Optimization of Extraction Conditions and Fiber Selection for Semivolatile Analytes Using Solid-Phase Microextraction

Robert E. Shirey
Supelco Inc., Supelco Park, Bellefonte, PA 16823

Abstract

A group of 15 large volatile and semivolatile analytes (MW 92–499 amu) representing 13 organic classes are extracted with 9 different solid-phase microextraction fibers. The extraction efficiencies of the fibers for each of the analytes are compared. The influence of modifying the pH of the sample on the extraction efficiency of the fibers is shown. The effects of the size of the analytes with respect to fiber coating thickness and the relationship between fiber coating polarity and analyte polarity are discussed. In addition to fiber polarity and coating thickness, the different mechanisms by which the fibers extract are presented. A comparison of immersion and heated headspace extraction techniques for these analytes is shown.

Introduction

Recently, an article (1) was published on the optimization of extraction conditions for low molecular-weight analytes (< 90 amu) using solid-phase microextraction (SPME). The same goals used in that study were applied to this study, which evaluated the extraction of larger molecular-weight analytes (92–499 amu). The analytes in this study (with the exception of toluene and *o*-xylene) are typically classified as semivolatile compounds. Analytes that are not usually concentrated by dynamic headspace but have sufficient vapor pressure below 270°C (i.e., > 10^{-7} mm Hg at 25°C) and are thermally stable are classified as semivolatile organic compounds (2).

The ongoing evolution of SPME fiber coatings has created some problems for analysts in selecting the proper fiber for their application. There are numerous factors that must be considered when selecting the proper fiber coating. These factors include the desired detection limits, linear concentration range, size, and polarity of the analytes. When these factors are considered, the analyst is more capable of selecting an adsorbent- or absorbent-type fiber coating with the proper polarity.

In addition to the fiber selection, it is important to know how to modify the sample, and the type of extraction technique (i.e., headspace or immersion) must also be taken into consideration. The goal of this study was to determine the best fiber choice with extraction conditions that would provide the best extraction efficiency for each class of analytes.

There have been numerous papers written on the extraction of semivolatile analytes using SPME. Polyaromatic hydrocarbons (PAHs) have been extracted from both water (3,4,5) and soil (6) samples using several different SPME fibers. SPME has been used for the extraction of nitroaromatic explosives (7,8,9) and aromatic amines (10). Buchholz and Pawliszyn (11) demonstrated that phenols could be extracted by SPME. Other analysts have verified the extraction of phenols from water (12,13) and from other substrates (14,15).

Generally, the term "semivolatile compounds" refers to environmental analytes; however, there are many semivolatile analytes that are analyzed in food products. The extraction of nitrosamines in smoked ham has been accomplished using SPME (16). The extraction of pyrazines (17,18), monoterpenes (19), and some polar flavor analytes such as eugenol, 2,4-dimethylphenol, and 2-phenylethanol (20) in food and beverage products has been demonstrated using SPME.

There are two books (21,22) on SPME that cover the optimization of specific applications in detail. However, this is the first study that focuses specifically on the optimization of the SPME extraction conditions and fiber selection for a variety of semivolatile analytes.

Experimental

Chemicals

The chemicals used as analytes and the organic solvents used to prepare the mixtures (ACS-certified grade) were purchased from Aldrich Chemicals (Milwaukee, WI) with the exception of 1,3,5-trinitrobenzene (TNB) and decachlorobiphenyl (DCBP), which were obtained from Supelco (Bellefonte, PA). Potassium phosphate and sodium chloride salts were obtained from Sigma Chemicals (St. Louis, MO). Solutions used in this study were prepared with deionized water.

Instrumentation

A Varian (Walnut Creek, CA) 3400 gas chromatograph (GC) in

combination with a Varian Saturn II ion trap were used to analyze the samples. The GC injection port contained a low volume liner (0.75-mm i.d.), and a Merlin Microseal (Supelco) was used instead of a septum to seal the inlet. A 0.25-mm PTE-5 capillary column (30 m × 0.25-mm i.d) and a bonded 5% phenyl polydimethylsiloxane (PDMS) phase (Supelco) were used to resolve the components. Data were gathered with the Saturn II software, which was standard with the equipment.

