the concentrations tested. The calibration curve was then used to examine quantitation of amines for the fiber injection. The

correlation coefficients of the syringe calibration curves ranged from 0.99334 to 0.99931 for all the NPTFA derivatives tested. The masses of solutes extracted by the fiber coatings were all within the calibration range to ensure accuracy.

Derivatization of amines with NPTFA in air was carried out with in-matrix derivatization technique. The detailed set-up of in-matrix derivatization for air analysis was presented in the experimental section of Chapter 3. Two microliters of the standard amine mixture were spiked into 40 mL silanized amber vials. After amines were fully vaporized, 2 μL of 80 mg/mL of the NPTFA/CH_2Cl_2 solution was injected into the sample vial. The mixture was allowed to mix well and react at room temperature for 10 minutes before the fiber was placed in the reaction vial to isolate reaction derivatives.

With PFBAY: Reactions of PFBAY with amines at concentrations ranging from 10 to 1000 ppm in MeCN were used to carry out the syringe calibration and the external calibration for the fiber extraction and analyzed by GC/FID. The above reaction mixture was sealed in a reaction vial. The vial was heated at 80°C for 2 hours in a water bath. After the reaction, the vial was taken out of the water bath and cooled to room temperature. Exactly 0.2 μL of this mixture was then injected onto the GC column. The correlation coefficients were between 0.99367 and 0.99942 for all the amine/PFBAY imines. Masses extracted by fiber coatings were also within the mass range of the calibration curve.

Derivatization of amines in water with PFBAY was also carried out with in-matrix derivatization technique. The detailed set-up of in-matrix derivatization for aqueous sample analysis was presented in the experimental section of Chapter 3. Before the reaction, 2 μL of 2.25 mg/μL PFBAY/MeCN and microliter amounts of amine stock solution were spiked into the 10 mL aqueous solution in a 15 mL vial. The vial was then sealed and placed in a water bath kept at 80°C for 15-30 minutes. After the reaction mixture cooled to room temperature, a fiber was introduced in the headspace above the aqueous solution to extract reaction derivatives.

5.3 Determination of Amines in Air

5.3.1 Extraction of Free Amines with Direct SPME

The initial work for the analysis of free amines was performed by direct SPME. Three fiber coatings were examined: CAX(DVB), PA and PDMS. Like fatty acids, amines are also very polar; therefore, the sample vials were acid washed and silanized prior to sampling.

Examination of the exposure time profiles for the isolation of 25 ng/mL each of methyl, ethyl, diethyl, isobutyl, butyl, amyl, and hexyl amines from air using the above three fibers was first carried out. The results indicated that under the procedure used, methyl and ethyl amines could not be extracted in amounts large enough for FID detection. Therefore, the data for these two amines could not be reported. The exposure time profiles indicated that the equilibrium was reached within 20 minutes for diethyl, isobutyl, butyl, amyl, and hexyl amines with the PDMS coated fiber, and 30 minutes for the CAX(DVB) and PA coated fibers.

The amounts of the amines isolated with the above three fiber coatings from air samples were then compared and the results are shown in Figure 5-1. Sample concentration was 25 ng/mL. The extraction time was 30 minutes with all three fibers. The results reveal that the amounts extracted by the fiber followed a trend: CAX(DVB) > PA > PDMS. Among the above three fiber coatings, the PDMS is non-polar, whereas the CAX(DVB) and PA are both polar polymers. Therefore the polar amine compounds have lower affinity for the PDMS coating. As a result, the amounts of amines extracted by the PDMS coating were the lowest. The CAX(DVB) fiber is a polar porous polymer which has hydroxyl groups on the surface. It has stronger interactions with amines through hydrogen bonding than the PA fiber coating. As a result, CAX(DVB) fiber coating extract larger amounts of amines than the PA coating. Therefore, the CAX(DVB) coated fiber was chosen for the remainder of experiments.

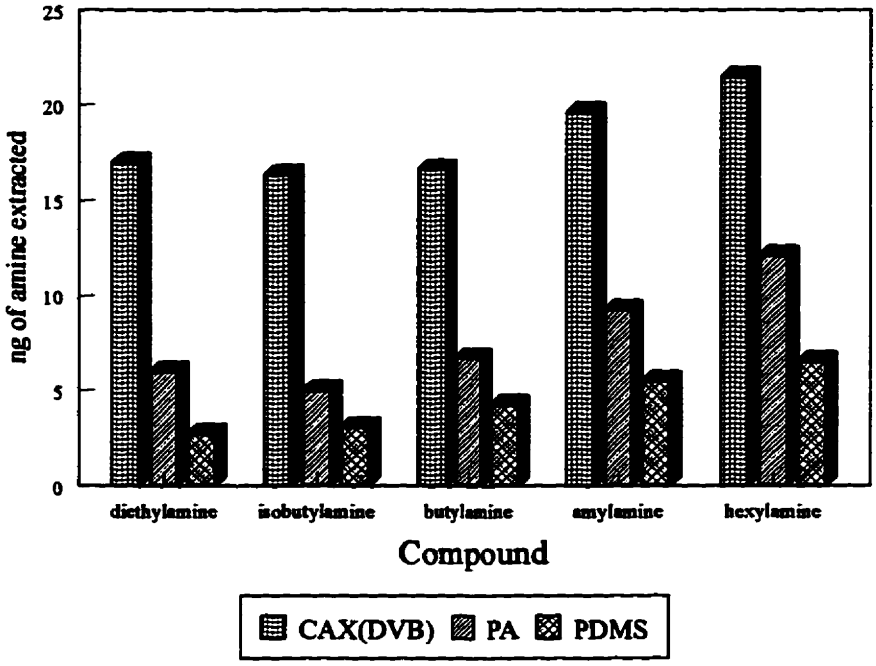

Figure 5-1. Coating evaluation for the extraction of volatile amines in air with the CAX(DVB), PA and PDMS coated fibers.

Table 5-1 summarizes the fiber linear ranges, the LODs and the RSDs (%) for the extraction of free amines from air using the CAX(DVB) coated fiber. The fiber linear ranges were found to be linear between 5 and 50 ng/mL for all the amines tested, except methyl and ethyl amines which could not be detected under the above conditions. The correlation coefficients were between 0.99277 and 0.99797. The %RSD values were calculated with 3 replicate extractions and they were between 2.1 and 6.1 for all the amines examined (except methyl and ethyl amines), which is considered to be satisfactory. The LOD was estimated to be the concentration of the amine which produces a signal five times that of the background noise. It can be seen that LODs were in the low ng/mL to the high pg/mL levels for the diethyl, isobutyl, butyl, amyl and hexyl amines.

Table 5-1. Fiber linear ranges, LODs and RSDs (%) using direct SPME sampling for the extraction of volatile amines from air with the CAX(DVB) coated fiber.

Compound	fiber linear range (ng/mL, FID)	r^2	LOD (pg/mL, FID)	%RSD (n=3, 10 ng/mL)
Methylamine	ND	-	-	-
Ethylamine	ND	-	-	-
Diethylamine	5-50	0.99658	1,010	6.1
iso-Butylamine	5-50	0.99277	672	2.1
Butylamine	5-50	0.99291	474	5.5
Amylamine	5-50	0.99797	327	4
Hexylamine	5-50	0.99605	261	5.1

ND = not detected

In the NIOSH guide,[2] the exposure limits to these small amines are around 10-30 ng/mL for industrial air and about 100-1000 times lower (10-300 pg/mL) for indoor air exposure. The experiments indicated that direct SPME could be used to monitor diethyl, isobutyl, butyl, amyl and hexyl amines in the industrial atmosphere. However, for the analysis of free methyl and ethyl amines in both the industrial and indoor air, as well as for the diethyl, isobutyl, butyl, amyl, and hexyl amines in the indoor air, more sensitive alternatives are needed.

5.3.2 Determination of Amines by Derivatization/SPME

The above experiments illustrated that free amines were too polar to be effectively isolated in very low concentrations from air by direct SPME sampling using the existing fiber coatings. Derivatization of these polar compounds to less polar and more volatile derivatives is essential to

overcome the above problems. As mentioned in Chapter 1, many derivatizing reagents have been used to derivatize amines. In this study, 4-nitrophenol trifluoroacetate (NPTFA) synthesized by Dr. Chong's group at the University of Waterloo, was used to convert methyl, ethyl, diethyl, isobutyl, butyl, amyl, and hexyl amines into the less polar trifluoroacetamides for easy GC separations. This reagent has the advantages of reacting with the volatile amines almost instantly at room temperature upon contact and generating volatile and thermally stable amide derivatives of the amines tested. This reagent also has the advantage of reacting with both of the primary and secondary amines at the same time.

