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Headspace solid-phase microextraction of analytes important to biofuels

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HEADSPACE SOLID-PHASE MICROEXTRACTION OF ANALYTES IMPORTANT TO BIOFUELS

By

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ANALYTES IMPORTANT TO BIOFUELS

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Biodiesel is a renewable, biodegradable, clean burning fuel, produced from vegetable oils and animal fat. It is a mixture of fatty acid alkyl esters, products that result from the catalytic transesterification of lipids.

The first part of this research describes the development of a new and direct method used to rapidly and quantitatively determine the amount of free methanol in biodiesel samples. The analytical method developed is different from the current standards for methanol determination, and it is the first headspace-SPME method used to extract methanol from biodiesel as matrix.

The second part of this research describes the direct analysis of acetic acid and 2-furaldehyde in an aqueous mixture using headspace SPME. The direct and accurate determination and quantitation of these two analytes is very important, as they can be inhibitors or food sources for microorganisms capable of producing lipids or ethanol.

DEDICATION

I would like to dedicate this research to my loving parents, Adriana and Costel Paraschivescu, and to my husband Ovidiu.

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CHAPTER I

INTRODUCTION

The goal of this research was to develop new, direct, and quantitative analytical methods for analyzing methanol in biodiesel and acetic acid and 2-furaldehyde in aqueous samples. Methanol is one of the reagents used in the transesterification reaction and is also a contaminant of the final product, biodiesel. Residual methanol in biodiesel decreases the flash point and damages the rubber components of the vehicle's fuel system[1], so an accurate, fast, and direct method to determine the free methanol in biodiesel was desired. Similar goals were set for the analysis of acetic acid and 2-furaldehyde. These two compounds are among the products of acid hydrolysis of lignocellulosic biomass.[2] The aqueous mixture obtained from the acid hydrolysis of switchgrass may be fed to oleaginous microorganisms. The oil produced by these microorganisms can be converted to biodiesel. The acetic acid and 2-furaldehyde present in the aqueous mixture can act as inhibitors or food sources for the oleaginous microorganisms, so their direct analysis and quantitation is important. The methods developed employed headspace solid-phase microextraction and gas chromatography with flame ionization detection.

Solid-phase microextraction (SPME) was developed by J. Pawliszyn and co-workers in 1989.[3] SPME uses a polymer coated fused silica fiber on which the analytes

are adsorbed. The fiber is then directly inserted into the injector of a gas chromatograph where the analytes are thermally desorbed and then the fiber can be reused. This technique has multiple advantages: no solvent is required, it is simple and fast, and there are two possible modes of analyte extraction, headspace and direct immersion. Also, SPME can be automated and can be coupled with GC or HPLC.

One disadvantage is the limited lifetime of the fiber, which degrades with usage and can cause analyte peak tailing and co-elution.[4] Headspace extraction is preferred over direct immersion because it prolongs the lifetime of the fiber due to the absence of contact between fiber and solution. A valuable feature of SPME is that different fiber coatings provide selective extraction of analytes from a mixture based on matching the properties of the fiber coating relative to the analyte polarity. [4] The importance and use of this technique is increasing daily in many fields. SPME has been applied to analysis of environmental samples, flavor and food products, surfactants, forensic, and toxicological analysis.[5 - 10]

CHAPTER II

HEADSPACE SPME DETERMINATION OF METHANOL IN BIODIESEL

2.1 Literature review

Biodiesel is a renewable, biodegradable, clean burning fuel, produced from vegetable oils and animal fat. It is non-toxic, it produces less carbon and sulfur oxides than petroleum diesel and it is non-flammable. Biodiesel is a mixture of fatty acid alkyl esters, products resulting from the transesterification of saponifiable lipids. This process can be performed with acidic, basic or enzymatic catalysis (see Figure 2.1), and under supercritical conditions.[11] Sodium methoxide, one of the most widely used basic catalysts, is very efficient in transesterifying glyceride-bound fatty acids. The base-catalyzed transesterification takes place quickly (few minutes) and at room temperature. Anhydrous reaction conditions are required in order to avoid saponification as a side reaction.[12] If the lipid sample to be transesterified contains a considerable amount (more than 1%) of free fatty acids, then the acid catalysis route must be chosen. The most frequently used reagents for acid-catalyzed transesterification are sulfuric acid in methanol and boron trifluoride in methanol. This reaction requires a longer time (2-3 hours) and higher temperatures (100 °C).[12]

Badings[13] proposed a mixed transesterification procedure, the first step being a base catalyzed transesterification of the lipid-bound fatty acids and the second one being the methanolic sulfuric acid treatment of the remaining free fatty acids.

The most important experimental factors in the transesterification process are: molar ratio of alcohol to lipid, type of catalyst, temperature, and time.[1] The stoichiometry of the transesterification reaction requires a 3:1 molar ratio of alcohol (usually methanol) to lipid, but in practice a higher amount of methanol is used. The optimum molar ratio of methanol to glycerides for base-catalyzed transesterification is considered to be 6:1 and at this value a 98% conversion to fatty acid alkyl ester is observed.[14] With molar ratios lower than 6:1 the conversion to alkyl ester decreases to 82% and the amount of mono-, di- and triglycerides increases, and ratios higher than 6:1 do not improve the alkyl ester yield, complicate ester and glycerol separation, and increase the cost of methanol recovery.[14] The transesterification reaction is reversible and takes place stepwise. The initial triglycerides are transformed into diglycerides, then monoglycerides, and lastly glycerol. The excess of alcohol helps shift the equilibrium towards formation of fatty acid alkyl esters and glycerol.[1] The final reaction mixture contains fatty acid alkyl esters (biodiesel), tri-, di- and monoglycerides, glycerol, residual alcohol, and catalyst. Most of these contaminants of the biodiesel need to be removed in order for the biodiesel to meet the specifications.[15]

Figure 2.1 General transesterification reaction.[1]

Acid catalyzed transesterifications require larger quantities of methanol, with molar ratio of alcohol to vegetable oil as high as 30:1.[14] The methyl ester formation can be enhanced by increasing the methanol to oil ratio, increasing the amount of acid catalyst used, decreasing the amount of water present in the vegetable oil mixture, and using alcohols with high boiling temperatures.[16,17] Zheng et al.[17] used methanol: oil molar ratios from 50:1 to 250:1, 1.5 to 3.5 mol% sulfuric acid and high temperatures, 70 °C and 80 °C. They reported quantitative oil to FAME conversion (99±1%).[17]

Enzyme catalyzed transesterifications are not yet commercially feasible, mainly due to the low reaction yields and long reaction times.[11] Lipase was reported[18] as a suitable biocatalyst for the synthesis of biodiesel from vegetable oils.

Another type of transesterification is the one in supercritical methanol. The advantages of this method are the fact that it does not require any kind of catalyst, the reaction time is shorter, the energy consumption is lower and it is environmentally friendly.[19] The supercritical methanol transesterification requires high temperatures (250 – 400 °C) and pressures (100 – 350 atm)[19]– see Table 2.1. Water present in the reaction mixture does not have negative effects on the fatty acid alkyl ester yield.[11]

Table 2.1 Catalytic versus supercritical transesterification of vegetable oils.[19]

	catalytic methanol method	**supercritical methanol method**
methylating agent	methanol	methanol
catalyst	alkali or acid	none
reaction temperature (°C)	30 - 70	250 - 400
reaction pressure (atm)	1	100 - 350
reaction time (min)	60 - 360	7 - 15
methyl ester yield (wt %)	97	98
to be removed for purification	methanol, catalyst, glycerol, soaps	methanol
free fatty acids	saponified products	methyl esters, water

Methanol is the most used alcohol for the transesterification reaction due to its low cost and ability to allow glycerin separation. Ethanol can also be used but water should be removed as much as possible from the oil and alcohol. The disadvantage of using methanol is that it is very flammable and in the presence of more than 2% water, methanol is corrosive towards aluminum alloys. Even if ethanol is more environmentally friendly and is obtained from renewable and biodegradable agricultural products, it contains some acetic acid traces, so it also corrodes aluminum alloys.[11]

The quality of biodiesel depends on numerous factors, flash point being one of them. The flash point is directly affected by the quantity of alcohol in the biodiesel. Residual methanol in biodiesel can cause a lower flash point and can also induce a rapid deterioration of the rubber components of the vehicle's fuel system.[1] The amount of methanol that can be present in biodiesel is regulated by the European Biodiesel Standards EN 14214 (test method EN-14110), the limit being 0.2 % mass/mass[15], and by

the ASTM D6751-07a (test method D93 or EN-14110), the limit being at least 130 °C (flash point method) or 0.2 % by volume.[20]

The unique feature of this research is the fact that it provides a direct method to rapidly and quantitatively determine the amount of free methanol in biodiesel samples.[21] The analytical method developed is different from the current US standard for methanol determination, ASTM D6751 which uses the Flash Point (closed cup) measurement (test method D93)[22] and from the European standard which uses the headspace GC method (test method EN-14110).[23]

One research group[24] reported the determination of methanol in biodiesel by derivatizing it with N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA). They directly injected a mixture of rape-seed oil methyl esters and BSTFA in N,N-Dimethylformamide into a gas chromatograph fitted with a DuraBond-5 capillary column and flame ionization and mass spectrometric detection. Other groups have used headspace solid phase microextraction (SPME) to determine the amount of methanol in other matrices, such as: pectin[5], plant polysaccharides[6], aspartame sweeteners[7], body fluids[8,9], and air[10]. While most of the headspace SPME analyses of methanol were performed with a Carboxen-Polydimethylsiloxane (CAR-PDMS) fiber assembly[5,8,10], one research group[20] used pencil lead and reported good overall recoveries.

2.2 Experimental

2.2.1 Chemicals and Reagents

Optima-grade methanol was purchased from Fisher (Fair Lawn, NJ), 4A activated molecular sieves were acquired from Sigma-Aldrich (St. Louis, MO), anhydrous sodium sulfate (Na_2SO_4) was obtained from Merck (Darmstadt, Germany), and the biodiesel (B100) was supplied by the Petroleum Products Laboratory (Mississippi State, MS). The 10 mL SPME vials, fiber assembly, 75-µm carboxen-polydimethylsiloxane (CAR-PDMS), 70-µm carbowax-divinylbenzene (CAR-DVB), 100-µm polydimethylsiloxane (PDMS), 85-µm polyacrylate (PA), and 60-µm polyethylene glycol (PEG) fibers, were purchased from Supelco (Bellefonte, PA).