Materials

The SPME fibers (100-µm PDMS, 30-µm PDMS, 7-µm PDMS, 85-µm Polyacrylate (pacrylate) PDMS–Divinylbenzene (DVB) StableFlex (SF), Carbowax (CW)–DVB SF, Carboxen–PDMS SF, DVB–Carboxen–PDMS SF, and bare or uncoated fused-silica) were obtained from Supelco. All of the fiber outer needles used in the study were 23 gauge instead of the normal 24 gauge. All fibers were conditioned according to the manufacturer's specifications prior to the extraction of the samples.

Preparation of standard stock mixtures

The 15 components were combined into 1 mixture at a concentration of 2 mg/mL in methylene chloride (Figure 1). Part of the stock mixture (stock mixture A) was further diluted to a second stock mixture (stock mixture B) with a final concentration of 100 µg/mL (100 ppm) for each analyte. Both mixtures were stored at –4°C in amber vials.

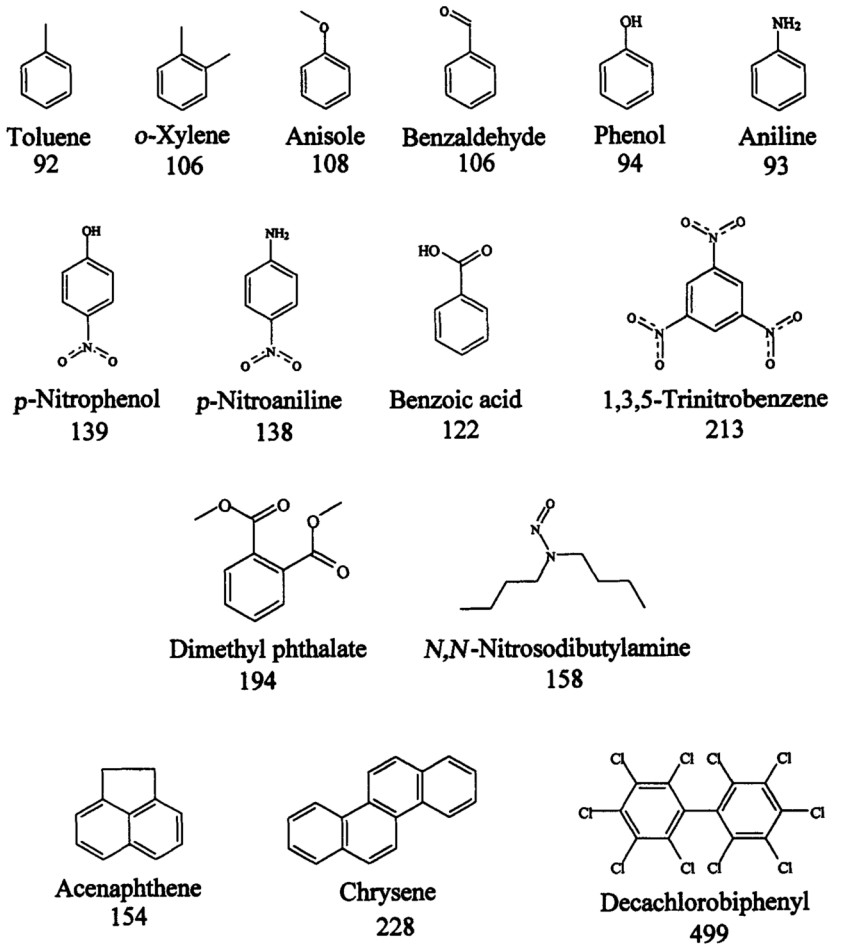

Figure 1. The analyte structures, molecular weights, and names used in this study.

Preparation of buffers and final solutions

One liter of each buffer solution at pH levels of 2, 7, and 11 were prepared at a concentration of 0.05M with various combinations of tribasic, dibasic, and monobasic potassium phosphate salts. To reach a pH of 2, less than 0.5 mL of HCl was required in addition to monopotasium phosphate. The Henderson-Hasselbach equation was used to ensure that the ionic strength was consistent in all of the solutions. In addition to the buffer, 25% ± 0.05% NaCl was added to the buffered water that was used as the sample media for the extraction of the analytes.

Conditions for extraction of samples

Immersion

The samples were prepared by placing 4 mL of the buffer solutions into a nominal 4-mL vial and spiking it with 3 mL of stock mixture B for a sample concentration of 75 ppb for each analyte. Each of the 9 SPME fibers extracted the analytes in the three pH buffers in duplicate.