Since the derivatizing reagent and the reaction derivatives are volatile, the derivatization of amines was carried out directly in the gaseous phase by spiking microliter amounts of NPTFA/CH_2Cl_2 into the sample vial. The reaction scheme for the derivatization of amines with NPTFA is shown in Figure 5-2. The pair of nonbonding electrons on the nitrogen of an amine allow the amine to function not only as a base but also as a nucleophile. They attack the carbonyl carbon of NPTFA, then the $O_2NC_6H_4O^-$ species is displaced (leaving as nitrophenolate) and an amide is formed.

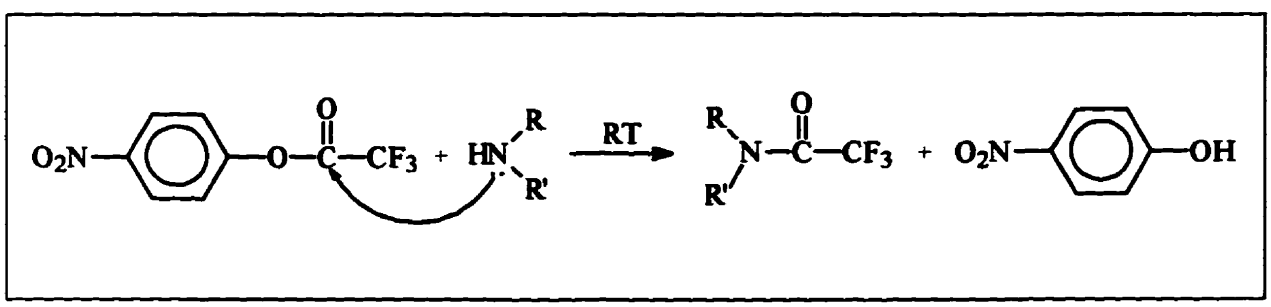

Figure 5-2. Reaction scheme for the derivatization of amines with NPTFA.

The effect of the amount of reagent used on the yield of derivatization was first examined. It was found that 160 μg (2 μL of 80 mg/mL stock solution) of NPTFA was able to derivatize all of the amines to their NPTFA amides in air. Higher concentrations did not produce higher yields

of the reaction derivatives. Therefore, 2 μL of 80 mg/mL of NPTFA/CH_2Cl_2 was used for subsequent experiments.

The exposure time profiles for the extraction of 25 ng/mL of the amines as their NPTFA amide derivatives from air were monitored using the CAX(DVB) coated fiber and the results are shown in Figure 5-3. It was noticed that the NPTFA derivatives of diethyl and isobutyl amines co-eluted and could not be separated under the conditions used. Therefore, the results for these two analytes are reported together. Before the CAX(DVB) was placed in the reaction vial, amines and NPTFA were allowed to mix well and react at room temperature for 10 minutes. This figure illustrates that the extraction equilibrium could be reached within 20-30 minutes. Similar experiments were also performed using the PA and PDMS coated fibers and the results (data not shown) indicated that the extraction could reach equilibration within 20 minutes for both fibers.

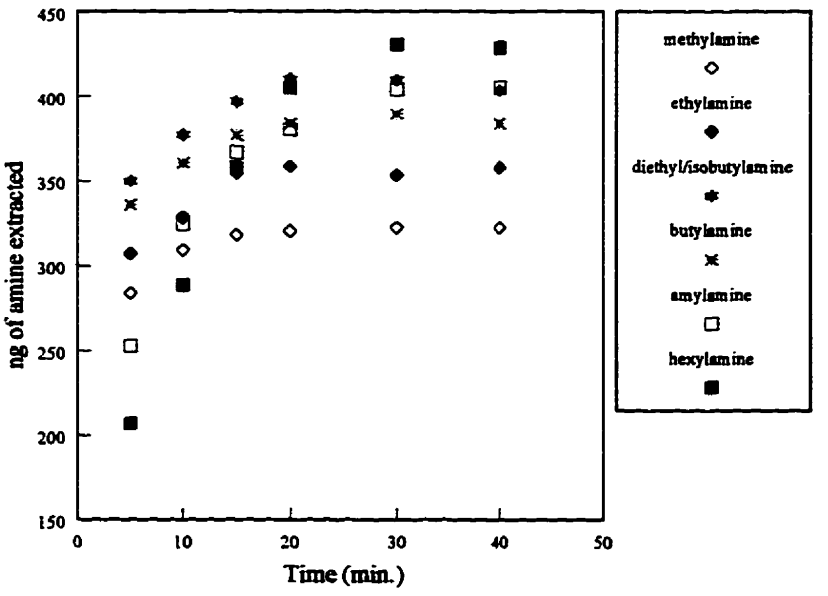

Figure 5-3. Exposure time profiles for the extraction of volatile amines as their NPTFA amides with the CAX(DVB) coated fiber from air.

Fiber coating evaluation for the extraction of 25 ng/mL of the volatile amines as their NPTFA derivatives from air was then examined using the three selected fiber coatings. The comparison of the amounts extracted is illustrated in Figure 5-4. The extraction time used was 30 minutes for all three fibers. This figure indicates that after the derivatization technique was incorporated, methyl and ethyl amines (as their NPTFA derivatives) extracted by all three fiber coatings could be detected with FID. The results also illustrate that the CAX(DVB) coated fiber extracted the highest amount of the amine/NPTFA amides among the three fibers tested. Therefore, the CAX(DVB) coated fiber was chosen to carry out subsequent experiments.

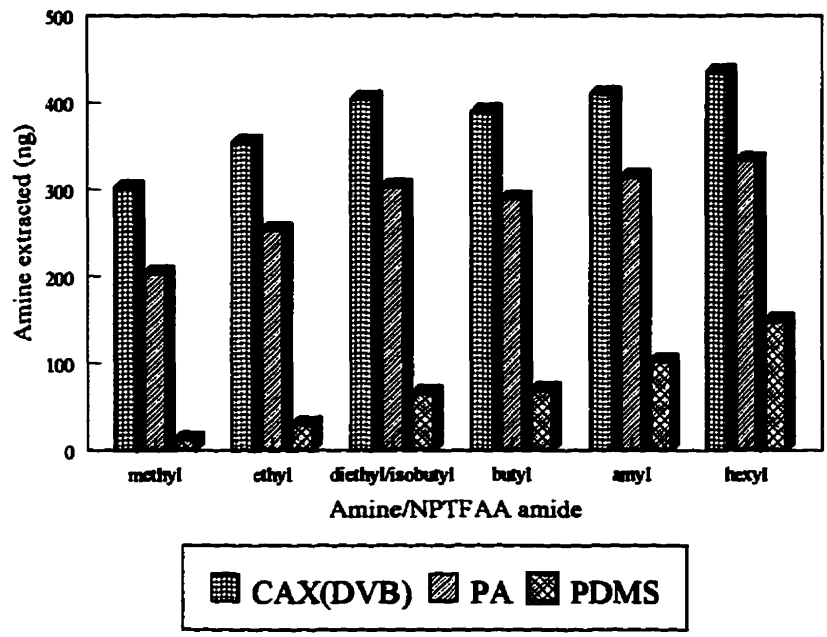

Figure 5-4. Coating evaluation for the extraction of amine/NPTFA amides from air using the CAX(DVB), PA and PDMS coated fibers.

Table 5-2 presents the fiber linear range, LOD and %RSD for the extraction of the amine/NPTFA amides from air using the CAX(DVB) coated fiber. The fiber linear ranges were linear between 1.25-125 ng/mL for methyl and ethyl amines, and 0.125-125 ng/mL for diethyl and isobutyl, butyl, amyl, and hexyl amines with the correlation coefficients between 0.99376 and 0.99975 for all the amines examined. It can be seen that the lower ends of the fiber linear ranges

for methyl and ethyl amines were an order of magnitude higher than for the rest of the amines tested. This was caused by the interfering peak of trifluoroacetic acid in the region of the peaks of NPTFA amides of methyl and ethyl amines. Trifluoroacetic acid is one of the starting materials for the synthesis of NPTFA and it is also one of the degradation product of NPTFA when it contacts with water. The batch of NPTFA used for these experiments was not freshly made. Since NPTFA is moisture sensitive, this interfering problem would always happen when aged derivatizing reagent is used. This problem could be overcome by using a mass spectrometer as a detector. This situation could also be improved by using freshly synthesized derivatizing reagent. The LODs were determined by the reagent blank and estimated to be between low pg/mL and low ng/mL for all the amines tested. The %RSD values were calculated with 3 replicate extractions using the CAX(DVB) coating and they were between 3.1 and 10 for all the amines tested, which is considered to be satisfactory.