2.2.2 Instrumental Analysis

The analyte was adsorbed onto the SPME fiber and then thermally desorbed in the inlet of a Hewlett-Packard (HP) 6890N gas chromatograph (Palo Alto, CA) equipped with a split/splitless injection port and flame ionization detection (FID) system. The injector and detector temperatures were held constant during the analysis (200 °C and 300 °C, respectively). The fused silica capillary column used for separation was a 30-m, 0.32-mm i.d., 0.25-µm film thickness Hewlett-Packard HP-5 (5%-phenyl)-methylpolysiloxane. The GC oven was programmed as follows: the initial temperature of 40 °C was held for 4.0 min, increased to 120 °C at 10 °C/min, and then increased to 200 °C at 20 °C/min and held at 200 °C for 1 min. Helium was used as carrier gas at a

constant flow rate of 1.5 mL/min. The injector was operated in split mode (20:1 split ratio). The data were acquired using a HP-CORE ChemStation system.

2.2.3 Solid-Phase Microextraction

An automated SPME system (CombiPAL, LEAP Technologies) was used with the 75-µm CAR-PDMS fiber assembly. CAR-PDMS is recommended for gases and low molecular weight compounds (MW 30-225). When new SPME fibers were installed they were conditioned in the gas chromatograph (GC) injection port at 300 °C for 2 hours, according to the manufacturer's recommendations.

The biodiesel (B100) used for calibration was washed three times with distilled water and dried with anhydrous sodium sulfate (Na_2SO_4) and molecular sieves. This procedure insured a practically complete elimination of methanol present in the initial biodiesel (B100). Standard solutions for calibration were prepared by spiking different amounts of methanol into the washed and dried biodiesel.

The 10 mL SPME vials each containing 1 mL of biodiesel solution were capped with Teflon lined septum caps and heated at 50 °C for 20 min, with constant stirring (500 rpm). The SPME fiber was then exposed to the headspace of the vial and the volatile compounds were adsorbed onto the fiber for 20 min at 50 °C, at constant temperature and stirring. Then the fiber was exposed for 2 min at 200 ºC in the GC injection port for complete desorption and GC analysis of the analytes.

2.3 Results and Discussion

The optimization of the SPME extraction conditions was performed to achieve the highest adsorption in the shortest amount of time. Five different types of SPME fibers were investigated to determine the one that yields the highest methanol adsorption. These fibers were: 75-µm carboxen-polydimethylsiloxane (CAR-PDMS), 70-µm carbowax-divinylbenzene (CAR-DVB), 100-µm polydimethylsiloxane (PDMS), 85-µm polyacrylate (PA), and the newly released 60-µm polyethylene glycol (PEG). Three methanol in biodiesel solutions were used (see Table 2.2 and Figure 2.2) and each analysis was run in triplicate and the results were averaged.

Table 2.2 SPME fibers - experimental data.

	CAR-PDMS 75-µm	**CW-DVB 70-µm**	**PDMS 100-µm**	**PA 85-µm**	**PEG 60-µm**
conc. (% mass)	avg. (*n*=3) area (pA · s)	avg. (*n*=3) area (pA · s)	avg. (*n*=3) area (pA · s)	avg. (*n*=3) area (pA · s)	avg. (*n*=3) area (pA · s)
0.0165	121.8	50.1	19.3	46.5	79.7
0.021	162.8	64.8	22.7	58.2	94.6
0.033	211.1	99.9	36.0	85.5	157.1

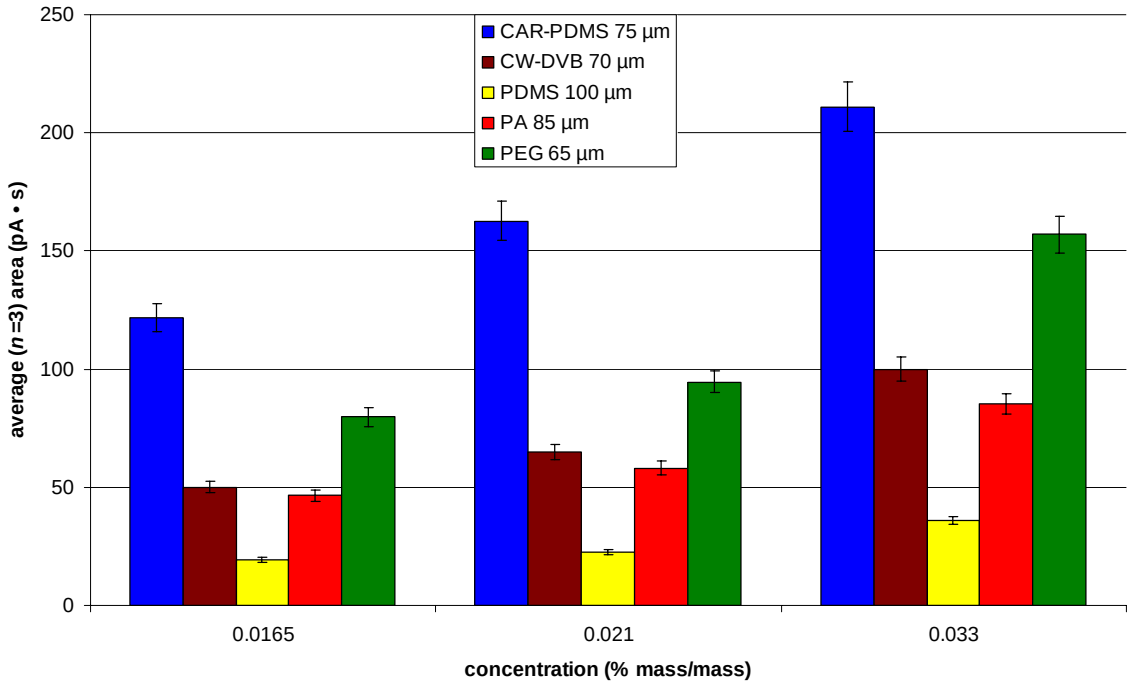

Figure 2.2 Comparison of extraction efficiency of different SPME fibers.

The experimental data indicate that the best extraction of methanol in biodiesel was achieved with the 75-µm CAR-PDMS SPME fiber. A second order polynomial calibration curve was obtained for each of the SPME fibers tested.

The next step in the optimization procedure was to vary the extraction temperature and time, using the 75-µm CAR-PDMS SPME fiber and the 0.19 mg/mL (0.02 % mass/mass) methanol in biodiesel concentration. Three extraction temperatures were investigated: 35 ºC, 50 ºC and 65 ºC and three extraction times: 10 min, 20 min and 30 min. The sample volume used for the optimization studies was 1.0 mL. The experimental results are listed in Table 2.3 and Figure 2.3.

Table 2.3 Variation of extraction temperature with time – experimental data.

	35 ºC	**50 ºC**	**65 ºC**
time (min)	avg.(*n*=6) area (pA · s)	avg.(*n*=6) area (pA · s)	avg.(*n*=6) area (pA · s)
10	123.8	145.2	84.4
20	155.0	182.8	96.8
30	134.4	122.3	75.7

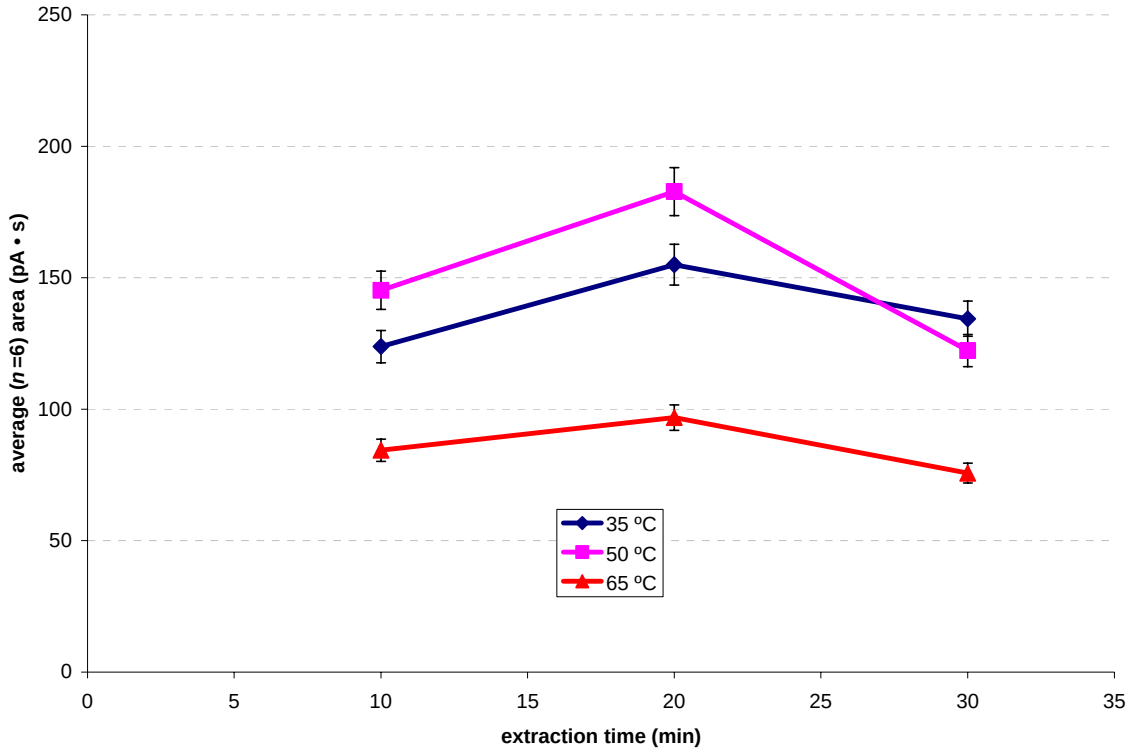

Figure 2.3 Response versus time at 35 ºC, 50 ºC, and 65 ºC.

Based on the optimization studies, the following parameters were chosen for all subsequent experiments: a 75-µm CAR-PDMS fiber assembly, 50 °C extraction temperature, and 20 minutes extraction time. It appears that at higher temperatures and

longer extraction times, the analyte vapors leaks from the SPME vial and a lower amount is absorbed by the fiber (see Figure 2.3).

The retention time of methanol (*Rt* = 2.15 min) was determined by direct injection of neat methanol. Washing the biodiesel (B100) three times with distilled water and drying it over anhydrous Na_2SO_4 and activated molecular sieves removed more than 99% of the initial methanol. The actual amount of methanol present in biodiesel after this procedure was $2.2x10^{-4}$ % mass/mass. This determination was performed using the above described headspace SPME and GC conditions. This washed and dried biodiesel was considered methanol–free and will be referred to as “reference biodiesel”.

Calibration curves were constructed by using reference biodiesel spiked with methanol. The concentration of these standard solutions is expressed in mass percent (% mass/mass). Solutions were prepared by mass/volume and then converted to mass/mass units. One mg/mL methanol/biodiesel corresponds to 0.11 % mass/mass (European standards). The average density of the biodiesel (B100) employed in this study (6 replicates, relative standard deviation 0.12 %) was determined to be 0.880 g/mL. The responses for calibration concentrations (shown in Table 2.4) were made with six replicates and the reproducibility was expressed as relative standard deviation (RSD). The range of RSD for these calibration points was from 3.41 % to 10.36 % and the average RSD was 7.06 %.