All of the samples were extracted by immersing the various fibers for 30 min. To enhance extraction efficiency, all of the samples were stirred at a constant rate.

Heated headspace

The samples were also extracted using the heated headspace technique. For these samples, 3 mL of the buffer solutions was placed in a 4-mL vial. The samples were spiked with 2.25 µL of stock mixture B for a final concentration of 75 ppb. The solutions were heated to 60°C for 10 min and then extracted with the fiber for 30 min by inserting the fiber into the vial headspace. All of the samples were stirred at a constant rate.

Desorption and analysis of samples

The samples were desorbed for 3 min into a splitless/split injection port. The desorption temperature varied depending upon the fiber type, as shown in Table I. The oven of the GC was programmed to start at 45°C, hold for 2.5 min, ramp at 10°C/min to 215°C, ramp at 20°C/min to 320°C, and then hold for 7 min. The injection port was first set in the closed splitless mode for the initial 75 s, then opened and split at a 50:1 ratio. The column was inserted directly into the ion source with the transfer line set at 300°C. Helium was used as a carrier gas and maintained at a constant pressure of 15 psi throughout the oven program. This was equivalent to a linear velocity of 25 cm/s at 45°C or 1.5 mL/min.

The ion trap was set to collect ions with a mass-to-charge range of 45–525 at 0.6 s/scan. The ion source was heated at 250°C. Selected ions were used to quantitate the analytes. The ions used for quantitation are listed in Table II.

Determination of response factors

Response factors were determined by making 5 direct injections (0.5 µL) of stock mixture B into the GC using the program conditions previously listed. Under these conditions, approximately 50 ng of each analyte was delivered to the column. Direct injections were also made daily in order to monitor for changes in the response factors. No change greater than 5% was observed throughout the study. The ion counts from the average of 5 injections for each analyte were determined using the quantitating ion. Response factors were determined by dividing the average ion counts for each analyte into the average ion count response for acenapthene (ACE). This resulting quotient was the relative response factor used for each analyte (Table III).

Results and Discussion

Selection of mixture and stability

There were 2 goals in this study. One goal was to determine the effect of analyte polarity and functionality in relationship to fiber polarity and type, and the other goal was to determine the effect of analyte molecular size with respect to the type of fiber coating. The analytes chosen for the study started with a phenyl ring with one substitution group having varied functionalities. There were 6 analytes selected that met this criteria: toluene, anisole, benzaldehyde (BZAL), phenol, aniline, and benzoic acid (BZA). Four additional analytes chosen consisted of a phenyl ring with 2 functional groups or substitutions. These analytes were xylene, *p*-nitrophenol (PNP), *p*-nitroaniline (PNA), and dimethyl phthalate (DMP). One analyte, TNB, contained 3 substitutions on the phenyl ring. *N*,*N*-nitrosodibutylamine (NDBA) provided both polar and nonpolar functionalities and were the only nonaromatic analyte in this study. The remaining nonpolar analytes were 2 PAHs (ACE and chrysene), and the other analyte was DCBP, a polychlorinated biphenyl (PCB). Chrysene and DCBP expanded the molecular weight range of the analytes in the mixtures. All of the analytes were resolved in one chromatographic run using the conditions listed in the Experimental section.

There were some concerns about the stability of these analytes in one mixture. The combination of acids and bases can create certain interactions. The mixture was monitored on a daily basis with direct injections. There was one extraneous peak that increased slightly in size with time. This peak, identified as 3- or 4-nitro-*N*-phenylmethylene-benzenamine, is either an interaction of phenol with PNA or PNP, or TNB interacting with aniline (23). However, there was not a loss of any of the components that was greater than 5% throughout the study.

Overview of fiber types used in this study

The SPME fiber coatings can be classified by polarity, extraction type (absorbent or adsorbent), or size exclusivity. Table I lists the types of fibers and classifies them by polarity and extraction type.

The absorbent-type fibers used in this study consisted of 2 phases, nonpolar PDMS and moderately polar pacrylate. Three different PDMS-coated fibers with coating thicknesses of 100, 30, and 7 µm, along with the 85-µm pacrylate fiber, were evaluated.

Absorbent-type fibers extract by the partitioning of analytes into a liquid-like coating, acting somewhat like a sponge. The analytes migrate freely in and out of the coating. The ability of the coating to retain analytes is dependent primarily on the thickness of the coating and the size of the analyte. The polarity of the fiber coating may enhance the attraction of an analyte to that particular coating, but it is the thickness of the fiber that retains the analytes. There is virtually no competition between analytes (24).