Table 5-2 Fiber linear ranges, LODs and RSDs (%) for CAX(DVB) coated fiber, derivatization/SPME of amines with NPTFA in air.

Compound	fiber linear range (ng/mL, FID)	r^2	LOD (pg/mL, FID)	%RSD (n=3, 5 ng/mL)
Methylamine	1.25-125	0.99975	1,000	10
Ethylamine	1.25-125	0.99444	1,000	4.3
Diethyl/isobutylamine	0.125-125	0.99672	23.3	4.7
Butylamine	0.125-125	0.99843	19.1	5.1
Amylamine	0.125-125	0.99376	9.8	3.8
Hexylamine	0.125-125	0.99779	7.2	4.5

5.3.3 Comparison of Direct SPME with Derivatization/SPME

Compared to direct SPME, derivatization combined with SPME significantly improved the method sensitivity. After derivatization/SPME was applied, methyl and ethyl amines (as their NPTFA amides) could be detected in air samples at relatively low concentrations. The linearity of the CAX(DVB) coated fiber for the extraction of volatile amines (as NPTFA amides) from air was two times wider than for direct SPME. The LODs for diethyl, isobutyl, butyl, amyl, and hexyl amines were 2 orders of magnitude lower than for direct SPME. This technique was sensitive enough to monitor diethyl, isobutyl, butyl, amyl, and hexyl amines in indoor air. For methyl and ethyl amines, this technique can only be used to test their presence in industrial atmosphere.

5.4 Analysis of Amines in Aqueous Solutions

5.4.1 Background

Free amines are difficult to extract from water and are not easily chromatographed due to their high polarity. In the literature, the determinations of amines present in water have generally utilized three approaches: (1) direct GC analysis of the aqueous samples; (2) concentration of the amines followed by separation and detection; (3) alteration of the amines by derivatization.

Direct analysis of aqueous samples minimizes sample preparation, thereby lowering analysis time and reducing systematic errors and sample contamination. Direct methods have used GC coupled with an amine-deactivated column[3] and ion chromatography.[4] Both techniques were limited to parts-per-million (ppm) level detection limits.

Detection limits can be improved by concentrating the target analytes before their measurement. Some sample enrichment techniques such as steam distillation,[5] vacuum distillation,[6] liquid-liquid extraction,[7] supercritical fluid extraction,[8] and solid-phase extraction[9] have been used. These methods lowered the detection limits to about 0.01-0.1 ppm, but also concentrated impurities leading to possible false positives or high results.[10]

Amines have been derivatized to fluoroacetamides,[11] boron chelates,[12] and *m*-toluamides[13] to improve their chromatographic separation and detection limits. However, the reagents used in these derivatizations have the disadvantage of reacting with water, which restricts their application to organic solvent extracts of amines only. Derivatization of amines with reagents such as *o*-phthalaldehyde,[14] dinitrofluorobenzene,[15] and 2-methoxy-2,4-diphenyl-3(2H)-furanone[16] in the presence of water has been reported. These methods lack one or both of the following desirable features: sensitivity and chromatographic resolution. Recently, pentafluorobenzaldehyde has been reported to derivatize primary amines directly in a mixture of acetonitrile and water followed by GC/ECD and GC/MS analysis. The method provided relatively high sensitivity (derivatives contained the good electron capturing pentafluorophenyl moiety) and good chromatographic resolution.[10] Soild-phase reagents (prepared on a solid support such as silica, alumina, crosslinked polystyrene, etc.) containing a benzoyl- group, such as 3,5-dinitrobenzoyl[17] and pentafluorobenzoyl,[18] have also become popular for heterogeneous solid-phase derivatization of amines coupled with HPLC and GC/MS analysis.

Since SPME techniques have been successfully used for the analysis of very polar and volatile fatty acids in both gaseous and aqueous phases, they were utilized for the analysis of polar amine compounds as well.

5.4.2 Headspace SPME Technique

Amines are bases, and therefore the aqueous matrix containing these compounds has to be alkalized so that the amines are remain in their non-ionic form and consequently can be effectively extracted from water. However, most of the fiber coatings are not stable in very basic solutions; therefore direct SPME would be undesirable. Since smaller amines are very volatile, headspace SPME technique was chosen to carry out the initial experiments.

In the previous experiments for the analysis of fatty acids in aqueous solutions (presented in Chapter 3), strong mineral acid and salt were added into the aqueous matrices to decrease the polarity and solubility of the polar fatty acids, which significantly enhanced the isolation of the target fatty acids from water. Therefore, a similar matrix modification was also applied to the analysis of amines in water. Since the pK_a values of the smaller amines are around 10 to 11, the

aqueous solution was alkalized to pH 10 along with the addition of salt. The pK_a values for the target amines in water are listed in Table 5-3.[1,19]

The coating evaluation for the isolation of volatile amines from air indicated that the CAX(DVB) coating extracted the target analytes in the highest amounts among the fiber coatings tested. Therefore, the CAX(DVB) coated fiber was chosen for this study. However, further experiments indicated that only when the amine concentration was above 250 ppm, were the amounts extracted by the CAX(DVB) coating using headspace SPME technique detectable by FID. This result suggested that headspace SPME was not sensitive for the analysis of very polar amines in water at very low concentrations. The sensitivity could be improved by using GC/MS. Shirey *et al.* reported that the LODs for the analysis of free methyl, dimethyl, diethyl, and trimethyl amines in water were in the mid-ppb levels when headspace SPME coupled with GC/MS was used.[20] However, FID is a very frequently used detector for general analytical purposes. The development of a new technique suitable for FID application would be helpful.

Table 5-3. The pK_a values of methyl, ethyl, butyl, amyl, and hexyl amines in water.

Compound	pK_a (25°C)[1,17]
Methylamine	10.66
Ethylamine	10.64
Butylamine	10.63
Amylamine	10.63
Hexylamine	10.56

5.4.3 Derivatization/SPME Technique

Determination of free amines in water using headspace SPME sampling was not sensitive enough for trace analysis of small amines when FID was used. Therefore, derivatization coupled

with SPME was investigated for the determination of small amine compounds. Previous studies showed that derivatization coupled with SPME has been successful for the analysis of very polar short-chain fatty acids in aqueous solutions. The sensitivity and selectivity for the analysis of short-chain fatty acids were significantly improved after derivatization/SPME had been applied. Since 1,2-naphthoquinone-4-sulfonate[21] (NQS) and pentafluorobenzaldehyde[10] (PFBAY) have been used to derivatize amines in the presence of water, these reagents were chosen for this study.

(a) With 1,2-naphthoquinone-4-sulfonate (NQS)

The reaction of NQS with amines was first tested in CH_2Cl_2 to check the GC performance of the amine/NQS derivatives. The reaction was performed in CH_2Cl_2 at room temperature for 10-30 minutes. The GC analysis showed nice, sharp and well separated peaks of the target NQS derivatives indicating good GC detection and quantitation. The derivatization of NQS with 100 ppm of the amines was then tested in water under a pH 10 condition. The reaction was monitored for 10 to 240 minutes with both the CAX(DVB) and PA coated fibers. However, no amine/NQS derivatives were seen from the fiber injections of the headspace extraction. This might be due to high water solubility but low volatility (the reagent was originally designed as a tagging reagent for HPLC analysis) of the amine/NQS derivatives, preventing them from being efficiently extracted by the CAX(DVB) and PA coated fibers from the headspace. Therefore, no further investigation with this reagent was made.

(b) With 2,3,4,5,6-pentafluorobenzylaldehyde (PFBAY)

Since PFBAY can only react with primary amines, it was used to derivatize methyl, ethyl, isobutyl, butyl, amyl, and hexyl amines directly in water during this preliminary study. Headspace SPME sampling was used for these experiments.