Table 2.4 Average (n=6) values of RSD for calibration concentrations.

methanol in biodiesel concentration (% mass/mass)	**average RSD*** (%)
0.0057	9.94
0.011	9.64
0.023	9.92
0.034	10.36
0.045	10.33
0.057	5.52
0.11	4.42
0.23	3.41
0.57	3.43
1.14	3.67
1.70	6.67
2.27	7.47

*The replicates were acquired over a period of five days.

In the concentration range from 0.0057 to 0.23 mass percent, the results fit a second order polynomial curve ($y = 1x10^{-6}x^2 – 1x10^{-4}x + 0.0121$) (see Figure 2.4). For higher methanol concentrations, a third order polynomial curve should be used. (see Figure 2.5).

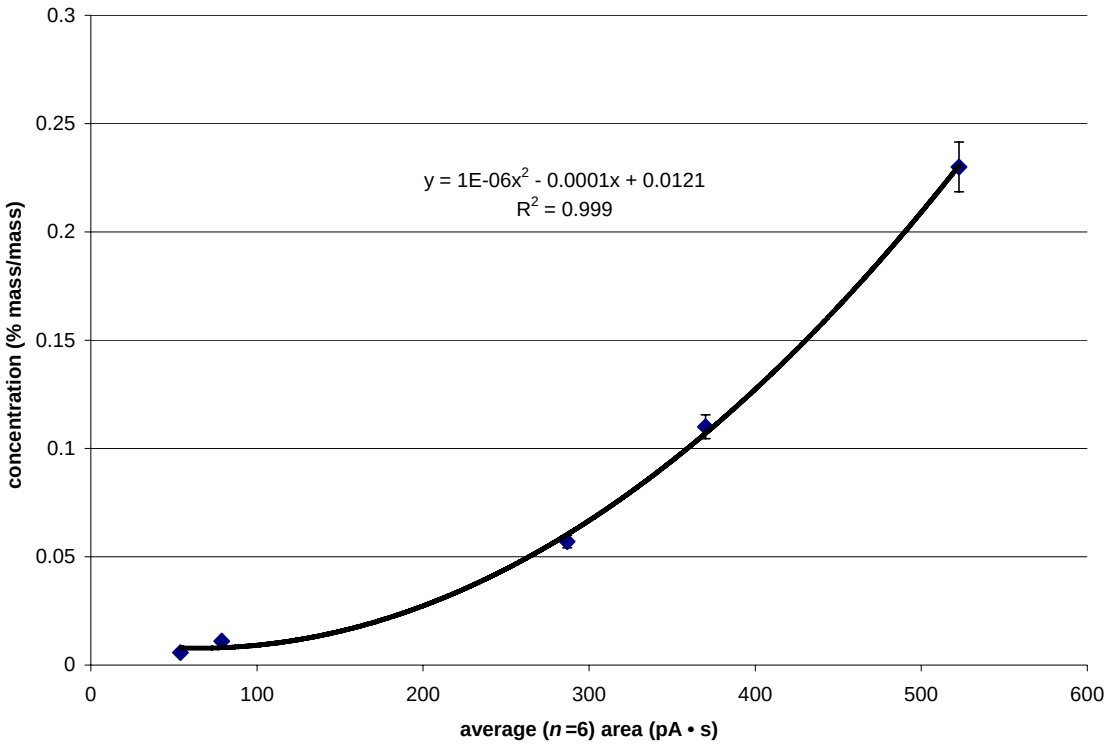

Figure 2.4 Calibration curve for methanol in reference biodiesel – 0.0057 % to 0.23 % mass/mass.

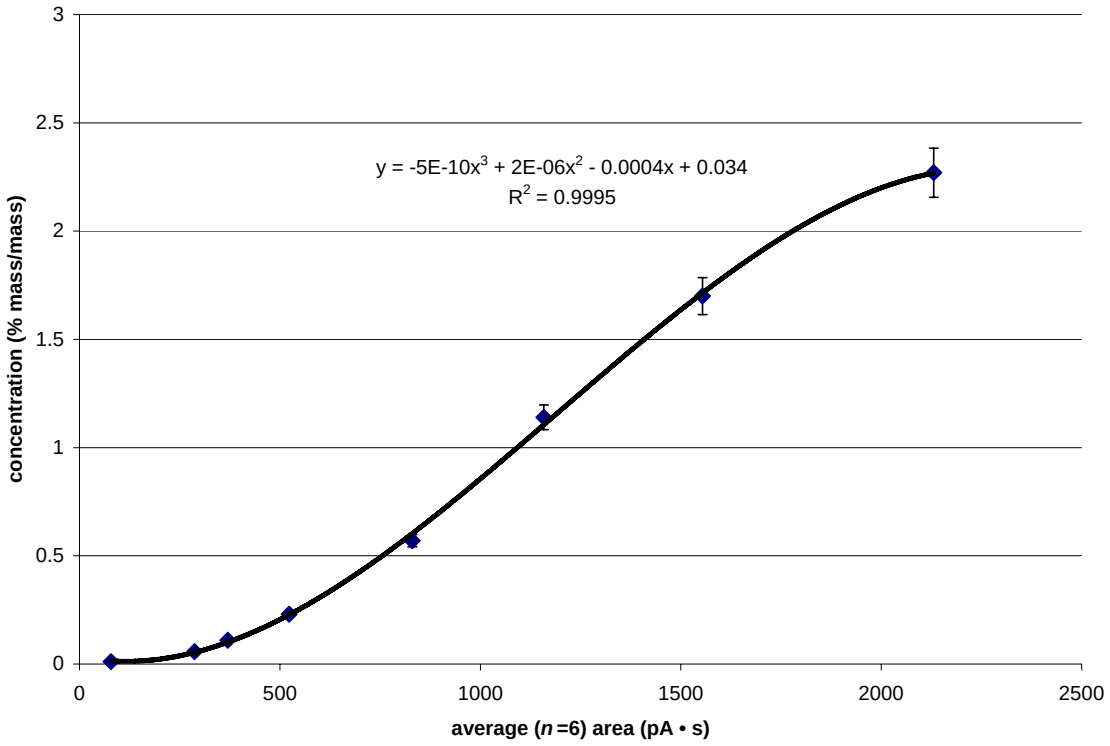

Figure 2.5 Calibration curve for methanol in reference biodiesel– average of six series.

Three spiked samples with different concentrations of methanol in reference biodiesel were used to check the reproducibility of this headspace SPME method. The concentrations were 0.057, 0.11, and 0.23 mass percent. For each concentration, a set of six replicates was recorded and the relative standard deviation was 1.00 % for the 0.057 mass percent solution, 1.52 % for the 0.11 mass percent solution, and 2.01 % for the 0.23 mass percent solution. The recovery was also excellent, ranging from 97.43 % to 101.55 % with an average of 100.20 % and a relative standard deviation of 1.60 % (n=6).

This headspace SPME method was used to analyze 13 actual biodiesel (B100) samples obtained from the Petroleum Products Laboratory (Mississippi State, MS). The

results are summarized in Table 2.5. According to the headspace SPME method, all the samples in this table, except number 10, 11 and 13 would pass the European EN-14110 standard. The European EN-14110 test method uses biodiesel (B100), which is heated at 80 °C in a hermetically sealed vial for 45 minutes and then a defined amount of gas phase is injected into the GC/FID by means of a preheated syringe. This headspace method can be manual or automated and it uses 2-propanol as internal standard. An external calibration can also be used if the headspace procedure is automated.[23]

Table 2.5 Methanol concentration in biodiesel samples from Petroleum Products Lab.

sample no.	**sample name**	**methanol concentration (% mass/mass)**
1	2006-93A	0.0085
2	2006-93H	0.12
3	2006-95A	0.015
4	2006-107A	0.081
5	2006-108A	0.043
6	2006-108B	0.0022
7	2006-108C	0.073
8	2006-108D	0.16
9	2006-108E	0.095
10	2006-109B	0.61
11	2006-111A	0.72
12	2006-111B	0.15
13	2006-111C	0.25

The following alcohols in biodiesel were analyzed by headspace SPME, using the previously described optimum SPME and GC conditions: ethanol, 1-propanol,

2-propanol, 1-butanol, 2-butanol, iso-butanol, and tert-butanol. They were spiked in reference biodiesel at five different concentrations: 0.01, 0.05, 0.11, 0.2, and 0.4 mass percent. For each of the alcohols, the results fit a second order polynomial curve and are summarized in Table 2.6. Six replicates were run for the 0.2 mass percent concentration for each of the above alcohols and the relative standard deviation (%) is also reported in the table below.

Table 2.6 C_2-C_4 alcohols in biodiesel.

alcohol	***Rt*** **(**min**)**	$\mathbf{R^2}$	**RSD** (%) for 0.2 % m/m
ethanol	2.25	0.9999	1.431
2-propanol	2.35	0.9998	2.028
tert-butanol	2.45	0.9999	1.056
1-propanol	2.61	0.9977	2.931
2-butanol	2.88	0.996	0.672
iso-butanol	3.13	1.000	1.542
1-butanol	3.58	0.9994	0.630

2.4 Conclusions

A new and direct method for determining the amount of methanol in biodiesel was developed.[21] Headspace SPME proved to be reproducible and sensitive, allowing analysis for concentrations of methanol in biodiesel well below those imposed by the standard specification. There is no need for using an internal standard and derivatization. This method was used for analyzing biodiesel samples from various producers. The sample volume used was 1.00 mL as compared to the flashpoint method that requires at least 70.00 mL.

The new analytical method developed is a simple, direct and reliable alternative to the two standard methods for methanol determination in biodiesel, and is the first headspace SPME method used to extract methanol from biodiesel as substrate.