The adsorbent-type fibers contain either DVB, a porous polymer, or Carboxen 1006, a porous carbon molecular sieve, or both. When DVB is suspended in PDMS, the polarity is relatively nonpolar; however, it has been demonstrated that this fiber will extract polar amines (1). When DVB is suspended in CW, the resulting fiber coating is moderately polar. Carboxen is essentially bipolar because the pores are the primary mechanism for extracting and retaining analytes. One evaluated fiber contained 2 layers, DVB–PDMS layered over Carboxen PDMS. Bare fused-

Table I. Fiber Types and Desorption Temperatures

Fiber coating	Desorption temperature	Coating type	Polarity
Bare fused-silica	270°C	adsorbent	unknown
7-µm PDMS	270°C	absorbent	nonpolar
30-µm PDMS	270°C	absorbent	nonpolar
100-µm PDMS	270°C	absorbent	nonpolar
85-µm Pacrylate	290°C	absorbent	polar
PDMS–DVB SF	260°C	adsorbent	bipolar
CW–DVB SF	260°C	adsorbent	polar
DVB–carboxen SF	270°C	adsorbent	bipolar
Carboxen–PDMS SF	310°C	adsorbent	bipolar

Table II. Quantitating Ions for Analytes

Analyte	*m/z*	Analyte	*m/z*
Toluene	91	DMP	163
Xylene	91	ACE	154
Anisole	108	PNP	139
BZAL	105	PNA	138
Aniline	93	TNB	75
Phenol	94	Chrysene	228
BZA	105	DCBP	499
NDBA	159		

Table III. Response Factors for Analytes

Analyte	Response factor*	Analyte	*m/z*
Toluene	0.72	DMP	0.42
Xylene	0.83	ACE	1.00
Anisole	1.13	PNP	3.87
BZAL	2.28	PNA	3.16
Aniline	0.83	TNB	4.64
Phenol	0.87	Chrysene	0.69
BZA	3.93	DCBP	3.16
NDBA	2.53		

* Responses were relative to ACE response.

silica is listed as an adsorbent-type fiber because the surface of the fused-silica interacts with the analytes.

Adsorbent-type fibers extract analytes by physically interacting with the analytes. Adsorbents are generally solids that contain pores or high surface areas. The extraction can be accomplished by trapping the analytes in either the internal or external pores. Internal pores (micro- and mesopores) are ideal for trapping small and midsize analytes and usually retain the analytes until energy is applied. Macropores, primarily on the surface of the material, can also trap larger analytes. Suspension of the adsorbents in a liquid phase can enhance selectivity based on polarity of the phase. The phase bonds the adsorbent to the fiber.

The physical properties of DVB and Carboxen 1006 are described in detail elsewhere (25). Both DVB and Carboxen 1006 have similar surface areas; however, this Carboxen has a higher degree of micropores, and the average diameter of its micropores is less than the average diameter of the micropores in DVB. This makes the Carboxen-coated fiber better for extracting small analytes (< 150 amu), and DVB containing mostly mesopores is better for extracting larger analytes (> 100 amu). It is difficult to release larger analytes from the smaller pores of Carboxen.

To overcome this problem, a DVB–Carboxen dual-coated fiber was created to enable the smaller analytes to migrate through the DVB layer and be retained by the micropores in the inner layer of Carboxen, while the larger analytes are retained mostly in the DVB layer (26).

