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Determination of amines in air and water using derivatization combined with solid-phase microextraction

Lin Pan, J. Michael Chong, Janusz Pawliszyn*

The Guelph-Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry, University of Waterloo, Waterloo, Ont. N2L 3G1, Canada

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Abstract

Solid-phase microextraction (SPME) is a simple, fast, economic and solvent-free sample preparation technique. The selectivity and sensitivity of this technique for the determination of amines in gaseous and aqueous matrices can be significantly improved by coupling derivatization with SPME during sampling. Derivatization has advantages in enhancing coating–gas and coating–water partition coefficients for amines, thus improving SPME extraction efficiency and method sensitivity. For air analysis, 4-nitrophenyl trifluoroacetate effectively derivatized volatile and polar amines into less polar amides in the gaseous phase, with limits of detection (LODs) at the low pg/ml level when gas chromatography–flame ionization detection (GC–FID) was used. For aqueous sample analysis, pentafluorobenzylaldehyde was utilized to convert polar primary amines into their less polar imines directly in aqueous solutions. Derivatization-SPME provided LODs at low ng/ml to pg/ml levels with GC–FID. Compared to direct SPME, derivatization-SPME lowered LODs by two–three orders of magnitude for the analysis of amines in air and aqueous solutions.

Keywords: Derivatization, GC; Sample handling; Solid-phase microextraction; Amines

1. Introduction

The determination of organic amines present in various matrices is an important problem. These compounds can be found in biological fluid [1,2], environmental samples [3] and industrial process streams, often at trace levels. Low-molecular-mass amines are important air pollutants due to their odorous and toxic characteristics [4]. 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 [5].

Free amines are difficult to extract from sample matrices and cannot be chromatographed well, due to their high polarity. In the literature, the determination of amines has generally used three approaches: (1) Direct GC analysis of the aqueous samples; (2) concentration of the amines followed by separation and detection and (3) alteration of the amines by derivatization.

Direct analysis of aqueous samples minimizes sample preparation, thereby lowering the analysis time and reducing systematic errors and sample contamination. Direct methods have used GC coupled with an amine-deactivated column [6] and ion

*Corresponding author.

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chromatography [7]. Both techniques were limited to detection limits at the parts-per-million (ppm) level.

Detection limits can be improved by concentrating the target analytes before their measurement. Some sample enrichment techniques, such as steam distillation [8], vacuum distillation [9], liquid–liquid extraction [10], supercritical fluid extraction [11] and solid-phase extraction [1], have been used. These methods lowered the detection limits to about 0.01–0.1 ppm, but also concentrated impurities, leading to false positives or high results [12].

Amines have been derivatized to fluoroacetamides [13], boron chelates [14] and *m*-toluamides [15] 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 amines in gaseous samples or organic solvent extracts of amines from water. Derivatization of amines with reagents such as *o*-phthalaldehyde [16], dinitrofluorobenzene [17] and 2-methoxy-2,4-diphenyl-3(2H)-furanone [18] 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–electron-capture detection (ECD) and GC–MS analysis. The method provided relatively high sensitivity and good chromatographic resolution [10]. Solid-phase reagents (prepared on a solid support such as silica, alumina, cross-linked polystyrene, etc.) containing a benzoyl group, such as 3,5-dinitrobenzoyl [19] and pentafluorobenzoyl [20], have also become popular for the heterogeneous solid-phase derivatization of amines coupled with high-performance liquid chromatography (HPLC) and GC–MS analysis.

Solid-phase microextraction (SPME) is a novel solvent-free sample preparation technique. When coupled with derivatization, it leads to a significant improvement in sensitivity for the determination of polar fatty acids in various sample matrices [21,22]. Derivatization-SPME has the advantages of enhancing the coating–gas and coating–water partition coefficients of target analytes, thus improving SPME extraction efficiency and method sensitivity. In this paper, the determination of polar amines in gaseous and aqueous matrices using derivatization-SPME is presented.

2. Experimental

2.1. Chemicals and materials

Methyl-, ethyl-, diethyl-, isobutyl-, butyl-, amyl- and hexylamines were obtained from Supelco Canada (Mississauga, Canada) and were used as received. The derivatizing reagents were 2,3,4,5,6-pentafluorobenzylaldehyde (PFBAY), which was obtained from Aldrich (Milwaukee, MI, USA), and *p*-nitrophenyl trifluoroacetate (NPTFA), which was synthesized according to the method described previously [23]. All solvents used in this study were of analytical-reagent grade. Deionized water (NANOpure, ultrapure water system, Barnstead, IA, USA) was used to prepare aqueous samples.