The range of the relative standard deviation for this work was 3.4 to 10.4 %. The reproducibility of an interlaboratory study of the European EN-14110 test method was between 12-18 %.[23]

CHAPTER III

HEADSPACE SPME DETERMINATION OF ACETIC ACID AND 2-FURALDEHYDE IN AQUEOUS SAMPLES

3.1 Literature review

Acetic acid and 2-furaldehyde have been frequently analyzed by solid-phase microextraction (SPME), a technique developed and patented in 1989 by J. Pawliszyn and his co-workers.[3] These two compounds have been found present in various substrates, such as raw cane and sugar beets,[26] palm sugar,[27] cheese,[28] wine,[29, 30, 31] beer,[32] bread,[33] whisky,[34] air,[35] water,[36, 37, 38] and other matrices.[39, 40] 2-Furaldehyde was detected as a volatile component of oak,[41] wine bouquet,[29] beer,[32] and Italian vinegars,[42] and is responsible for the characteristic flavor and aroma (caramel like) of the above mentioned materials. Some groups analyzed acetic acid and 2-furaldehyde directly by using headspace SPME,[26, 41, 42, 44] others derivatized them and extracted them from the aqueous samples by direct immersion.[36, 37, 38] The extraction of acetic acid and 2-furaldehyde was achieved with different SPME fibers, among the most used being the 75-µm CAR-PDMS (carboxen-polydimethylsiloxane),[32-35, 38, 44] the 85-µm PA (polyacrylate),[28, 29, 36-39] and the 65-µm CW-DVB (carbowax-divinylbenzene).[30,43] Most of the chromatography

involved SPME-GC/MS or SPME-GC/FID, DB-5 (5%-phenyl-methylsiloxane) or DB-Wax (Innowax) capillary column. Sodium chloride was also added to enhance the acetic acid and 2-furaldehyde extraction, a practice called the "salting out effect".[29-34, 36-38, 41-44] The salt solvates the water molecules to a higher extent than the analytes and allows the fiber to better extract the analyte molecule in its neutral form. It was observed that the peak area increases as the salt concentration increases.[31] Derivatization decreases the polarity of the analyte and the derivative has a better chromatographic behavior and is easier to detect.[36-39]

The direct analysis of acetic acid and 2-furaldehyde in an aqueous mixture is important because they are among the products of acid hydrolysis of lignocellulosic biomass such as switchgrass, alfalfa, and wood.[2, 45] The accurate determination and quantitation of these two analytes is very important, as they can be inhibitors or food sources for oleaginous microorganisms. The oil produced by these microorganisms can be converted to biodiesel by catalytic transesterification.

3.2 Experimental

3.2.1 Chemicals and Reagents

Optima-grade water, glacial acetic acid, and sulfuric acid were purchased from Fisher Scientific (Fair Lawn, NJ), 2-furaldehyde, 2,4-dinitrophenylhydrazine, hydroxylamine hydrochloride, and sodium bisulfite were acquired from Sigma-Aldrich (St. Louis, MO), and sodium chloride was supplied by Chempure (Houston, TX). The 10 mL SPME vials, fiber assembly, 75-µm carboxen-polydimethylsiloxane (CAR-PDMS), and 60-µm polyethylene glycol (PEG) fibers, were purchased from Supelco (Bellefonte, PA).

3.2.2 Instrumental Analysis

The analytes were adsorbed onto the SPME fiber and then thermally desorbed in the inlet of a Hewlett-Packard (HP) 6890N gas chromatograph (Palo Alto, CA) equipped with a split/splitless injection port and flame ionization detection (FID) system. The injector and detector temperatures were held constant during the analysis (200 °C and 300 °C, respectively). The capillary column used for separation was a 30-m, 0.32-mm i.d., 0.25-µm film thickness Hewlett-Packard HP-5 (5%-phenyl-methylpolysiloxane) fused silica. The GC oven was programmed as follows: the initial temperature of 40 °C was held for 4.0 min, increased to 120 °C at 10 °C/min, and then increased to 200 °C at 20 °C/min and held at 200 °C for 1 min. Helium was used as carrier gas at a constant flow rate of 1.5 mL/min. The injector was operated in split mode (20:1 split ratio). The data were acquired using a HP-CORE ChemStation system.

3.2.3 Solid-Phase Microextraction

An automated SPME system (CombiPAL, LEAP Technologies) was initially used with a 75-µm CAR-PDMS fiber assembly. CAR-PDMS is recommended for gases and low molecular weight compounds (MW 30-225). New SPME fibers were conditioned in the gas chromatograph (GC) injection port at 300 °C for 2 hours, according to the manufacturer's recommendations. The 75-µm CAR-PDMS fiber was used for the determination of alcohols in biodiesel, and it proved to be very efficient towards extracting polar analytes. Also, literature[44] reports the successful use of CAR-PDMS for the determination of free volatile fatty acids in wastewater.

The 10 mL SPME vials each containing 1 mL of solution were capped with Teflon lined septum caps and heated at 50 °C for 20 min, under constant stirring (500 rpm). The SPME fiber was then exposed to the headspace of the vial and the volatile compounds were adsorbed onto the fiber for 20 min at 50 °C, at constant temperature and stirring. Then the fiber was exposed for 2 min at 200 ºC in the GC injection port for complete desorption and GC analysis of the analytes.

3.3 Results and Discussion

The retention time of acetic acid (*Rt* = 3.10 min) and of furfural (*Rt* = 7.10 min) were determined by spiking 5 mg/mL of pure compounds in water.

The goal of this research was to be able to directly detect both acetic acid and 2-furaldehyde in an aqueous mixture, because this method will be used to analyze the products of the acid hydrolysis of lignocellulosic biomass.[2, 45]

A calibration curve for acetic acid in distilled water was obtained, using the above mentioned headspace SPME and GC conditions and a second order polynomial fit was calculated. The reproducibility was expressed as relative standard deviation RSD = 2.80% (n = 5). The same experiments and calculations were performed for acetic acid in a 30% sodium chloride aqueous solution (30 g NaCl / 100 g distilled water). A second order polynomial calibration curve was also obtained (see Figure 3.1) and the relative standard deviation RSD = 3.21% (5 replicates, n = 5). A 57% increase in the peak area was observed for acetic acid when the 30% sodium chloride aqueous solution was used instead of distilled water.

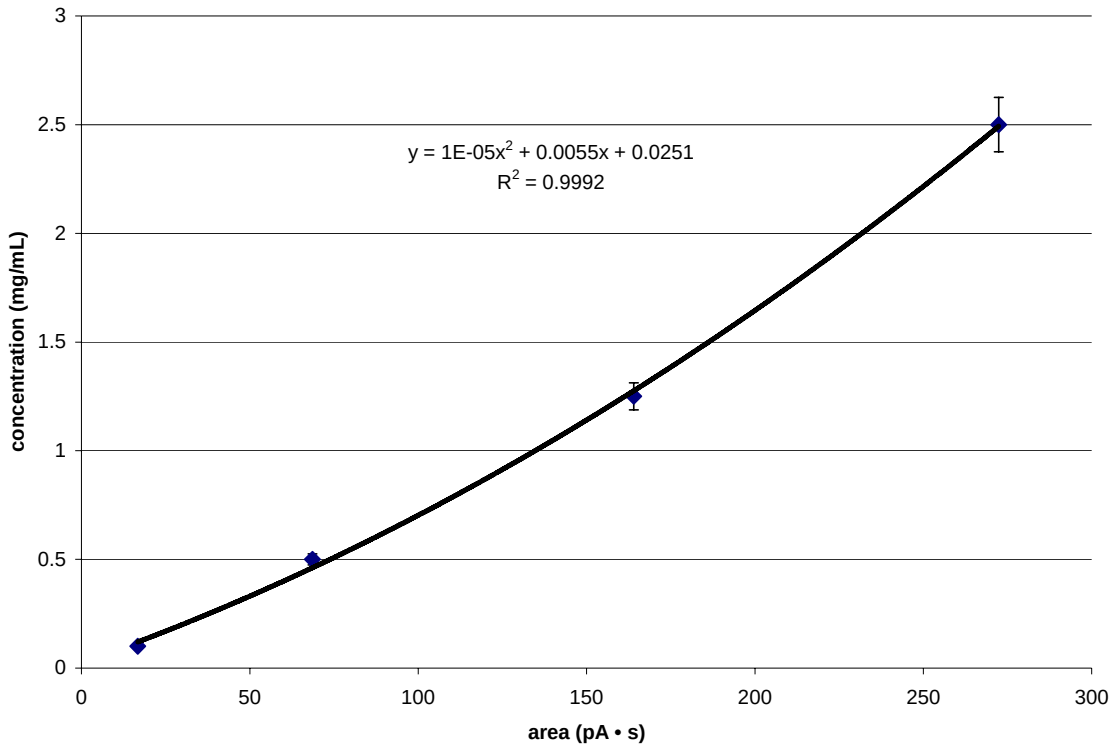

Figure 3.1 Calibration curve for acetic acid in 30% sodium chloride aqueous solution.

2-Furaldehyde was analyzed by spiking different amounts in 30% sodium chloride aqueous solution and a second order calibration curve was also obtained (see Figure 3.2). The reproducibility was expressed as relative standard deviation RSD = 1.34% (n = 5). It was observed that under the same experimental conditions and for the same concentration values, 2-furaldehyde gives a much larger peak area than acetic acid does.

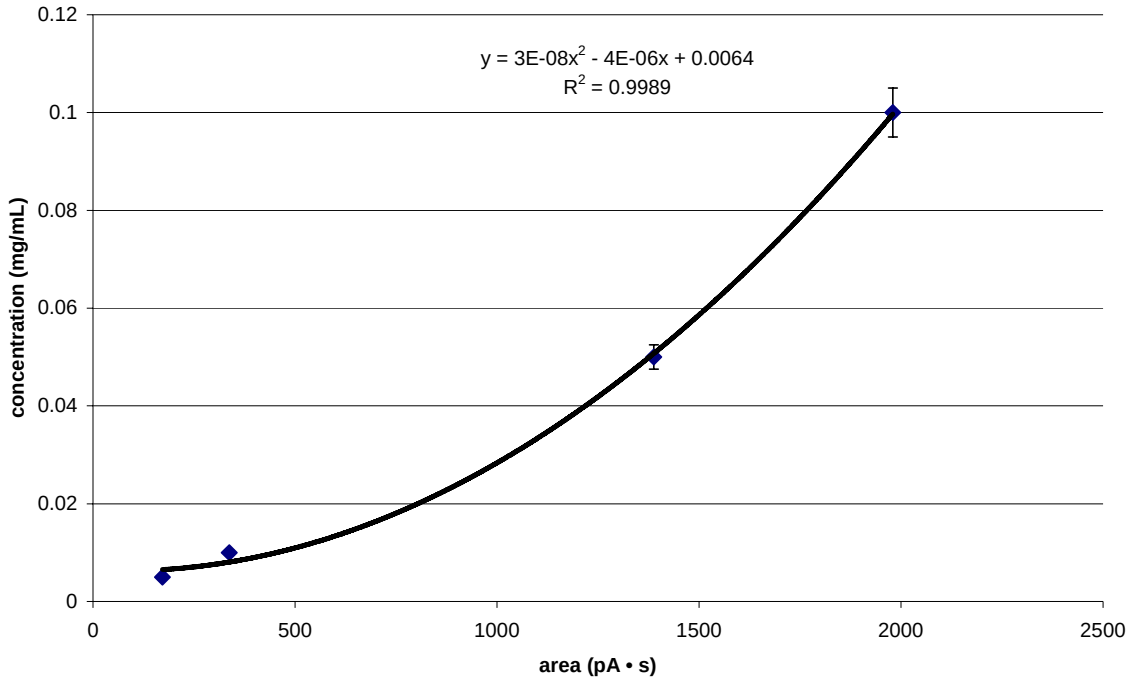

Figure 3.2 Calibration curve for 2-furaldehyde in 30% sodium chloride aqueous solution.