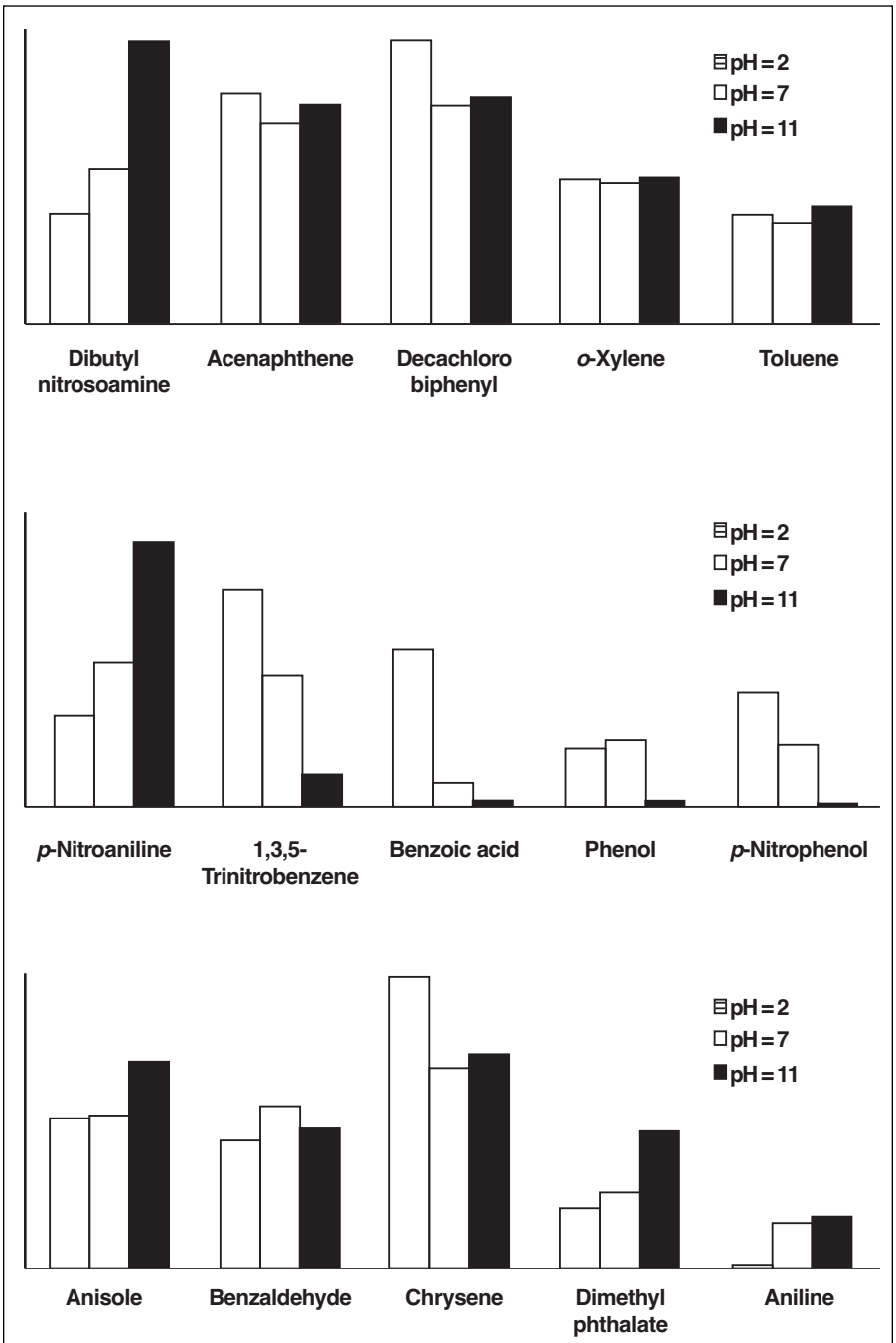

Figure 2. The effects of sample pH on the extraction of analytes. The graphs show average adjusted area responses from all of the SPME fibers at each pH level for each analyte. Analytes were extracted by immersion.

Reasons for response factors

Because the degree of fragmentation varied greatly when the analytes were ionized and only selected ions were used for quantitation, response factors were needed for mass spectrometry discrimination. For example, the response for DCBP in the total ion chromatogram was greater than the response for ACE (the reference analyte), but DCBP was highly fragmented with respect to ACE. As a result, DCBP obtained a response factor of 3.16, as shown in Table III.

Because some of the highly polar analytes had low vapor pressures and were reactive with inlets, columns, and the ion source, their area responses were low. These analytes also required high response factors. Using response factors better represented how much of the analytes, relative to each other, the fibers extracted.

Effects of pH

When comparing fibers, it is best to show results using optimized conditions. It was important to determine the best solution pH for optimum fiber extraction efficiency. In this study, three pH levels were used to determine the effects on extraction efficiency. The three pH levels of 2, 7, and 11 were selected to maximize the pH range and remain within the pH stability range of the fibers. All buffers contained 25% ± 0.05% NaCl to enhance analyte recovery.