The SPME devices were purchased from Supelco (Bellefonte, PA, USA). The detailed set-up and the manipulation of the SPME device were described previously [24]. Three types of fibers were used for this work: Carbowax divinylbenzene [CAX(DVB)], poly(dimethyl-siloxane) (PDMS) and poly(acrylate) (PA) and they were obtained from Supelco Canada, and had film thicknesses of 65, 100 and 85 μm, respectively.

For air sample analysis, 40 ml amber vials were used along with 2.5×0.8 cm stirring bars (VWR Scientific, West Chester, PA, USA) to agitate the samples during extraction. The vials were sealed with PTFE-faced silicone septa and phenolic hole caps. For aqueous sample analysis, 15 ml sample vials and 1.2×0.8 cm stirring bars were used.

2.2. Sample preparation

A stock standard containing 2000 μg/ml each of methyl-, ethyl-, diethyl-, isobutyl-, butyl-, amyl- and hexylamines 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 bicarbonate buffer solution, pH 10 (0.1 M), was prepared according to Ref. [25].

2.3. Derivatization procedure

(1) With NPTFA: Reactions of amines with NPTFA in methylene chloride (CH_2Cl_2) were carried out for syringe calibration. A 500-μl volume of NPTFA–CH_2Cl_2 (10 mg/ml) was added to 500 μl of CH_2Cl_2 containing amines with concentrations ranging from 20–5000 ppm. The solution was mixed using a magnetic stirring bar at room temperature for 30 min, at which time the gas chromatography–flame ionization detection (GC–FID) area counts of target peaks were maximal. The solution (0.2 μl) was injected onto the GC column. Calibration was linear for the concentrations tested. The calibration curve was then used to quantitate amines for fiber injection. To ensure accuracy, the masses of analytes extracted by the fiber coatings were all within the calibration range.

For air analysis, 2 μl of the standard amine mixture was injected into 40 ml silanized amber vials. Prior to sampling, the sample vial was heated in a drying oven (100°C) to vaporize the amines. After the samples were cooled to room temperature, 2 μl of the NPTFA–CH_2Cl_2 solution (80 mg/ml) was injected into the sample vial. The mixture was allowed to sit at room temperature for 10 min to ensure proper mixing and complete reaction. The SPME fiber was then placed in the reaction vial to sample the reaction derivatives.

(2) 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. The above reaction mixture was sealed in a reaction vial. The vial was heated at 80°C for 2 h 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. Masses extracted by fiber coatings were within the range of the calibration curve.

For aqueous sample analysis, 6 μl of PFBAY–MeCN (2.25 mg/μl) and microliter amounts of amine stock solution were spiked into water (10 ml). The vial was then sealed and placed in a water bath at 80°C for 15–30 min. After the reaction mixture cooled to room temperature, a 10-ml aliquot was transferred into a 15-ml sample vial. A SPME fiber was introduced into the headspace above the aqueous solution to extract reaction derivatives.

2.4. Instruments

A Varian 3400 GC system coupled with a septum-equipped programmable injector (SPI) and a FID system (Varian Canada, Georgetown, Canada) or coupled with a Varian Saturn I ion-trap mass detector (ITMS) (Varian Canada) were used. The GC system was also equipped with cryogenics in both the injector and the oven for temperature programming. LB-2 septa (Supelco Canada) were used in the injector. For GC–FID analysis, the column used was a 30 m×0.25 mm I.D. SPB-5 with a 1-μm stationary phase (Supelco Canada). For GC–ITMS analysis, the column used was a 30 m×0.25 mm I.D. SPB-5 with a 0.25-μm stationary phase (Supelco Canada).

2.5. Chromatography

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 min, then programmed to 135°C at a rate of 15°C/min, and from 135 to 280°C at a rate of 20°C/min with a final hold time of 2 min; the injector temperature was programmed from 40 to 275°C at a rate of 250°C/min for solvent injections and it was held constant at 275°C for SPME fiber injections. The fiber desorption time was 5 min. The FID temperature was 300°C. For the GC–ITMS analysis of PFBAY derivatives, the same GC conditions as for the GC–FID analysis were used, with the transfer line held at 250°C. A mass range of 45–450 u was scanned for GC–ITMS analysis.