Figure 5-5 shows the reaction time profiles for the derivatization of 10 ppm each of the 6 selected amines in water. The pH of the sample solution was adjusted to 10. Reaction was carried out at 80°C and monitored for 15, 30, 60 and 120 minutes. At each time point, the sample vial was taken out of the water bath and cooled to room temperature. A CAX(DVB) coated fiber (with 65 μm film thickness) was placed in the headspace above the aqueous solution to isolate

PFBAY imines and the extraction time was 20 minutes. It can be seen that reactions of methyl, ethyl, isobutyl and butyl amines with PFBAY in water were faster than those of amyl and hexyl amines. At 15 minutes (reaction time), the yield of imines for C_1-C_4 amines had reached their peak and dropped afterwards. For amyl and hexyl amines, the formation of their imines had a peak at a 30 minute reaction time, then decreased after 30 minutes. The experiment on the reagent blank indicated that PFBAY was quite stable in water even under alkaline conditions. The decrease of the formation of the amine/PFBAY imines after prolonged reaction times might be caused by the hydrolysis of the imine derivatives in the alkaline solution at elevated temperatures. This study suggested that if the shorter chain amines such as C_1-C_4 amines are the target analytes, a shorter reaction time of 15 minutes should be used. On the other hand, if the longer chain amines, such as C_5-C_6 amines, are the target analytes, a longer reaction time of 30 minutes should be used. In the subsequent experiments, 15 or 30 minute reaction times were used.

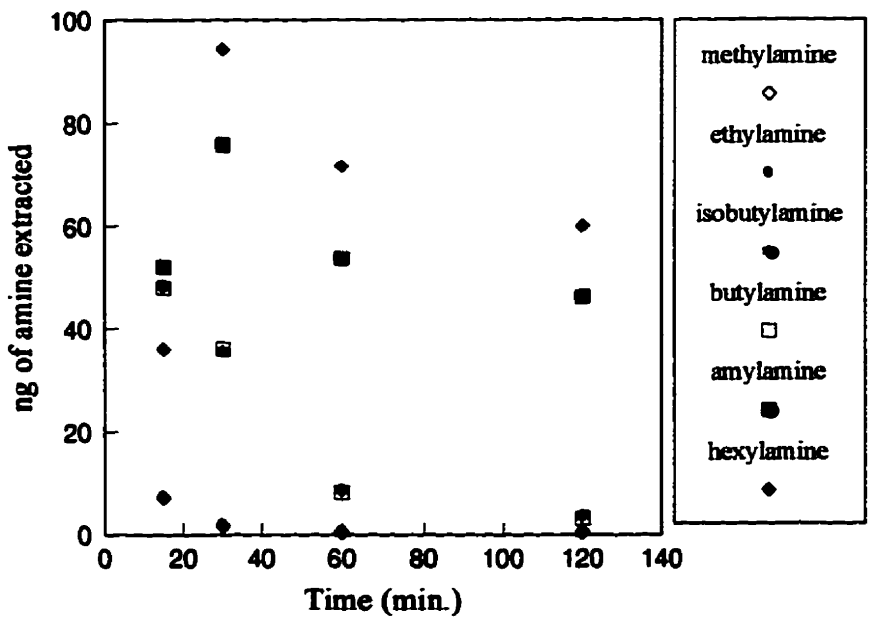

Figure 5-5. Reaction time profiles for the derivatization of small primary amines with PFBAY in water using the CAX(DVB) coated fiber to isolate PFBAY imines from water.

Figure 5-6 illustrates the amounts of amines (as their PFBAY imines) extracted from headspace above the aqueous phase with the CAX(DVB) and PA coated fibers. Samples contained 10 ppm each of the selected amines. Reaction time was 30 minutes and the extraction time was 20 minutes. This figure indicates that the PA coated fiber extracted more amines (as their PFBAY imine derivatives) from water than the CAX(DVB) coated fiber. Therefore, the PA coated fiber was employed for further experiments.

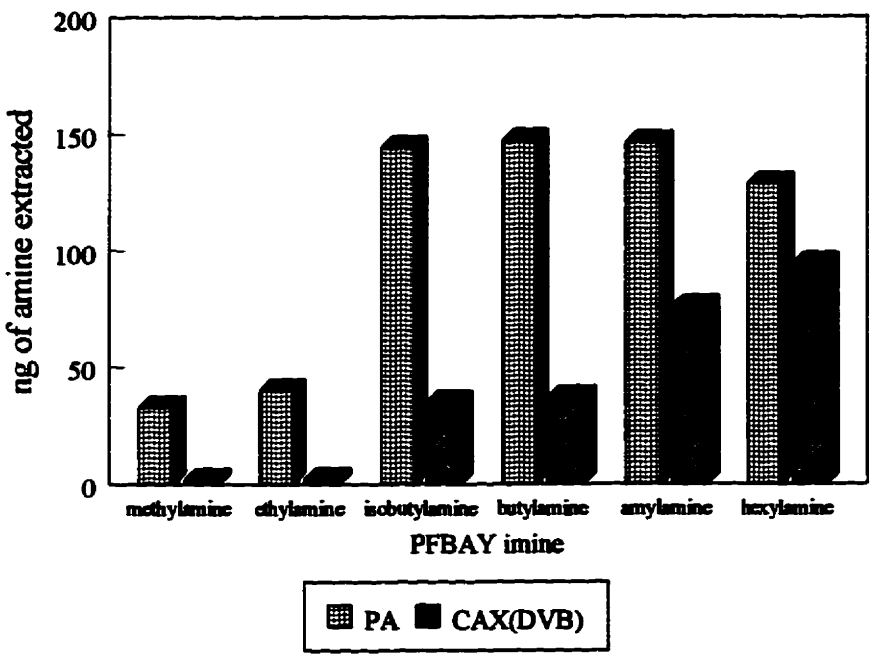

Figure 5-6. Comparison of the CAX(DVB) and PA coated fibers in the extraction of 10 ppm each of amines as their PFBAY imines from the headspace above water at pH 10 conditions.

The exposure time profiles for the extraction of amine/PFBAY imines were determined with the PA coated fiber. Reaction time was 15 minutes. Figure 5-7 shows that for the C_1-C_2 amine/PFBAY imines, the equilibrium can be reached within 20 minutes; however, for the C_3-C_6 amine/PFBAY imines, the equilibrium time was 40 minutes. This implies that C_1 and C_2 amine/PFBAY imines are more volatile than the C_3-C_6 amine/PFBAY imines.

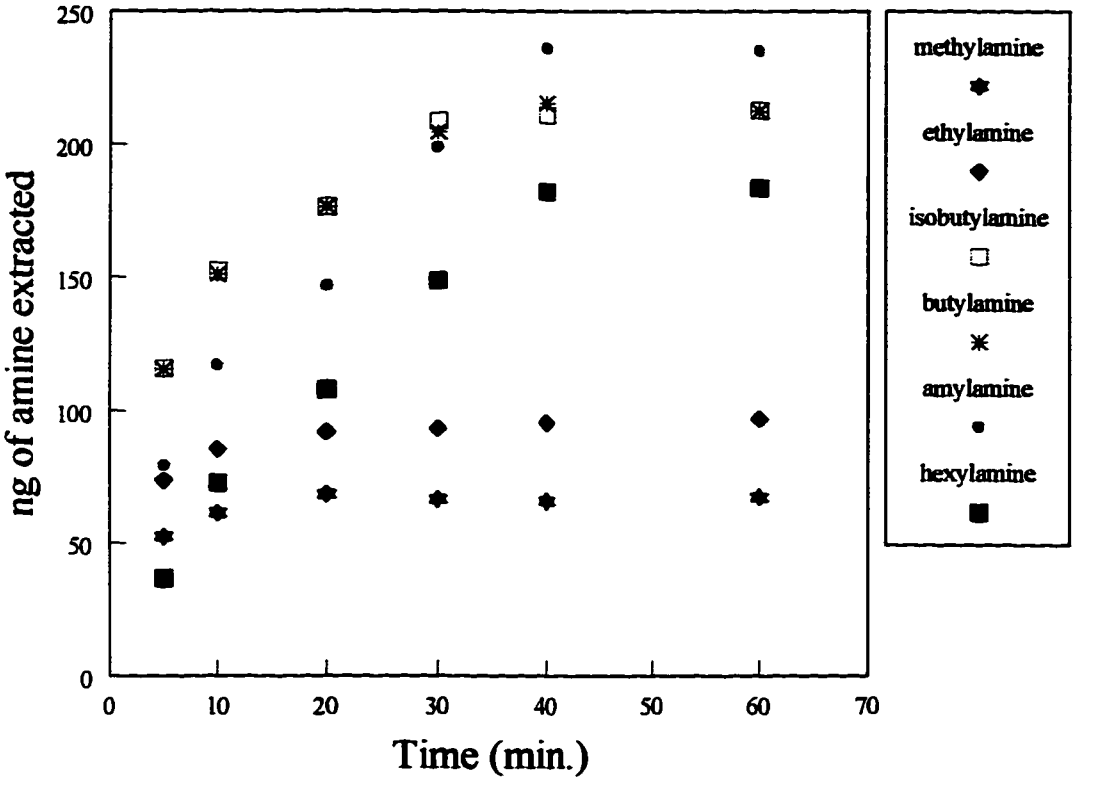

Figure 5-7. Exposure time profiles for the extraction of amines as their PFBAY imines from water with the PA coated fiber.