The responses for each analyte at the three pH levels were averaged from all of the fibers. The effects of pH were not fiber-dependent. The ratio of responses between pH levels was similar for all of the fibers for a given analyte. Figure 2 shows the comparison of responses for each analyte at the three pH levels. The charts show that pH affects the extraction efficiency of many of

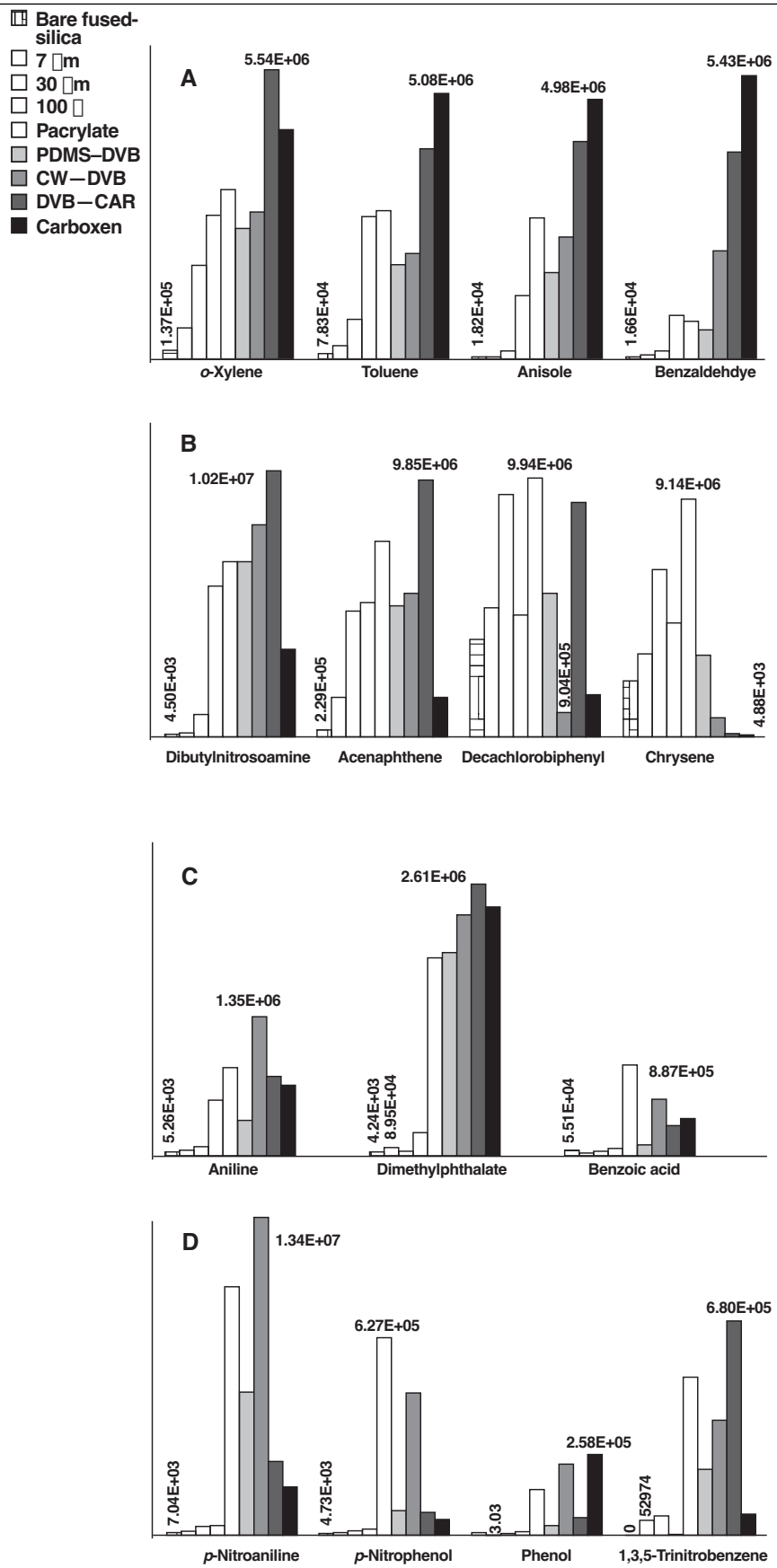

Figure 3. A comparison of adjusted area responses for each analyte extracted by the 9 SPME fibers. The analytes were extracted by immersion from the buffered solutions at the pH that provided optimum extraction efficiency.

the analytes. Polar analytes were affected more greatly by pH than nonpolar analytes. As expected, BZA and PNP were best extracted from a water solution that is acidic and aniline, PNA, and NDBA were best extracted from a basic water solution.