3. Results and discussion

3.1. Analysis of amines in air

3.1.1. Extraction of free amines with direct SPME

The initial work for the analysis of amines in air was performed by the isolation of free amines from a gaseous matrix with SPME sampling. Three fiber coatings were examined: CAX(DVB), PA and PDMS. As the previous study indicated that acid-

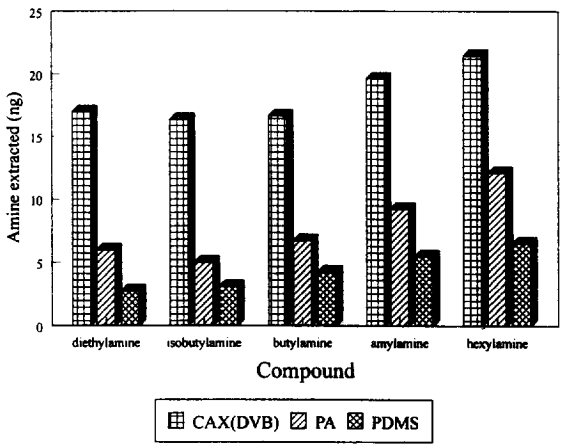

Fig. 1. Coating evaluation for the extraction of volatile amines from air with the CAX(DVB)-, PA- and PDMS-coated fibers. Sample concentration was 25 ng/ml for each amine. Extraction time was 30 min.

washed and silanized vials should be used for the analysis of polar analytes in air, e.g. fatty acids [21], to prevent them being adsorbed by the glass wall, acid-washed and silanized vials were also used for the current studies.

SPME is an equilibrium process with analytes partitioning between the fiber coating and the sample matrix. The equilibration time is determined by exposing the fiber to the sample matrix containing the target analytes for a variety of time periods until the amount extracted remains constant. The exposure time profiles indicated that the equilibrium was reached within 20 min for diethyl-, isobutyl-, butyl-, amyl- and hexylamines with the PDMS-coated fiber, and 30 min for the CAX(DVB)- and PA-coated fibers. The results also indicated that under the procedure used, methyl and ethyl amines could not be extracted in amounts large enough for FID; therefore, the data for these two amines could not be reported.

The amounts of the amines isolated from air samples with the above three fiber coatings were then compared and the results are shown in Fig. 1. The results reveal that the amounts extracted by the fibers followed a trend, i.e., CAX(DVB)>PA> PDMS. Polar amine compounds have lower affinities for the non-polar PDMS coating than for the polar CAX(DVB) and PA coatings, therefore, the amounts of amines extracted by the PDMS coating were the lowest. The CAX(DVB) coating is a porous polymer containing hydroxyl groups on its surface. It has stronger interactions with amines through hydrogen bonding than has the PA coating. As a result, the CAX(DVB) coating extracted larger amounts of amines than the PA coating. The CAX(DVB)-coated fiber was selected for the remainder of the experiments.

Table 1 summarizes the fiber linear ranges, limits of detection (LOD) and relative standard deviations (R.S.Ds.) for the extraction of free amines from air using the CAX(DVB)-coated fiber. The fiber linear ranges were found to be 5 and 50 ng/ml for all of the amines tested, except for methyl- and ethylamines, which could not be detected under the above conditions. The correlation coefficients were between 0.99277 and 0.99797. The R.S.D. values were between 2.1 and 6.1 ($n=3$) for all amines examined. The LOD was estimated to be the concentration of the amine that produces a signal that is five times

Table 1
Fiber linear ranges, LODs and R.S.Ds. 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)	R.S.D. (%) ($n=3$)
Methylamine	ND	–	–	–
Ethylamine	ND	–	–	–
Diethylamine	5–50	0.99658	1000	6.1
Isobutylamine	5–50	0.99277	670	2.1
Butylamine	5–50	0.99291	470	5.5
Amylamine	5–50	0.99797	330	4
Hexylamine	5–50	0.99605	260	5.1

ND=not detected.

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 hexylamines.

For small amines, the NIOSH guide [26] specifies exposure limits of 10–30 ng/ml for industrial air. The exposure limits for indoor air are usually 100–1000 times lower (10–300 pg/ml). The experiments indicated that direct SPME could be used to monitor diethyl-, isobutyl-, butyl-, amyl- and hexylamines in the industrial atmosphere. However, for the analysis of free methyl- and ethylamines in industrial air, as well as for all indoor air analysis, more sensitive alternatives are needed. Derivatization combined with SPME was investigated to address this need.