The effect of pH (pH 7 and pH 10) on the formation of PFBAY imines in water was also examined and the results are shown in Figure 5-8. Samples contained 10 ppm each of the selected primary amines. Reaction was carried out at 80°C for 15 minutes. The PA coated fiber was introduced into the headspace above the aqueous solution to isolate the imines. The extraction time was 20 minutes. It can be seen that the yields of imines at pH 10 were higher than at pH 7 for the smaller methyl and ethyl amines.

Reaction of primary amines with aldehydes has been described as a replacement of the original carbon-oxygen double bond in an aldehyde with a carbon-nitrogen double bond to form an imine.[22] The two step reaction mechanism for the formation of imines is illustrated in Figure 5-9.

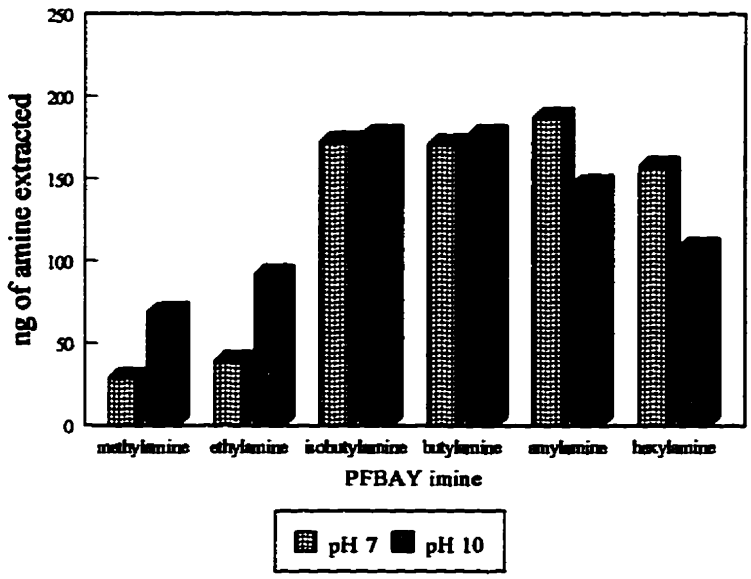

Figure 5-8. The effect of pH on the yield of derivatization of amines with PFBAY in water.

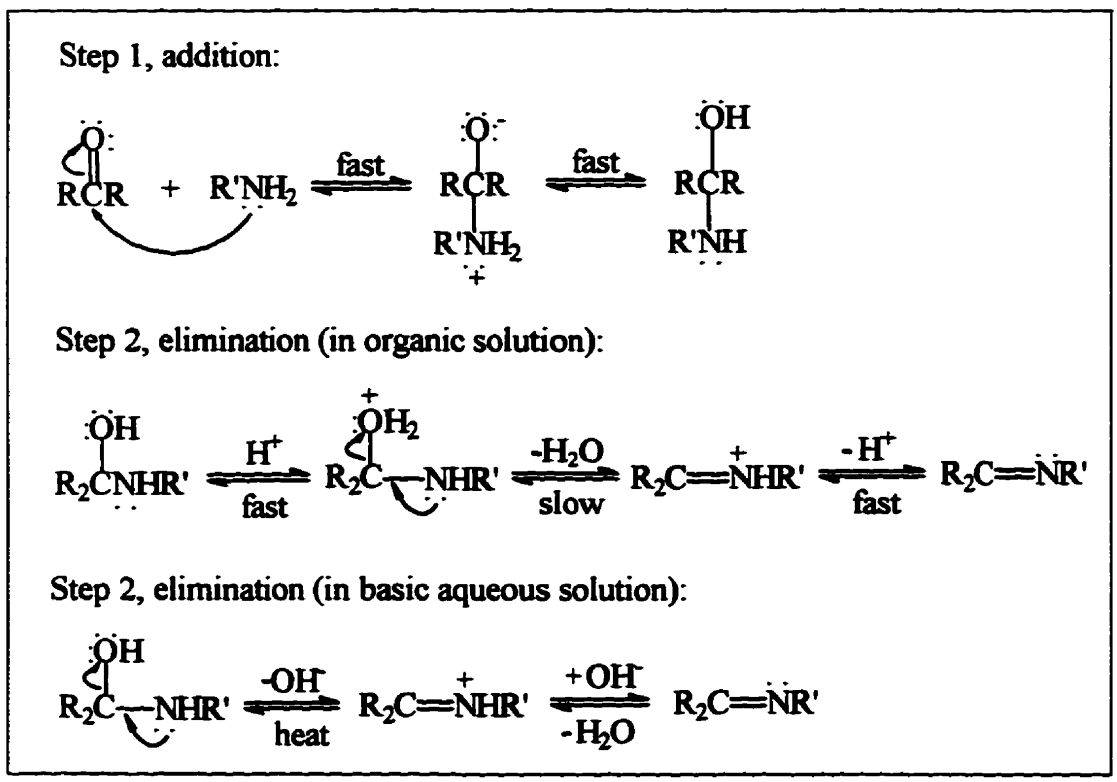

Figure 5-9. Reaction mechanism for the formation of imine.

Theoretically, imine formation is an acid catalyzed reaction when it is performed in an organic solution.[23] The first step is the addition of a nucleophilic, nonprotonated amine to the partially positive carbonyl carbon of an aldehyde, and it usually proceeds fast. Since this reaction is pH dependent, the pH conditions should be optimized so that the best reaction rate can be achieved. If the solution is too acidic, the concentration of the nonprotonated amines becomes negligible. The usually fast addition step becomes slow and actually becomes the rate-determining step in the sequence. The second step in the reaction is the elimination of the protonated -OH group as water. The rate of the second step increases with increasing acid concentration, since OH^- is a strong base and a poor leaving group while $-OH_2^+$ can leave as a weak base and is a good leaving group. Consequently, an increase in acidity causes Step 2 to go faster, but Step 1 to go slower. Conversely, decreasing acidity causes Step 1 to go faster, but Step 2 to go slower. The rate of the overall reaction is the greatest only when the pH is controlled between 3 and 4.[22] At these pH conditions, some of the amine is protonated, but some is free to initiate the nucleophilic addition. As well, at this pH, enough protons are present so that the second step of the reaction, the elimination of water, can proceed at a reasonable rate.

However, the situation is different when the reaction is performed directly in water. As bases, amines are easily protonated by water, especially for the very polar, smaller amines. To prevent the protonation happens, non acidic conditions would be preferred to facilitate the first step of the reaction in water. The required pH conditions to keep amines in their non-ionic form in water seems associated with their pK_b values or the pK_a values of their protonated conjugated ions in water (see Table 5-3). For the smaller methyl and ethyl amines, which have slightly higher pK_a values than the other longer chain amines, more basic conditions are needed to keep them in their neutral form. This is probably why the formation of imines for C_1-C_2 amines in water, in this experiment, produced higher yields at pH 10 than at pH 7. For the longer chain amines, pH 7 condition was enough to keep them in their non-ionic state. Under pH 7 or 10 conditions, there were not enough protons present in aqueous solutions. Therefore, the second step, the elimination of -OH group as water (as predicted for reactions in organic solution) was unlikely to take place. Since the nitrogen of the intermediate $R_2COHNHR'$ has a lone pair of electrons, these electrons can be added to the carbon atom which is directly connected with the -OH group. At

higher reaction temperatures, the -OH group would leave more readily after receiving a pair of electrons. The deprotonation of R_2CNHR' species in the basic conditions then generates imines.