When analyzing a mixture of acetic acid and 2-furaldehyde in 30% sodium chloride aqueous solution, it was observed that even though the concentration of 2-furaldehyde was 10 times lower than the concentration of acetic acid, at one point the response (peak area) for acetic acid was relatively constant and the peak area for 2-furaldehyde was increasing, as can be seen from Table 3.1.

Table 3.1 Acetic acid - 2-furaldehyde mixture in 30% sodium chloride aqueous solution.

acetic acid	**acetic acid**	**2-furaldehyde**	**2-furaldehyde**
area (pA · s)	concentration (µg/mL)	area (pA · s)	concentration (µg/mL)
1.3	50	164.2	5
9.3	100	316.0	10
41.8	500	1346.5	50
45.7	1000	1903.8	100

This matter was further investigated by analyzing a mixture of acetic acid and 2-furaldehyde in 30% NaCl aqueous solution, in which the 2-furaldehyde concentration was kept constant and the acetic acid concentration was varied. In the first set of experiments the concentration of 2-furaldehyde was kept constant at 50 µg/mL and the acetic acid concentration was varied from 100 µg/mL to 5000 µg/mL. In the second set of experiments the 2-furaldehyde concentration was kept constant at 100 µg/mL and the acetic acid concentration was varied from 100 µg/mL to 5000 µg/mL. It was observed that the peak area of the acetic acid decreases as the concentration of 2-furaldehyde increases. The response for acetic acid was lower in the second set of experiments than it was in the first set. As it can be seen from Table 3.2, 2-furaldehyde influences greatly the acetic acid absorption.

Table 3.2 The effect of 2-furaldehyde concentration on the acetic acid response.

acetic acid concentration	**acetic acid area (pA · s)**	**acetic acid area (pA · s)**	**acetic acid area (pA · s)**
(µg/mL)	(0 µg/mL 2-furaldehyde)	(in the presence of 50 µg/mL 2-furaldehyde)	(in the presence of 100 µg/mL 2-furaldehyde)
100	16.7	4.6	4.2
500	68.6	43.6	23.4
1000	139.8	75.5	45.4
2000	239.9	132.4	89.0
5000	480.4	259.0	187.8

The influence of high acetic acid concentration on the absorption of 2-furaldehyde on the SPME fiber was also investigated. For this experiment the concentration of 2-furaldehyde was varied from 5 µg/mL to 1000 µg/mL and the concentration of acetic acid was kept constant at 5000 µg/mL. It was observed that even though the concentration of acetic acid was kept constant, its peak area decreased as the concentration of 2-furaldehyde increased (see Table 3.3). It can be concluded that the 75-µm CAR-PDMS fiber has a much higher affinity for 2-furaldehyde than for acetic acid and that the affinity for acetic acid is strongly affected by the presence of 2-furaldehyde.

Table 3.3 The effect of acetic acid concentration on the 2-furaldehyde response.

2-furaldehyde concentration	**2-furaldehyde area (pA · s)**	**2-furaldehyde area (pA · s)**	**acetic acid area (pA · s)**
(µg/mL)	(0 µg/mL acetic acid)	(in the presence of 5000 µg/mL acetic acid)	5000 µg/mL
5	171.7	157.7	459.5
10	337.5	329.8	433.0
50	1380.2	1359.4	280.5
100	1979.5	1922.0	191.0
500	4075.2	4019.9	103.8

2-Furaldehyde concentrations higher than 50 µg/mL interfere with acetic acid absorption on the CAR-PDMS fiber, so different ways to inhibit 2-furaldehyde absorption were pursued. The first experiment was the reaction of 2-furaldehyde in 30% NaCl aqueous solution with an equivalent amount of sodium bisulfite. Sodium bisulfite forms an addition complex with aldehydes and ketones.[46] This approach did not work because 2-furaldehyde was still detected by headspace SPME. The next experiment was the reaction of 2-furaldehyde with 2,4-dinitrophenylhydrazine.[46] A saturated solution of 2,4-dinitrophenylhydrazine in 5% aqueous sulfuric acid was prepared and added to a 5 µg/mL solution of 2-furaldehyde in 30% NaCl aqueous solution. Even though an orange precipitate appeared, meaning a reaction occurred with 2-furaldehyde and the corresponding hydrazone formed, 2-furaldehyde can still be detected in the reaction mixture by headspace SPME. The last experiment was the reaction of 2-furaldehyde in 30% NaCl aqueous solution with hydroxylamine hydrochloride.[46] The corresponding reaction product (oxime) that was formed was strongly absorbed by the SPME fiber (large peak observed at 11.4 min). None of these reactions (see Figure 3.3) could

completely inhibit the 2-furaldehyde absorption on the SPME fiber, so other alternatives were considered.

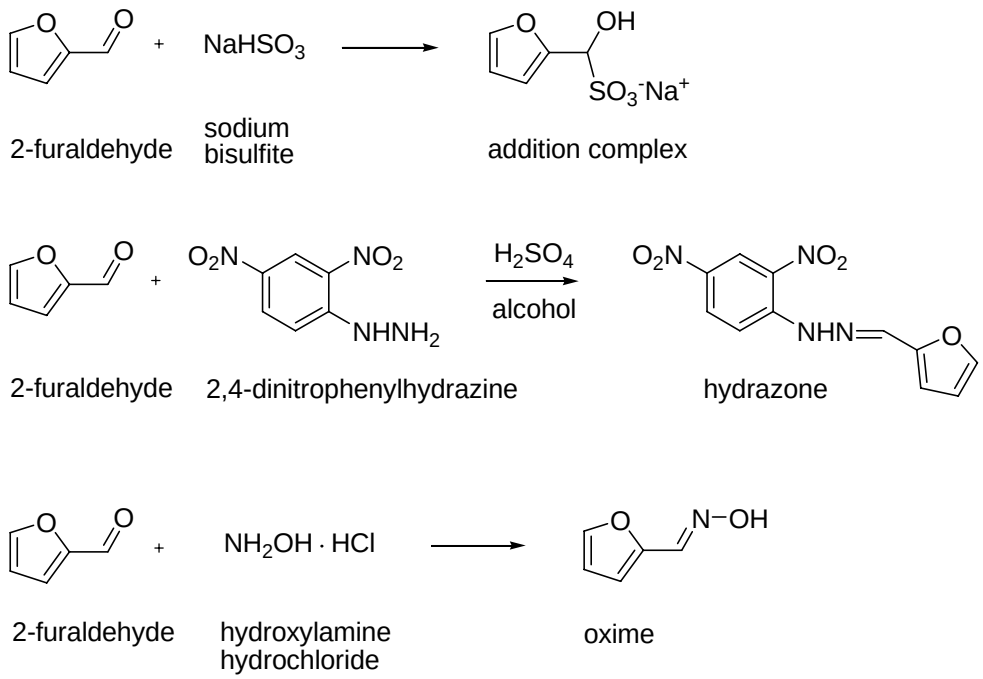

Figure 3.3 Specific reactions of aldehydes. Possible ways of inhibiting the absorption of 2-furaldehyde.

A different approach was considered, namely changing the SPME fiber. Literature reports that CW-DVB fiber has a high extraction capacity towards acetic acid, but has a very poor mechanical stability.[30, 36, 43] Its replacement, the 60-µm PEG fiber is more durable due to bonding of the fiber coating to a strong and inert metal core. It is recommended for alcohols and polar compounds with MW 40 – 275.[47] The following sets of experiments were performed with the newly released 60-µm PEG SPME fiber. Using

the same GC and SPME conditions described above, a set of calibration curves were run for acetic acid in the presence of 3000 µg/mL 2-furaldehyde (see Figure 3.4) and for 2-furaldehyde in the presence of 5000 µg/mL acetic acid in 30% NaCl aqueous solution (see Figure 3.5). A linear fit was obtained in both cases, with excellent correlation coefficients, $R^2 = 0.9993$ in the case of acetic acid and $R^2 = 0.9997$ in the case of 2-furaldehyde.

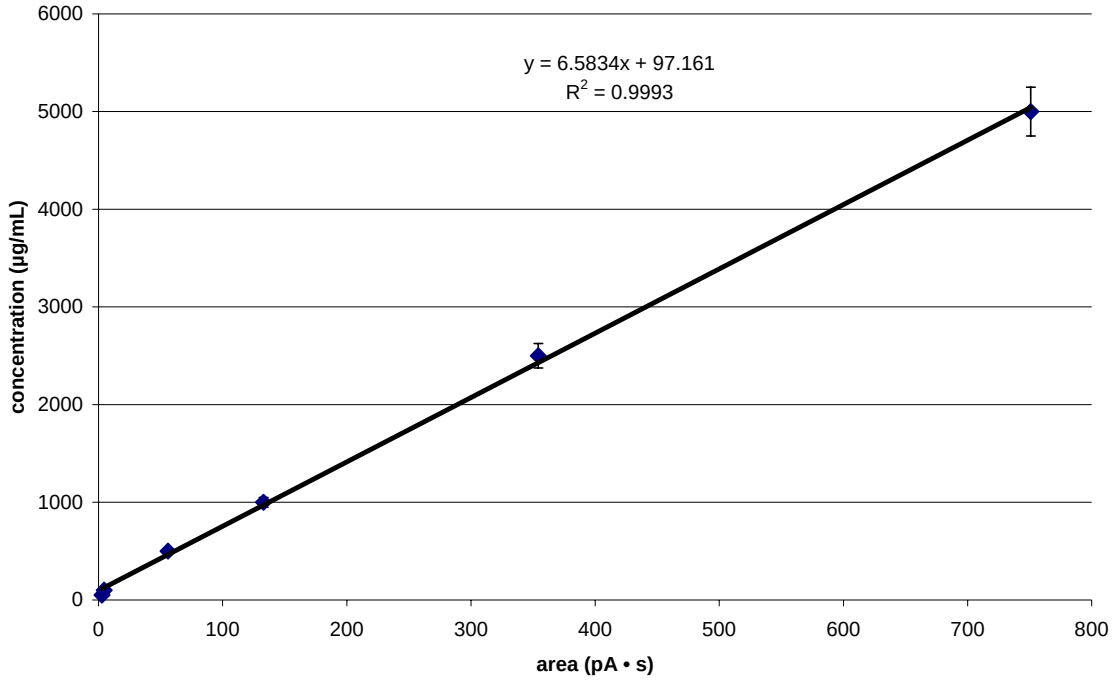

Figure 3.4 Calibration curve for acetic acid in the presence of 3000 µg/mL 2-furaldehyde in 30% NaCl aqueous solution, using the 60-µm PEG fiber.