3.1.2. Determination of amines with 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. Therefore, NPTFA was used to convert selected amines into their less polar trifluoroacetamides for GC separation. 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.

Since both the derivatizing reagent and the reaction derivatives are volatile, the derivatization of amines was carried out directly in the gaseous phase by injecting microliter amounts of amine–MeCN and NPTFA–CH_2Cl_2 into the sample vial.

The optimal amount of reagent used for the derivatization was first examined. It was found that 160 μg of NPTFA (2 μl of 80 mg/ml stock solution in CH_2Cl_2) 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, this amount was used for subsequent experiments.

Exposure time profiles were monitored using the CAX(DVB)-coated fiber and the results are shown in Fig. 2. It was observed that the NPTFA derivatives of diethyl- and isobutylamines co-eluted and could not be separated under the conditions used. Therefore, the results for these two analytes are reported together. This figure illustrates that the extraction equilibrium was reached within 20–30 min. Similar experiments were also performed using the PA- and PDMS-coated fibers and the results (data not shown) indicated that an equilibrium time of 20 min was required for both fibers.

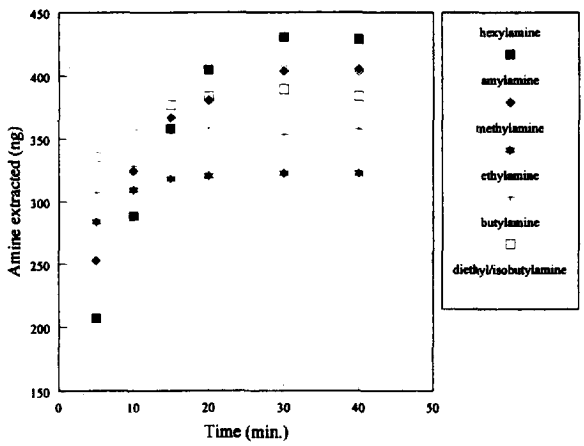

Fig. 2. Exposure time profiles for the extraction of 25 ng/ml each of volatile amines as their NPTFA amides with the CAX(DVB)-coated fiber from air.

Fiber coating evaluation was then examined using the three selected fiber coatings. A comparison of the amounts extracted is illustrated in Fig. 3. This figure

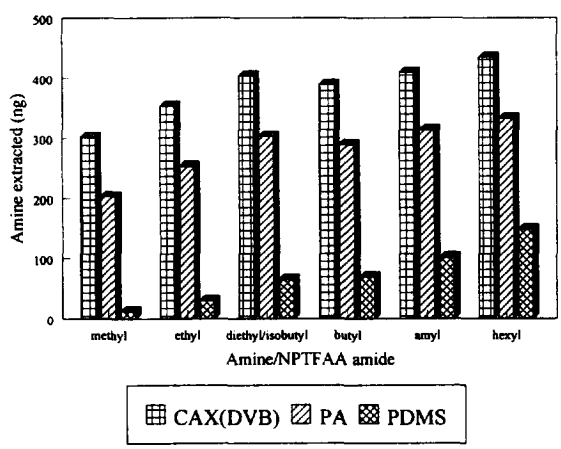

Fig. 3. Coating evaluation for the extraction of 25 ng/ml of amine/NPTFA amides from air using the CAX(DVB)-, PA- and PDMS-coated fibers. Extraction time was 30 min.

indicates that after the derivatization technique was incorporated, methyl- and ethylamines (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 used in subsequent experiments.

Table 2 presents the fiber linear range, LOD and R.S.D. (%) for the extraction of the amine/NPTFA amides from air using the CAX(DVB)-coated fiber. The fiber linear ranges were 1.25–125 ng/ml for methyl- and ethylamines, and 0.125–125 ng/ml for diethyl-, isobutyl-, butyl-, amyl- and hexylamines, with correlation coefficients of 0.99376 and 0.99975 for all of the amines examined. The linear range of the CAX(DVB) coating for the extraction of amines (as NPTFA amides) from air was two times wider than for direct SPME. The LODs were determined by the reagent blank and not by the detection ability of the detector. For the GC–FID analysis, the area counts, which were three times higher than the area counts in the reagent blank at the target regions, were used to calculate the detection limits. The LODs were estimated to be between low pg/ml and low ng/ml for all of the amines tested. The LODs for diethyl-, isobutyl-, butyl-, amyl- and hexylamines were two orders of magnitude lower than for direct SPME. This technique was sensitive enough to monitor diethyl-, isobutyl-, butyl-, amyl- and hexylamines in indoor air. The R.S.D. values were between 3.1 and 10% (n=3) for all of the amines examined.