In order to confirm that the effect of pH only has an impact on the reaction rather than on the extraction process, imines formed via the derivatization in MeCN solution were added into pH 7 and 10 aqueous solutions. Sample concentrations were the same for the two pH conditions tested. The PA coated fiber was then placed in the headspace to isolate target analytes and the extraction time was 20 minutes. Figure 5-10 illustrates that the amounts of amines extracted (as their corresponding imines) under the above two pH conditions were the same for all the amines tested. This confirmed the previous assumption that the pH only affects the formation of imines, not the extraction.

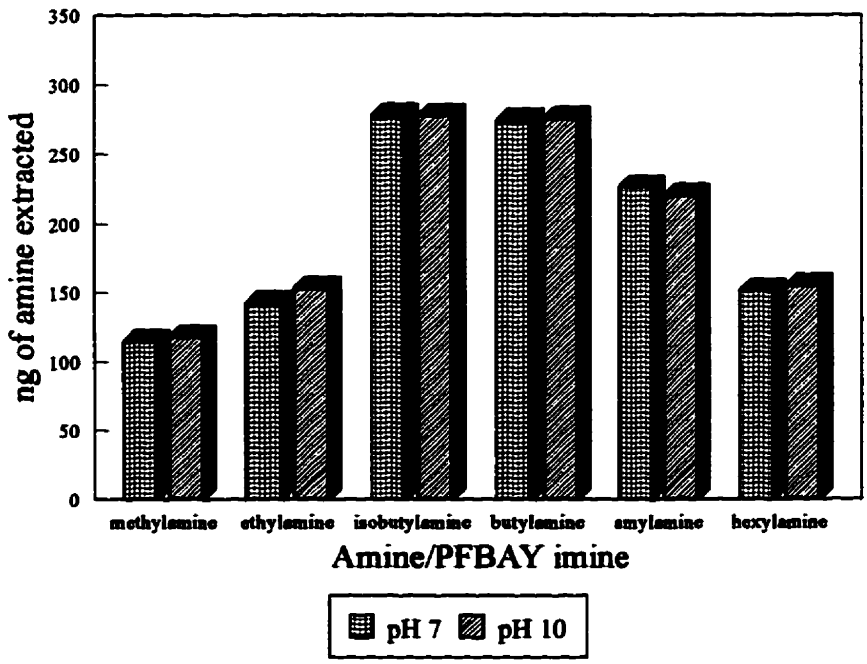

Figure 5-10. Effect of pH on the extraction of imines from water with the PA coating.

Table 5-4 presents fiber linear ranges, LODs and RSDs (%) for the analysis of amines in water using the PA coated fiber to isolate the PFBAY derivatives from water. The PA coated

fiber has a two orders of magnitude linearity for all the primary amines tested, it was between 0.1 and 10 ppm for methyl and ethyl amines, 0.05 or 0.01 and 1 ppm for the isobutyl or butyl amines, respectively, and 0.005 and 0.1 ppm for amyl and hexyl amines, with the correlation coefficients between 0.99496 and 0.99999 for all the amines tested. The LODs were determined by the reagent blank. They were in the low ppb and the high ppt levels for C_1-C_4 amines and C_5-C_6 amines, respectively. The %RSD values were determined by 3 replicate extractions of the amine/PFBAY imines from water and they were between 2.6 and 15.3 for all the amines examined. Compared to headspace SPME sampling, derivatization/SPME technique significantly reduced the detection limits for the analysis of primary amines in aqueous samples. The LODs were at least 3 orders of magnitude lower when derivatization of primary amines with PFBAY in water coupled with SPME was applied.

Table 5-4. The PA fiber linear ranges, LODs and RSDs (%) for the derivatization of amines in water with PFBAY using the PA coated fiber to isolate reaction derivatives from the headspace above the aqueous solution.

Compound	PA fiber linear range (μg/mL, FID)	r^2	LOD (ng/mL, FID)	%RSD (n=3, 0.1 μg/mL)
Mehylamine	0.1-10	0.99976	26	2.6
Ethylamine	0.1-10	0.99999	21	1.8
iso-Butylamine	0.05-1	0.99978	2	8.6
Butylamine	0.01-1	0.99496	2	7.5
Amylamine	0.005-0.1	0.99881	0.5	12.2
Hexylamine	0.005-0.1	0.99891	0.4	15.3

(c) Analysis of Wastewater Samples with PFBAY

A wastewater sample derivatized with PFBAY was determined using derivatization/SPME technique. Headspace SPME sampling was used. Standard addition was employed for quantitation. The pH of wastewater samples was adjusted to 10. A 10 mL of this solution was transferred to a 15 mL vial and 4 μL of 2.25 mg/μL PFBAY/MeCN was added to the real sample solution. The solution was heated at 80°C for 15 minutes. After the sample solution was cooled to room temperature, a PA coated fiber was placed in the headspace for sampling. The wastewater samples without the addition of the standard amines and the ones spiked with 1 and 10 μg/mL each of methyl and ethyl amines showed linear response with the correlation coefficients of 0.99846 and 0.99971 for methyl and ethyl amines, respectively. These experiments indicated the presence of 0.7 μg/mL of methyl amine in the wastewater samples. No ethyl amine was found during the analysis of real samples without the addition of standard amines. The results were confirmed by GC/ITMS and a extracted-ion GC/ITMS chromatogram for the analysis of wastewater samples is shown in Figure 5-11. The actual peak shown is the PFBAY imine of methyl amine.

The mass spectrum of methyl amine/PFBAY imine showed fragment ions at *m/z* 117, 181 and 208 and a molecular ion at *m/z* 209. Avery *et al.* indicated that electron impact mass spectra of imine derivatives of alkylamines are dominated by α-cleavage.[10] When the α-position of an amine is unsubstituted, the base peak will be *m/z* 208,[10] which was the case observed in this experiment. The *m/z* 181 fragment may result from the cleavage of the pentafluorophenyl group and the NCH_3 moiety.

5.5 Summary

This chapter presented the preliminary results for the analysis of amines with SPME techniques. For the analysis of volatile amines in air, both direct SPME and derivatization/SPME could be used to monitor these analytes in industrial air. The sensitivities can be improved by using more appropriate derivatizing reagents, such as compounds containing a pentafluorophenyl moiety, which can provide selective and sensitive electron capture detection.