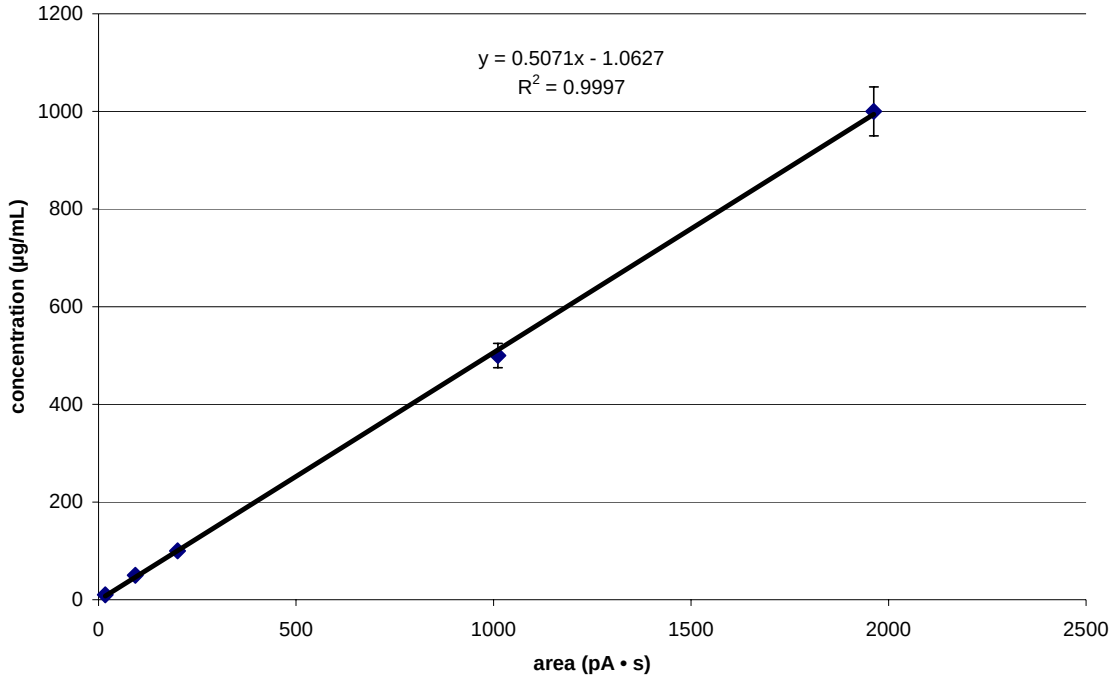

Figure 3.5 Calibration curve for 2-furaldehyde in the presence of 5000 µg/mL acetic acid in 30% NaCl aqueous solution, using the 60-µm PEG fiber.

A set of calibration curves was also run for the individual compounds, acetic acid in 30% NaCl aqueous solution (see Figure 3.6) and 2-furaldehyde in 30% NaCl aqueous solution (see Figure 3.7). Linear fit was obtained in both cases with good correlation coefficients, $R^2 = 0.9986$ in the case of acetic acid and $R^2 = 0.9998$ in the case of 2-furaldehyde.

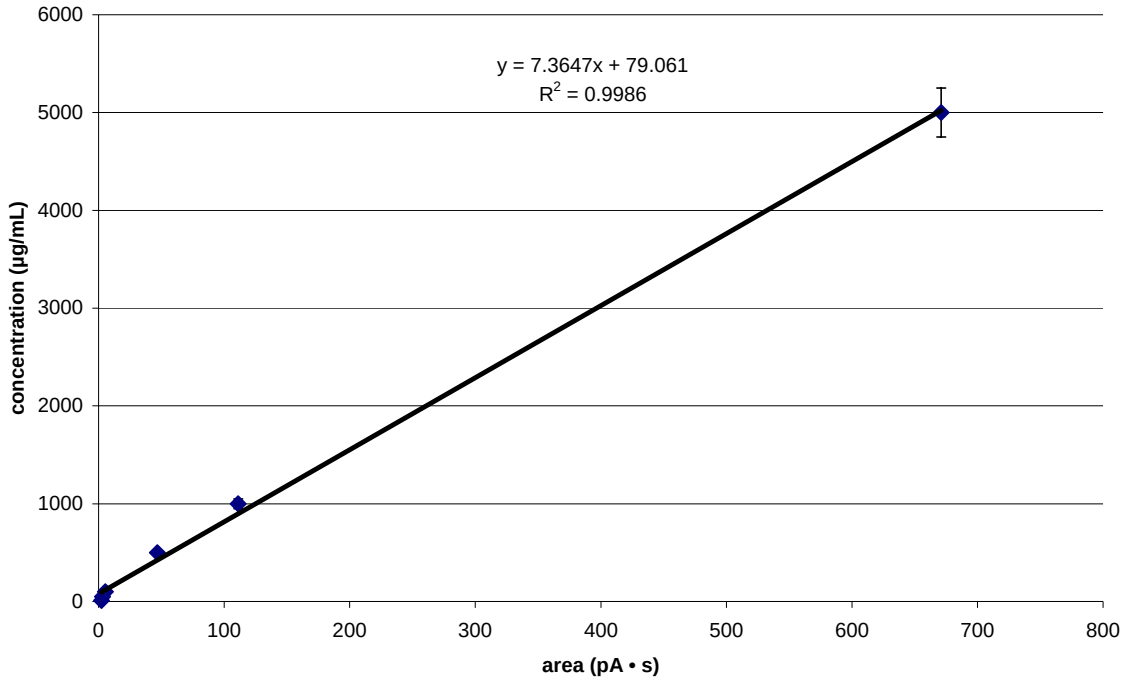

Figure 3.6 Calibration curve for acetic acid in 30% NaCl aqueous solution using the 60-µm PEG fiber.

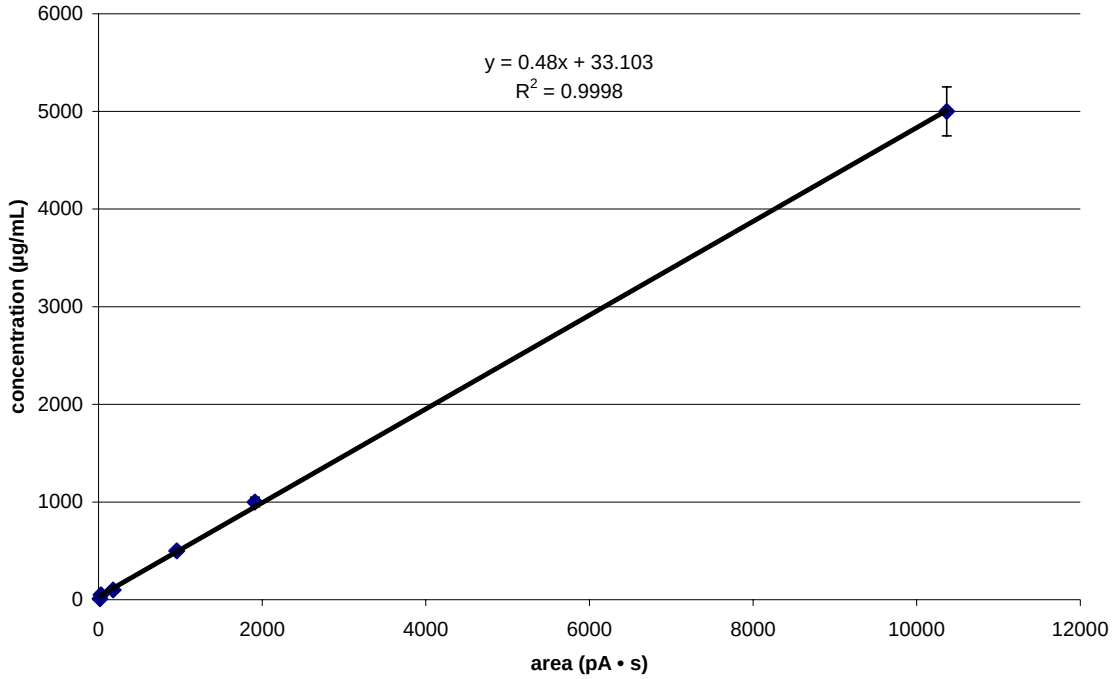

Figure 3.7 Calibration curve for 2-furaldehyde in 30% NaCl aqueous solution using the 60-µm PEG fiber.

These sets of experiments showed that the 60-µm PEG SPME fiber exhibits the expected behavior for both analytes and does not selectively extract 2-furldehyde. Linear calibration curve and good reproducibility were obtained when acetic acid was analyzed in the presence of high concentrations (3000 µg/mL and 1000 µg/mL) of 2-furaldehyde (see Table 3.4). The influence of acetic acid on the 2-furaldehyde absorption was also studied (experimental data showed in Table 3.5). The 60-µm PEG SPME fiber will be used for the detection of acetic acid and 2-furaldehyde in aqueous mixtures, and for the optimization of extraction conditions.

Table 3.4 The effect of 2-furaldehyde concentration on the acetic acid response using the 60-µm PEG fiber.

acetic acid concentration	**acetic acid area (pA · s)**	**acetic acid area (pA · s)**	**acetic acid area (pA · s)**
(µg/mL)	(0 µg/mL 2-furaldehyde)	(in the presence of 1000 µg/mL 2-furaldehyde)	(in the presence of 3000 µg/mL 2-furaldehyde)
50	3.0	2.1	2.8
100	4.9	4.0	4.6
500	56.1	56.8	56.0
1000	133.0	135.0	132.8
5000	775.6	780.9	771.9

Table 3.5 The effect of acetic acid concentration on the 2-furaldehyde response using the 60-µm PEG fiber.

2-furaldehyde concentration	**2-furaldehyde area (pA · s)**	**2-furaldehyde area (pA · s)**	**acetic acid area (pA · s)**
(µg/mL)	(0 µg/mL acetic acid)	(in the presence of 5000 µg/mL acetic acid	(5000 µg/mL)
10	17.0	17.2	710.0
50	99.4	93.2	716.6
100	193.7	200.0	730.9
500	1029.1	1011.0	723.3
1000	1983.5	1962.7	730.4

A reproducibility study was also performed for both acetic acid and 2-furaldehyde, using 5 replicate solutions of 1000 µg/mL concentration. The relative standard deviation (RSD %, *n*=5) was 0.76% for acetic acid and 0.33% for 2-furaldehyde.