3.2. Analysis of amines in aqueous solutions

3.2.1. Extraction of free amines with headspace SPME

To develop a new SPME method, the choice of sampling mode is the primary step. It is important to determine if direct or headspace SPME is a more appropriate sampling technique. The following parameters were considered: The volatility of the target analytes and the suitability of the sampling mode for real sample analysis. As a consequence, headspace SPME sampling was chosen, since the selected amines were volatile. 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 and this could interfere with the extraction. Headspace SPME avoids these adverse matrix effects.

Amines are bases and they are dissociated in neutral water solutions. SPME fiber coatings only extract polar analytes in their non-dissociated form from aqueous solutions. Therefore, the aqueous matrix, containing amines, has to be alkalized so that amines are present largely in their non-ionic form and consequently can be effectively extracted by SPME fiber coating from water. Since the pK_a values of the smaller amines are around 10 to 11, the aqueous solution was alkalized to pH 10. The pK_a values for the target amines are listed in Table 3 [1]. The addition of NaCl to aqueous matrices enhances the isolation of the target analytes from water [21]. Therefore, the addition of salt was also applied to the analysis of amines in water.

The coating evaluation for the isolation of free

Table 2
Fiber linear ranges, LODs and R.S.Ds. 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)	R.S.D. (%) (n=3)
Methylamine	1.25–125	0.99975	1000	10
Ethylamine	1.25–125	0.99444	1000	4.3
Diethyl/isobutylamine	0.125–125	0.99672	23	4.7
Butylamine	0.125–125	0.99843	19	5.1
Amylamine	0.125–125	0.99376	10	3.8
Hexylamine	0.125–125	0.99779	7	4.5

Table 3
The pK_a values of methyl-, ethyl-, butyl-, amyl- and hexylamines

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

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 enough for the analysis of free amines in water at very low concentrations. The sensitivity could be improved by using GC–MS. Shirey and Mani [27] have reported that headspace SPME coupled with GC–MS could be used to analyze free methyl-, dimethyl-, diethyl- and trimethylamines in water at mid-ppb levels. However, FID is a very frequently used detection method for general analytical purposes. The development of a new technique suitable for a FID application would be useful.

3.2.2. Determination of amines using the derivatization-SPME technique

Previous studies have shown that derivatization coupled with SPME is suitable for the analysis of very polar short-chain fatty acids in aqueous solution [21]. The sensitivity and selectivity for the analysis of these acids were significantly improved after derivatization. Therefore, derivatization coupled with SPME was investigated for the quantitation of small amines. Since PFBAY [12] has been used to derivatize amines in the presence of water, this reagent was selected for this study. As PFBAY can only react with primary amines, it was used to derivatize methyl-, ethyl-, isobutyl-, butyl-, amyl- and hexylamines directly in water.

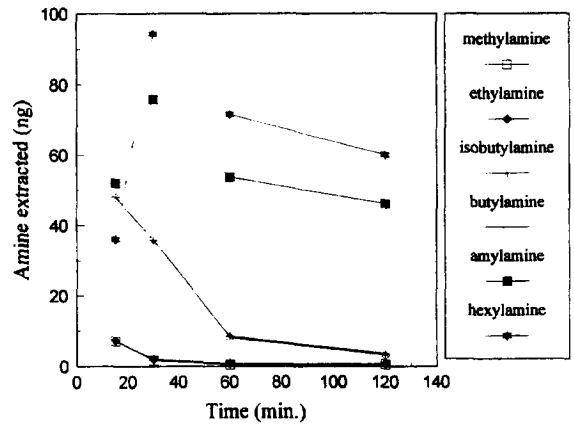

Fig. 4. Reaction time profiles for the derivatization of 10 μg/ml each of the six small primary amines with PFBAY in water using the CAX(DVB)-coated fiber to isolate PFBAY imines from water. The pH of the sample solution was 10, the reaction was carried out at 80°C and the extraction time was 40 min.