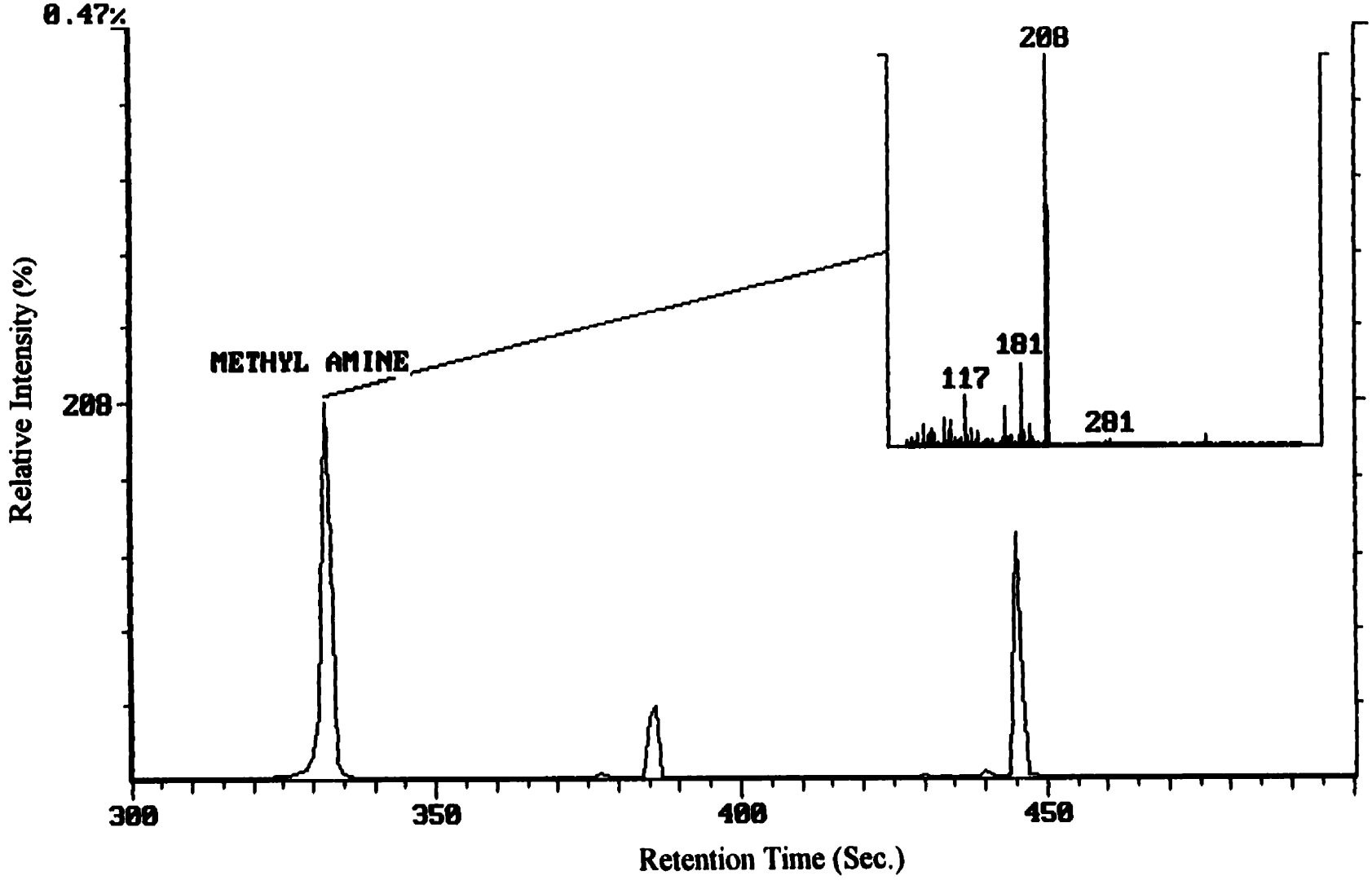

Figure 5-11. Extracted-ion GC/ITMS chromatogram for the derivatization of primary amine in a wastewater sample with PFBAY, indicating the presence of methyl amine.

For the analysis of amines in aqueous solution, headspace SPME had LODs in the mid to the high ppm ranges for the isolation of free amines from water when FID was used as the detector. Limits of detection was significantly reduced when derivatization of primary amines with pentafluorobenzaldehyde coupled with SPME was incorporated. The LODs for the primary amines tested were in the low ppb to the high ppt levels. This study presented a promising method for the analysis of very polar and volatile primary amines in water. When ECD or negative ion MS is used, more sensitive detection could be reached. If the compounds of interest are secondary or tertiary amines, other derivatizing reagents should be used, for example, pentafluorophenylacetaldehyde can be used to derivatize secondary amines.

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CHAPTER 6

CONCLUSIONS AND FUTURE RECOMMENDATIONS

6.1 Direct SPME

Direct SPME was successful for the analysis of C_6-C_{10} fatty acids in water. The optimum sensitivity of the technique was achieved when saturated salt at pH 1.5 matrix conditions were used to decrease the polarity and solubility of fatty acids. Under these conditions, the method had LODs in fg/mL levels when GC/FID was utilized. For the analysis of volatile and shorter chain C_2-C_5 acids and C_1-C_6 amines from water, direct SPME was less successful due to the characteristics of the currently available fiber coatings, which have insufficient affinities for them. Extraction of volatile C_2-C_5 acids and C_1-C_6 amines from air with direct SPME was also less effective since these compounds were too polar to be isolated in very low concentrations. For effective extraction of polar analytes using direct SPME, the development of more polar coatings and coating materials containing ion-exchange resin will definitely benefit the SPME technique.

6.2 Derivatization/SPME

SPME coupled with derivatization techniques was successful for the selective and sensitive determination of short-chain fatty acids and amines or long-chain fatty acids, in gaseous and/or aqueous matrices. Derivatization/SPME techniques were performed via in-matrix derivatization, in-fiber derivatization and in GC injector port derivatization. The derivatization efficiency can be dependent on the choice of derivatizing reagent, the concentration of the derivatizing reagent, and the matrix conditions. High selectivity and sensitivity can be achieved by the proper choice of the derivatizing reagent (in general, fluorinated reagents provided better sensitivity) and derivatization/SPME technique.

Derivatization/SPME of polar analytes in the sample matrix is the simplest way to improve SPME performance. By lowering the polarity of target analytes in the sample matrices, the analyte-matrix affinity was decreased and the analyte-fiber coating affinity was increased, thereby SPME extraction efficiency was improved.

For the analysis of fatty acids in air and water, derivatization/SPME offered LODs which were 1-3 orders of magnitude lower than direct SPME. The analysis of volatile fatty acids in

wastewater samples showed a good agreement between the results obtained with the two reagents, PFBBr and PFPDE used, indicating good accuracy of the methods.

For the derivatization of amines in air, NPTFA can be used to derivatize primary and secondary amines. This method decreased LODs by 2 orders of magnitude than direct SPME. However the sensitivity of this technique was not satisfactory for the analysis of methyl and ethyl amines due to the interfering peaks resulting from the decomposition of NPTFA during storage. Freshly made reagent is desirable to improve the effectiveness of the technique. Development of other reagents containing good electron capturing moieties (e.g. pentafluorophenyl moiety) for better detection of volatile amines in air is also recommended. PFBAY successfully derivatized primary amines in water. This method provided greater sensitivity than direct SPME. Further study on the analysis of secondary and tertiary amines is needed.

Simultaneous derivatization/extraction of analytes in a polymeric coating of the SPME device presented a novel concept to improve SPME extraction efficiency and method sensitivity. It demonstrated a new avenue to analyze polar analytes using SPME. This study revealed that with the derivatizing reagent in the fiber stationary phase, the SPME device can act as a concentrator, isolator and reactor to effectively derivatize and isolate polar analytes from sample matrices. This approach eliminated the need for pre-extraction and pre-concentration prior to derivatization, thus simplifying sample preparation procedure.

For air sample analyses of fatty acids, quantitative derivatization and extraction were achieved; therefore this technique could be used for integrating sampling. Compared to direct SPME, this method lowered LODs by 2-4 orders of magnitude. For aqueous sample analyses of fatty acids, the addition of salt and strong acid in the sample matrix enhanced the amounts of analytes extracted and reacted in the fiber coating. Increasing the temperature of sample solution from 25°C to 60°C was found to facilitate mass transfer of the analytes from the aqueous phase to the headspace which led to an increase in the amounts of fatty acids reacted in the fiber coating during in-fiber derivatization with PDAM. However, quantitative derivatization was not achieved, due to the decomposition of PDAM in an extremely humid environment combined with a high reaction temperature. In the future, the development of new derivatizing reagents which

can bind to the SPME polymeric coating, have high reactivity towards the target analytes, as well as have higher thermal and humid stabilities, is highly recommended. The development of other fluorine tagging reagents would also be helpful to increase the sensitivity of the technique. In-fiber derivatization can be employed for the determination of volatile and polar analytes in very complex sample matrices. This technique could also be used to analyze semi-volatile analytes when longer sampling time is used.

The techniques of post-derivatization, as well as derivatization in the GC injector port following SPME extraction of long-chain fatty acids from water, presented two simple alternatives to perform derivatization for convenient GC analysis of long-chain fatty acids. The application of these techniques to real samples can be helpful to understand the scope of these techniques.

Derivatization/SPME techniques were successfully used for quantitative analysis as well as fast screening of fatty acids in different types of sample matrices. Accurate quantitative analyses of real samples can be carried out by using standard addition as well as by using isotopically labelled analogues as internal standards.

Derivatization/SPME techniques were shown to be versatile for the analysis of volatile, semi-volatile and non-volatile analytes in various sample matrices. The potential of derivatization/SPME for the analysis of other groups of polar analytes, such as aldehydes, amino acids, etc., should be further explored.