To study the "salting out" effect often reported in literature,[29-34, 36-38, 41-44] two more aqueous solutions were considered, a 10% NaCl and a 0% NaCl. The results

obtained for acetic acid and 2-furaldehyde with these two solutions were compared with the ones obtained using the 30% NaCl aqueous solution. As it can be seen from the corresponding charts (see Figure 3.8 and Figure 3.9), using a 30% sodium chloride aqueous solution improved the extraction of both acetic acid and 2-furaldehyde. This phenomenon is due to the fact that water would rather solvate the salt ions than the analyte, so NaCl reduces the solubility of the analytes in water and they are readily extracted by the fiber.[31, 42]

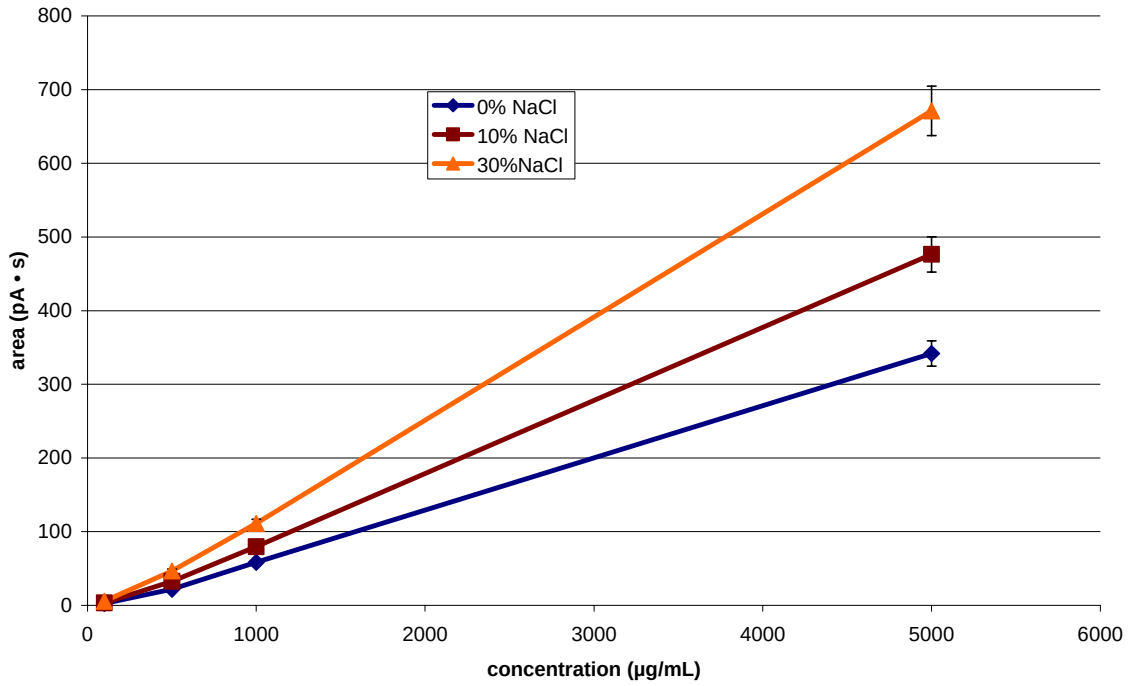

Figure 3.8 Effect of sodium chloride addition on the extraction of acetic acid using a 60-µm PEG fiber.

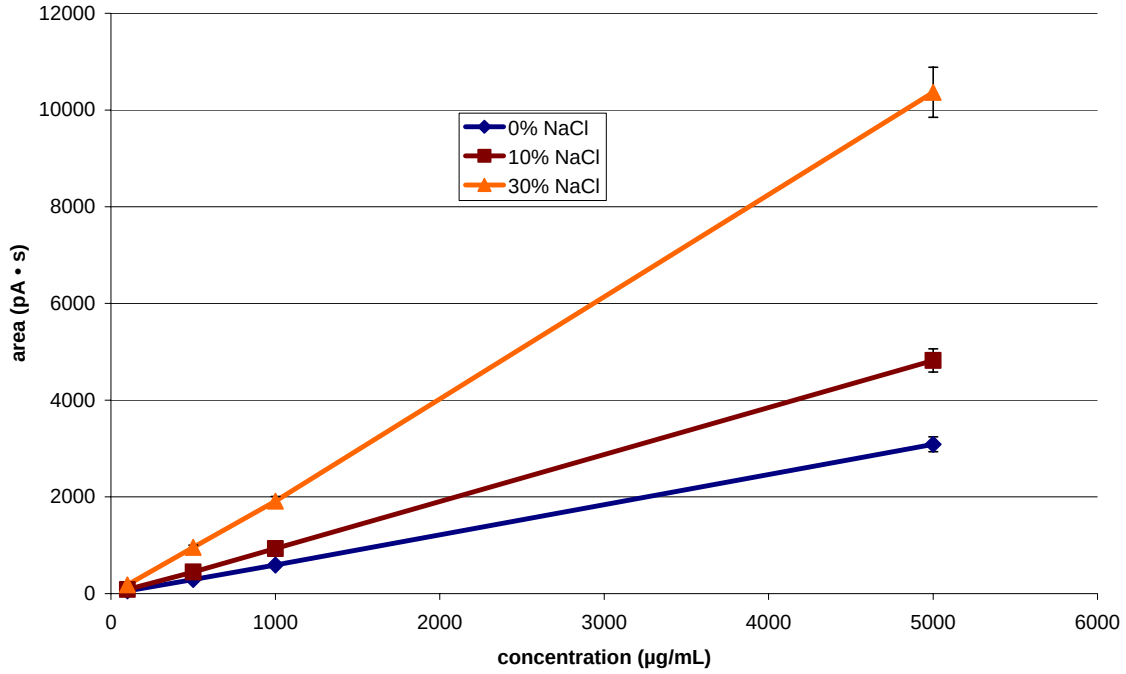

Figure 3.9 Effect of sodium chloride addition on the extraction of 2-furaldehyde using a 60-µm PEG fiber.

The optimization of the extraction temperature and time was performed in order to achieve the highest absorption (response) in the shortest amount of time. Four extraction temperatures (35 ºC, 50 ºC, 60 ºC, and 80 ºC) and three extraction times (10, 20, and 30 min) were investigated. The concentration of acetic acid and of 2-furaldehyde was 1000 µg/mL and each experimental point was analyzed in triplicate and averaged. The experimental data showed that the best extraction conditions for both analytes are

65 ºC and 20 minutes, as it can be seen from Figure 3.10 and Figure 3.11. The same phenomenon is observed here, there appears to be a leakage of analyte vapors from the SPME vials at higher temperatures and longer extraction times, as it can be seen in Figure 3.10).

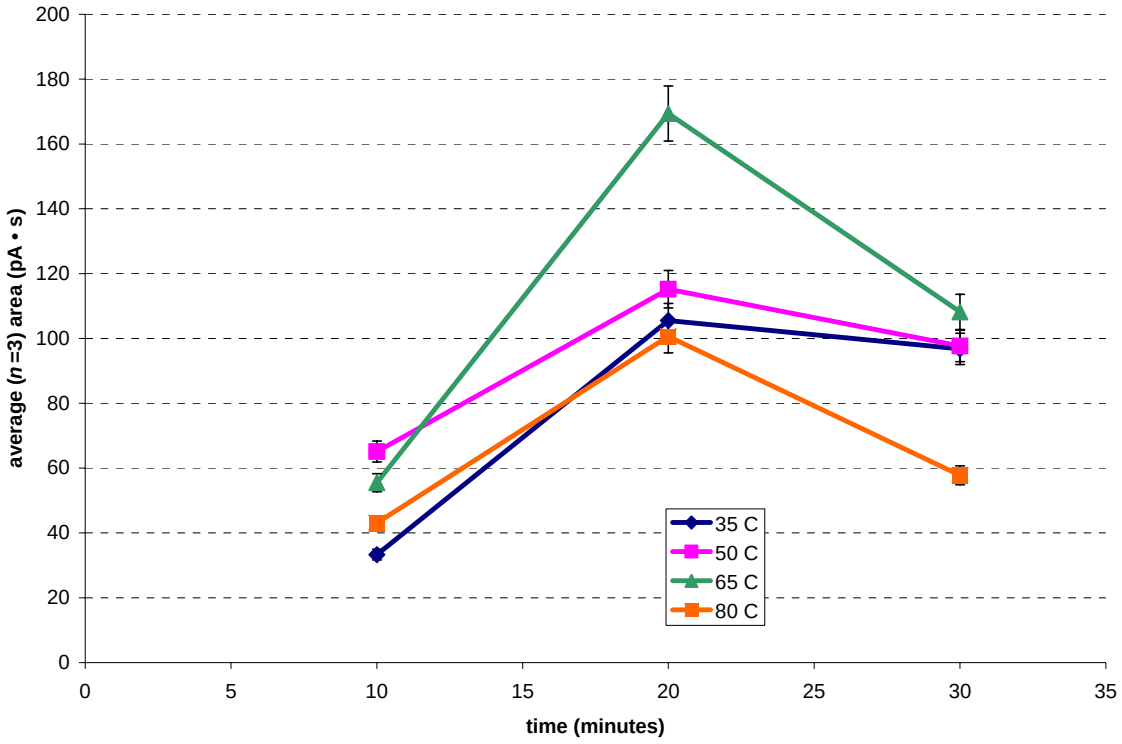

Figure 3.10 Optimization of SPME extraction conditions for acetic acid.

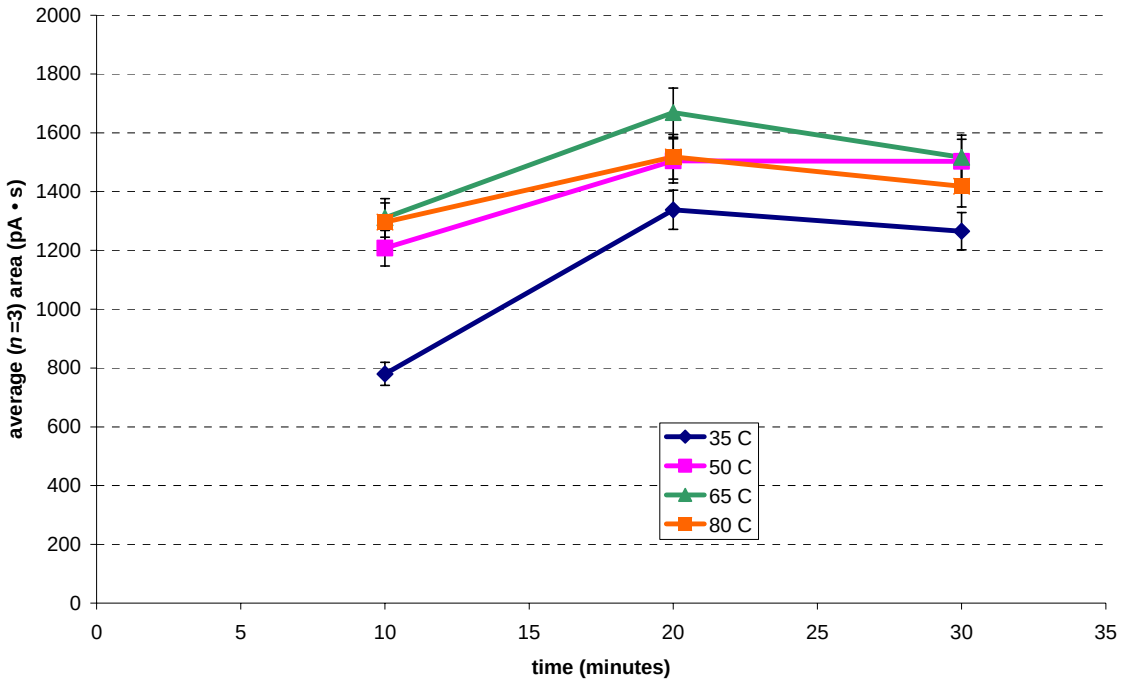

Figure 3.11 Optimization of SPME extraction conditions for 2-furaldehyde.