Fig. 4 shows the reaction time profiles for the derivatization of the six selected amines in water. It can be seen that the reactions of methyl-, ethyl-, isobutyl- and butylamines with PFBAY in water were faster than those of amyl- and hexylamines. At 15 min (reaction time), the recovery of imines for methyl-, ethyl-, isobutyl- and butylamines peaked and dropped thereafter. For amyl and hexyl amines, the formation of their imines peaked at 30 min and then decreased. Experiments with a reagent blank indicated that PFBAY was quite stable in water, even under alkaline conditions. The decrease in 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 (C_1–C_4) are the target analytes, a shorter reaction time of 15 min should be used. On the other hand, if the longer chain amines (C_5–C_6) are the target analytes, a longer reaction time of 30 min should be used. In the subsequent experiments, 15 or 30 min reaction times were used as indicated.

Fig. 5 illustrates the amounts of amines (as their PFBAY imines) extracted from water with the CAX(DVB)- and PA-coated fibers. This figure indi-

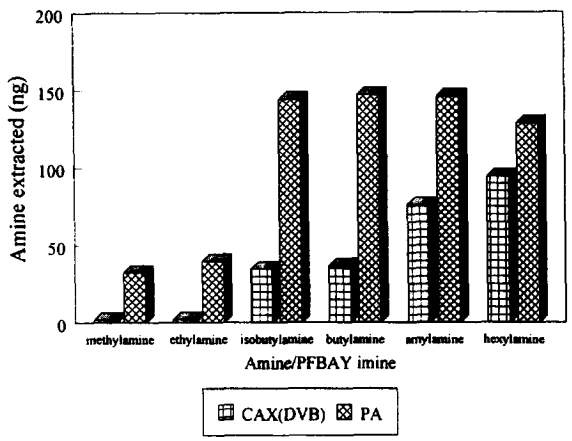

Fig. 5. Comparison of the CAX(DVB)- and PA-coated fibers in the extraction of 10 μg/ml of each amine as its PFBAY imine from the headspace above water under pH 10 conditions. The reaction time was 30 min and the extraction time was 20 min.

cates 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.

Exposure time profiles for the extraction of amine/PFBAY imines were determined with the PA coated fiber. Fig. 6 shows that for the C_1–C_2 PFBAY imines, equilibrium can be reached within 20 min; however, for the C_3–C_6 PFBAY imines, the equilibrium time was 40 min. This implies that C_1 and C_2 PFBAY imines are more volatile than the C_3–C_6 PFBAY imines.

The effect of pH (pH values of 7 and 10) on the formation of PFBAY imines in water was also examined and the results are shown in Fig. 7. The PA-coated fiber was introduced into the headspace above the aqueous solution to isolate the imines. 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.

The two-step reaction mechanism for the formation of imines [28] is illustrated in Fig. 8. Theoretically, imine formation is an acid-catalyzed reaction when it is performed in an organic solution [29]. The first step is the addition of a nucleophilic, non-protonated amine to the partially positive carbonyl carbon of an aldehyde, and it usually proceeds quickly. 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 non-protonated 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 $-OH_2^+$

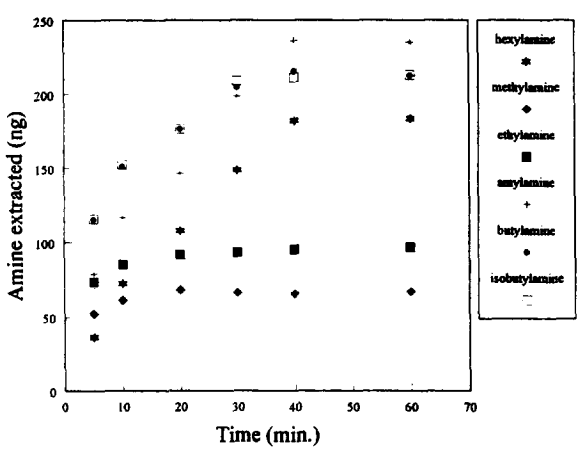

Fig. 6. Exposure time profiles for the extraction of amines as their PFBAY imines from water with the PA-coated fiber. The reaction time was 15 min.

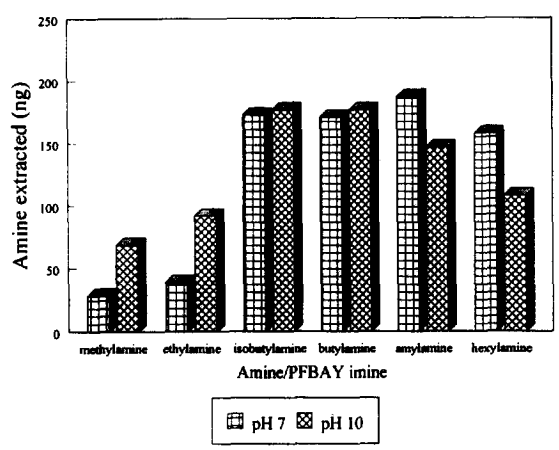

Fig. 7. The effect of pH on the derivatization yield of amines with PFBAY in water. Samples contained 10 μg/ml each of the selected primary amines. The derivatization was carried out at 80°C for 15 min.