APPENDIX A

Calculation of the coating/gas partition coefficient, K_1, for short-chain fatty acids:

The partition coefficient can be calculated according to the Equation 3-1:

$$K = \frac{nV_s}{C_o V_f V_s - nV_f} \tag{1}$$

where n is the amount of the analyte extracted at equilibrium and defined as in nanogram (ng); V_f and V_s are the volumes of the fiber coating and the sample, respectively; C_o, is the initial sample concentration and defined as in ng/mL, and

$$C_0 = \frac{n_0}{V_s} \tag{2}$$

where n_0 is the initial amount of the analyte in the sample. By combining Equations (1) and (2), we have

$$K_1 = \frac{nV_s}{(n_0 - n)V_f} \tag{3}$$

As shown in Figure 2-2, symbol a and b are the radii of the silica core and the fiber coating, respectively, L is the length of the fiber coating.

For 100 μm PDAM coated fiber: $a = 0.0055$ cm, $b = 0.015$ cm, $L = 1.1$ cm

the volume of the fiber coating $V_f = \pi\ (b^2\text{-}a^2)L = 3.14(0.015^2\text{-}0.0055^2)\times 1.1 = 6.7 \times 10^{-4}$ cm^3

For 85 μm PA coated fiber: $a = 0.0056$ cm, $b = 0.0141$ cm, $L = 1.1$ cm

the volume of the fiber coating $V_f = \pi\ (b^2\text{-}a^2)L = 3.14(0.0141^2\text{-}0.0056^2)\times 1.1 = 5.8 \times 10^{-4}$ cm^3

When the PA coated fiber was used to extract 0.5 ng/mL each of the gaseous C_2-C_5 acids in a 40 mL sample vial (the initial amount of each acid is 20 ng), the amount of C_2-C_5 acids extracted by the 95 μm PA coating was 6, 10.8, 12.5 and 16.2 ng, respectively, after the system

had reached equilibrium. Therefore, K_1 for the acetic acid can be calculated according Equation (3):

$$K_1 = \frac{6 \times 40}{(20-6) \times 5.8 \times 10^{-4}} = 30,000 \quad (4)$$

Similarly, K_1 for the C_3-C_5 acids was calculated to be 81,000; 115,000 and 294,000, respectively.

APPENDIX B

Following is the solution for the diffusion-derivatization process when SPME is used to extract-derivatize an analyte from air, based on the solution of the boundary value problem for heat transfer in a hollow cylinder.[1]

As shown in Figure 2-2, the system has a cylindrical geometry. Symbols a and b represent the inner and outer radii of the fiber coating, L is the length of the coating, D is the diffusion coefficient of the analyte in the coating, K is the partition coefficient between fiber coating and air ($K=[A]_f/[A]_g$), n_o is the initial amount of analyte in the vial, and V_f and V_g are the volumes of fiber coating and vial, respectively. The coating is assumed to contain excess reagent so that the analyte reacts everywhere and all the time in the coating at the rate $k[A](r,t)$, where $[A](r,t)$ represents analyte concentration at radius r and time t. Diffusion is assumed to be the only mass transport process in the coating with no adsorption or absorption at the fiber core or vial surfaces, and the analyte is assumed to be perfectly mixed in the gaseous phase.

The equations describing analyte diffusion in the coating are

$$\frac{\partial^2[A]}{\partial r^2}+\frac{1}{r}\frac{\partial[A]}{\partial r}=\frac{1}{D}\frac{\partial[A]}{\partial t}+\frac{k[A]}{D} \quad a<r<b,\ t>0 \tag{5}$$

$$\frac{\partial[A]}{\partial r}=0 \quad \text{at} \quad r=a,\ t>0 \tag{6}$$

and

$$\frac{K}{V_g}\left(n_0-2\pi bLD\int_0^t \frac{\partial[A]}{\partial r}_{r=b} dt\right)=[A] \quad \text{at } r=b,\ t>0 \tag{7}$$

Applying the Laplace transform to these equations and solving as in ref. 1 give

$$\Delta(p)v(r,p)=-k_3\{K_1(qa)I_0(qr)+I_1(qa)K_0(qr)\} \quad . \tag{8}$$

where $q=\sqrt{\frac{p+k}{D}}$ and

$$\Delta(p) = \Delta(q) = I_1(qa)[k_1 q K_1(qb) - k_2(Dq^2 - k)K_0(qb)]$$
$$- K_1(qa)[k_1 q I_1(qb) + k_2(Dq^2 - k)I_0(qb)] \qquad (9)$$

Substituting asymptotic expressions for the Bessel functions[2] shows the zeroes of $\Delta(p)$, which are the poles of $v(r,p)$, have bounded real parts so the criteria of the Laplace inversion theorem are fulfilled. By the Laplace inversion theorem the solution is

$$[A](r,t) = \sum\{residues \ \ of \ \ e^{\lambda t} v(r,\lambda)\}$$
$$= -2Dn_0 \sum_{\alpha_j} \frac{e^{(D\alpha^2 - k)t} \alpha\{K_1(\alpha a)I_0(\alpha r) + I_1(\alpha a)K_0(\alpha r)\}}{\frac{\partial}{\partial \alpha}\Delta(\alpha)\rfloor_{\alpha_j}} \qquad (10)$$

where the α_j are the positive roots of $\Delta(q)$. For $[A](r,t)$ to be real and bounded, the $D\alpha_j^2 - k$ must be real and non-positive (not proven here), so the α_j can be imaginary or real such that $D\alpha_j^2 - k < 0$. These roots can be determined by plotting Δ. When k is non-zero $\Delta(q)$ can be shown to have exactly one positive real root between 0 and $\sqrt{\frac{k}{D}}$ and an infinite number of imaginary roots. The amount of analyte reacted at time t is

$$2\pi Lk \int_0^t \int_a^b [A] r dr dt = 4\pi LDn_0 k \sum_{\alpha_j} \frac{\left[e^{(D\alpha^2 - k)t} - 1\right][b\{K_1(\alpha a)I_1(\alpha b) - I_1(\alpha a)K_1(\alpha b)\}]}{-(D\alpha^2 - k)\frac{\partial}{\partial \alpha}\Delta(\alpha)\rfloor_{\alpha_j}} \qquad (11)$$

Mathematical approximations show that when $K < 0.05\frac{V_g}{\pi Lb^2}$, the time to reach 90% extraction-derivatization from a vial of perfectly stirred sample obeys the following rule: if $\sqrt{\frac{k}{D}} < \frac{1.62}{b-a}$, then the time is $2.7\frac{V_g}{kKV_f} \pm 35\%$, otherwise it is $2.7\frac{V_g}{\sqrt{kD}\cdot KA_f} \pm 35\%$, where A_f is the surface area of the fiber, $A_f = 2\pi bL$.

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APPENDIX C

Procedure to calculate the relative humidity (R.H.) in a 40 mL sample vial after 0.5 μL of H_2O was added.

The following parameters are known: $V_s = 40$ mL, $V_w = 0.5$ μL, $d = 1$ mg/μL, $P_w = 24.326$ *mm Hg* (at 25.5°C), 1 atm = 760 *mm Hg*, $R = 0.08206$ $L{\cdot}atm{\cdot}mole^{-1}{\cdot}degK^{-1}$, $FW_w = 18$; where V_s and V_w are the volumes of sample vial and the water added to the sample vial, respectively, d is water density, P_w is the vapour pressure of water, R is gas constant, FW is the molecular weight of water. Therefore, the number of mole of 0.5 μL water, n, can be calculated as:

$$n = \frac{W}{FW} = \frac{dV_w}{FW} = \frac{1mg/L \times 0.5L}{18} \times 10^{-3} = 2.78 \times 10^{-5} mol \quad (1)$$

According to ideal gas law:

$$PV = nRT \quad (2)$$

where P is pressure (defined in atm here), V is the volume of the sample vial, n is the amount in mole, T is temperature (T = 273+25.5 = 298.5 K in this experiment). Therefore, the vapour pressure of water in a 40 mL vial contributed by the addition of 0.5 μL of water at a temperature of 25.5°C can be calculated as:

$$P = \frac{nRT}{V_s} = \frac{2.78 \times 10^{-5} mol \times 0.08206 L{\cdot}atm{\cdot}mol^{-1}{\cdot}K^{-1} \times 298.5K}{40 \times 10^{-3} L} = 0.017atm = 12.928mmHg \quad (3)$$

The R.H. can then be calculated as:

$$R.H. = \frac{P}{P_w} = \frac{12.928}{24.326} \times 100\% = 53\% \quad (4)$$