Linear calibration curves and good correlation coefficients were obtained under optimum conditions for acetic acid in the presence of 3000 µg/mL 2-furaldehyde (see Figure 3.12) and for each of the two analytes individually (see Figure 3.13 and Figure 3.14). The reproducibility (6 replicates) of a 5000 µg/mL acetic acid and 3000 µg/mL 2-furaldehyde mixture in 30 % NaCl aqueous solution was also investigated and the results are: 3.1% for acetic acid and 1.7% for 2-furaldehyde. Future tests and experiments will be conducted using the PEG fiber at the optimum extraction conditions.

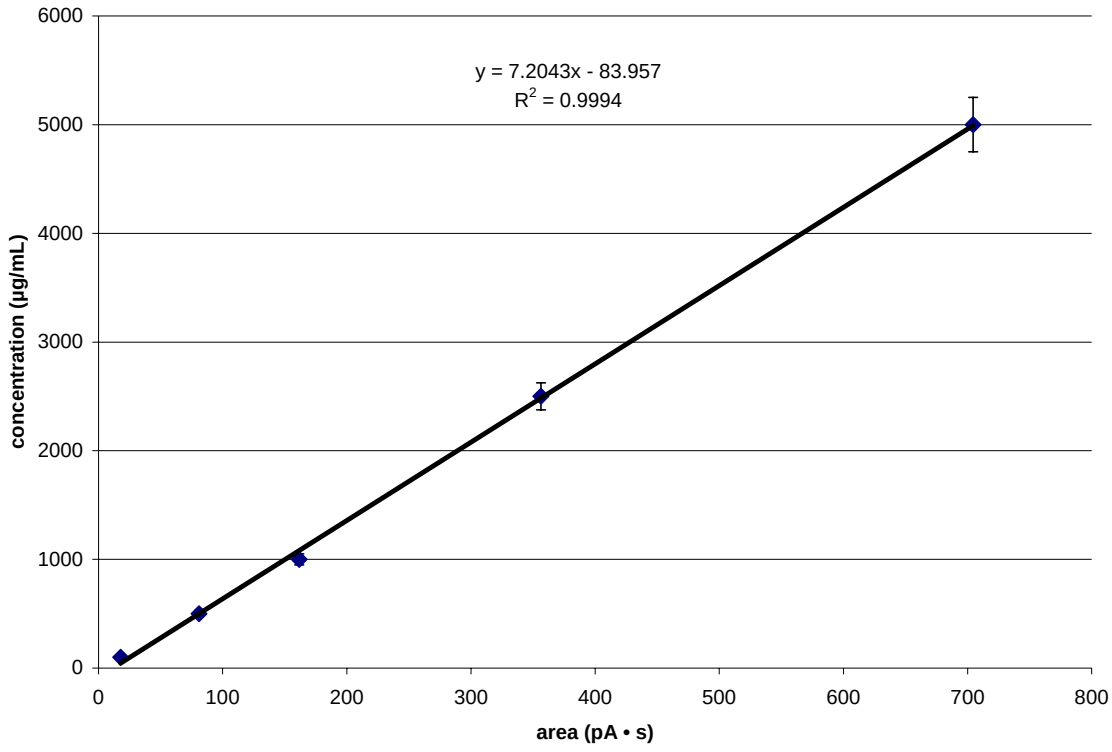

Figure 3.12 Calibration curve for acetic acid in the presence of 2-furaldehyde (3000 µg/mL) under optimum conditions.

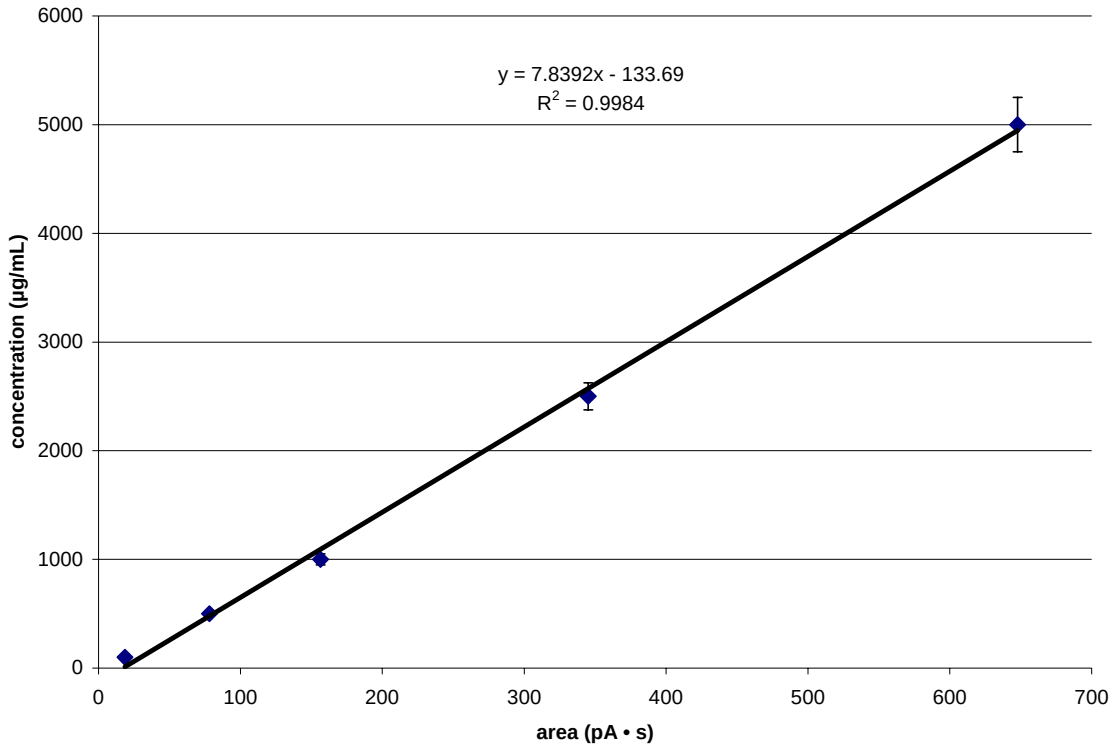

Figure 3.13 Calibration curve for acetic acid under optimum conditions.

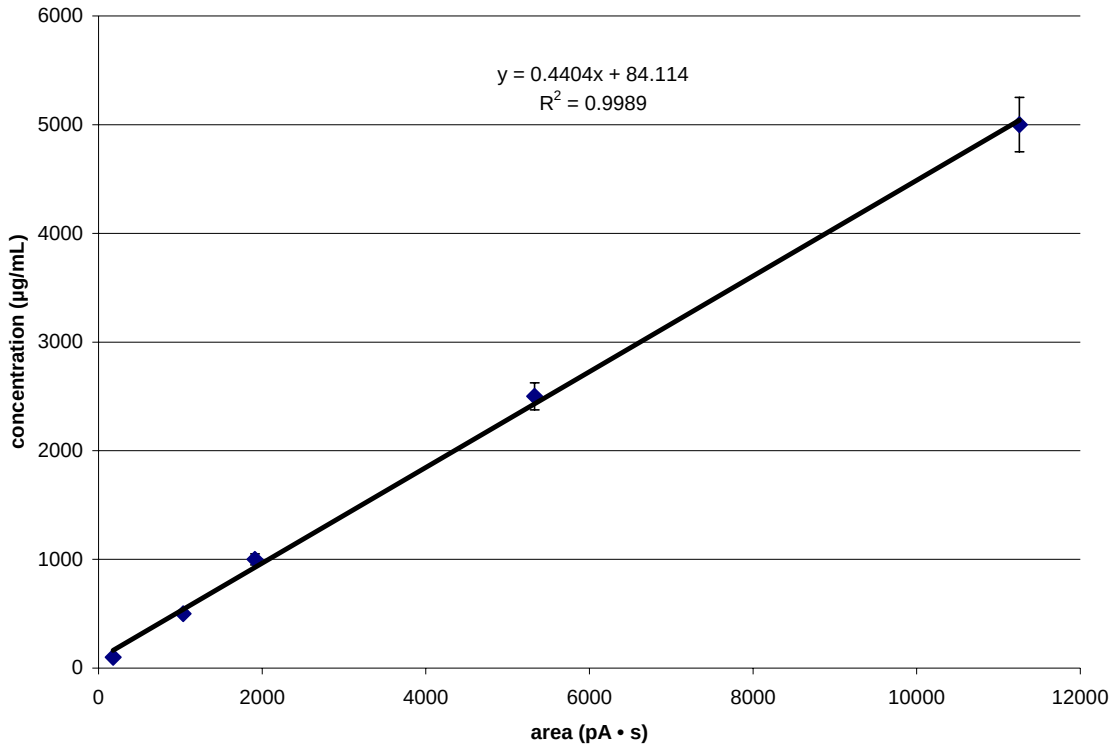

Figure 3.14 Calibration curve for 2-furaldehyde under optimum conditions.

3.4 Conclusions

Attempts to perform the direct analysis of both acetic acid and 2-furaldehyde in an aqueous mixture with the 75-µm CAR-PDMS SPME fiber showed that 2-furaldehyde concentrations higher than 50 µg/mL interfere with the acetic acid absorption. The accurate determination and quantitation of these two analytes was performed with the newly released 60-µm PEG fiber, which yielded linear calibration curves and good reproducibility. PEG fiber is a polar SPME fiber and it mainly extracts the polar analytes from the mixture. This simplifies the chromatographic analysis because there is no need for derivatization. Literature reports the determination of acetic acid and 2-furaldehyde in a complex mixture[29-34] or as individual components[42, 44] but no report studied their behavior as a mixture. The headspace SPME method developed can be used to determine directly and quantitatively both acetic acid and 2-furaldehyde in an aqueous mixture. This method ensures a shorter analysis time compared to the laborious HPLC[45] and GC[2] methods currently used. The analytes are directly analyzed and there is no need to extract them from the reaction mixture or to derivatize them, thus solvent expense, preparation time, and analysis time are minimized.

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