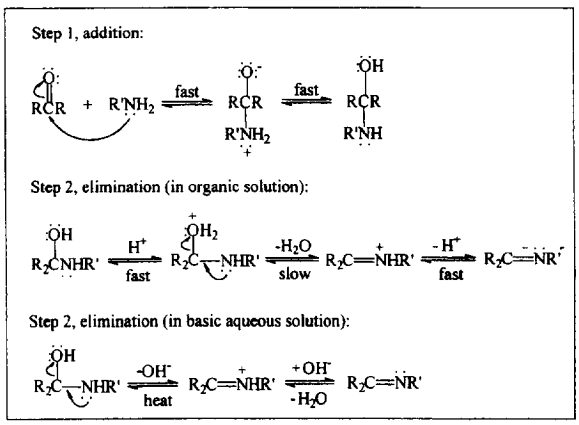

Fig. 8. Reaction mechanism for the formation of imine.

can leave as a weak base and is a good leaving group. The rate of the overall reaction is the greatest in a pH range of 3–4 [29]. Under 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, i.e. the elimination of water, can proceed at a reasonable rate.

The situation is different when the reaction is performed directly in water. As bases, amines are easily protonated by water. To prevent this protonation, 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 are very likely to be dependent on their pK_b values or the pK_a values of their protonated conjugated ions (see Table 3). For methyl- and ethylamines, which have slightly higher pK_a values than the other longer chain amines, more basic conditions are needed to force them into 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 (which are less basic), a pH value of 7 is enough to keep them in their non-ionic state. Under pH 7 or 10 conditions, there were not enough protons present in aqueous solutions. The second step, the elimination of the -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 that is attached to 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' under 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 derivatization in MeCN solution were added into aqueous solutions with pH values of 7 and 10. 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. Fig. 9 illustrates that the amounts of the amines extracted (as their corresponding imines) under the above two pH conditions were the same for all of the amines tested. This confirmed the previous assumption that the pH only affects the formation of imines and not the extraction.

Table 4 presents fiber linear ranges, LODs and R.S.Ds. for the analysis of amines in water using the PA-coated fiber to isolate the PFBAY derivatives from water. Linearity with the PA coating was two orders of magnitude for all of the primary amines tested. The LODs were determined by the reagent blank. They were in the low ppb and high ppt levels for C_1–C_4 amines and C_5–C_6 amines, respectively. The R.S.D. values ($n=3$) were between 2.6 and 15.3% for all amines examined. Compared to head-

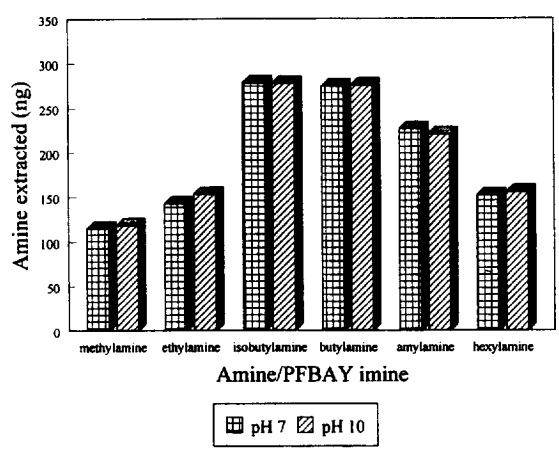

Fig. 9. Effect of pH on the extraction of imines from water with the PA coating. The extraction time was 20 min.

Table 4
PA fiber linear ranges, LODs and R.S.Ds. 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)	R.S.D. (%) (n=3)
Methylamine	0.1–10	0.99976	26	2.6
Ethylamine	0.1–10	0.99999	21	1.8
Isobutylamine	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	0.005–1	0.99891	0.4	15.3

space SPME sampling, the derivatization-SPME technique significantly reduced the detection limits for the analysis of primary amines in aqueous samples. The LODs were at least three orders of magnitude lower when derivatization of primary amines with PFBAY in water coupled with SPME was applied.

3.3. Analysis of wastewater samples with PFBAY derivatization

The ultimate test for any sample preparation technique is how it performs in the analysis of real samples. A wastewater sample was analyzed using derivatization-SPME. The pH of wastewater samples was adjusted to ten. The aqueous sample was derivatized with PFBAY. Headspace SPME sampling was used. Standard addition was employed for quantitation. A 10-ml sample of this solution was transferred to a 15 ml vial and 4 μl of 2.25 mg/μl PFBAY–MeCN was added. The sample vial was sealed and heated at 80°C for 15 min in a water bath. 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 solution containing methyl- and ethylamines and the ones spiked with 1 and 10 μg/ml each of methyl- and ethylamines, showed linear responses for fiber injection with correlation coefficients of 0.99846 and 0.99971 for methyl- and ethylamines, respectively. These experiments indicated the presence of 0.7 μg/ml of methylamine in the wastewater samples. No ethylamine was detected. The results were confirmed by GC–ITMS and an extracted-ion GC–ITMS chromatogram for wastewater samples is shown in Fig. 10. The actual peak shown is the PFBAY imine of methylamine.

The mass spectrum of methylamine–PFBAY imine showed fragment ions at m/z 117, 181 and 208 and a molecular ion at m/z 209. Avery and Junk [12] indicated that electron impact mass spectra of imine derivatives of alkylamines are dominated by α-cleavage. When the α-position of an amine is unsubstituted, the base peak will be m/z 208 [12], 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.

4. Conclusion

New SPME and derivatization-SPME techniques for the analysis of amines in gaseous and aqueous matrices were developed. For the analysis of volatile amines in air, direct SPME can be used to monitor free isobutyl-, butyl-, amyl- and hexylamines for industrial exposure. This technique cannot effectively isolate free methyl- and ethylamines from air samples as they are too polar. Derivatization coupled with SPME significantly improved the sensitivity over direct SPME. Derivatization-SPME decreased the limits of detection for the analysis of isobutyl-, butyl-, amyl- and hexylamines in air samples and can be used to monitor these amines for general indoor exposure levels. Derivatization-SPME also improved the limits of detection for monitoring these amines in industrial settings. To improve the sensitivities of the derivatization-SPME technique for the analysis of methyl- and ethylamines in air, more appropriate derivatizing reagents, such as reagents containing the pentafluorobenzyl moiety, which can provide selec-

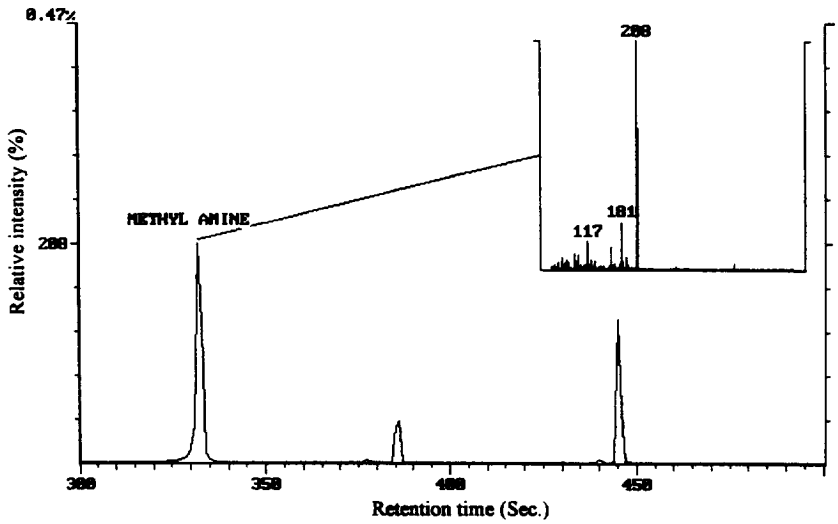

Fig. 10. Extracted-ion GC–ITMS chromatogram for the derivatization of primary amine in a wastewater sample with PFBAY, indicating the presence of methylamine. The actual peak shown is the PFBAY imine of methylamine.

tive and sensitive electron capture detection, should be used. For the analysis of amines in aqueous solutions, headspace SPME had LODs in the mid-to-high ppm ranges for FID. LODs were significantly reduced when derivatization of amines with pentafluorobenzaldehyde coupled with SPME was incorporated. The LODs for the primary amines tested were in the low ppb to 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 achieved.

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