For some analytes, it was not obvious if the pH would affect the extraction efficiency. For example, TNB was best extracted from acidic solutions; it was poorly extracted from basic solutions. In basic solutions, the Meisenheimer reaction that converts nitroaromatics into aromatic nitrosamines probably occurred (27). The low recovery was most likely a result of the instability of the analyte in a basic solution. Anisole appears to be best extracted from basic solutions, but the advantage was not great. It was surprising to see that DMP, a neutral analyte, was also extracted best in basic solutions. There was a definite trend showing that as the pH level increased, the extraction efficiency increased. It would be expected that phenol is extracted best in acidic conditions, but a neutral pH level was slightly better than an acidic pH level. This observation was noted in another study involving the extraction of phenol (28).

The remaining nonpolar analytes (toluene, xylene, BZAL, and ACE) were not affected by the pH of the solution. The results indicated that chrysene and DCBP were best extracted using acidic solutions, but the difference was probably within expected variation between extractions. In both cases, these analytes were better extracted from a basic solution than from a solution with a pH of 7. This indicated that there was probably no pH preference for these analytes because no trend in response from acid to base was observed.

Area responses of analytes

A comparison of the adjusted area responses for each analyte using the 9 SPME fibers is shown in Figure 3. The responses were obtained for each analyte extracted from solutions at the pH level that provided the best extraction efficiency for each particular analyte. The area counts for the fibers that extracted the most and the least of each analyte were listed so that a relative comparison could be made.

The smaller, less polar analytes in this series are shown in Figure 3A. As expected for these smaller analytes, the Carboxen–PDMS fiber was the best choice. However,

compared with the advantage of the Carboxen-coated fiber for the extraction of volatile analytes in a previous study (1), the advantage of the Carboxen for these analytes was much less. The DVB–Carboxen-coated fiber extracted these analytes similarly, and for xylene, it was slightly better than the Carboxen-coated fiber. These analytes were also extracted well by the DVB-containing fibers and the thicker absorbent fibers such as the 100-µm PDMS and the 85-µm pacrylate. The area counts obtained with most of these fibers were within the same order of magnitude as those for the Carboxen–PDMS.

The bare fused-silica and the 7-µm PDMS-coated fibers extracted these analytes poorly in comparison with the other fibers (up to 2 orders of magnitude less). This would be expected because of their small size. It was surprising that the bare fused-silica fiber could extract any of these smaller analytes. This demonstrated that there was probably an interaction between the analytes and the bare fused-silica fiber.

Figure 3B shows a comparison of the fiber for the extraction of larger nonpolar analytes and moderately polar NDBA. The effects of size and shape were greatly noted in this figure. The large planar chrysene molecule was easily extracted by the thinner coated fibers and the bare fused-silica fiber. Because of its planar configuration, the response for chrysene was poor when extracted by the Carboxen-coated fibers. This was probably because of the poor release of this molecule from the Carboxen surface when it was thermally desorbed. The layering of DVB over Carboxen improved the response by a factor of 10 in relation to the Carboxen-coated fiber.

The pacrylate coating (although moderately polar and thick) has a high affinity for aromatic compounds; therefore, it extracted the PAHs and DCBP well. Although DCBP is larger than chrysene, it was extracted better than chrysene with the Carboxen fiber. This indicated that the shape and size of the molecule was important. Apparently, the chlorine groups prevent the biphenyl rings from laying tightly on the surface of the Carboxen particles, which would allow DCBP to be released more efficiently during desorption.

All of the DVB-containing fibers extracted NDBA well (the best performing was the dual-layered fiber). CW–DVB extracted this analyte slightly better than PDMS–DVB because of the increased polarity of NDBA compared with the others in this figure. The advantage of the pacrylate fiber for polar analytes is less with this analyte because it is not aromatic. Pacrylate still extracted this analyte well, but it did not show a large advantage as it did for some of the polar aromatic analytes.

Bare fused-silica, which extracted all of the large nonpolar analytes, had difficulty extracting the more polar NDBA. Also, the precision with bare fused-silica was not favorable. As the fiber aged, it became less absorptive. Apparently, the surface for the fused-silica changed because of exposure to heat or water. Huret (29) reported that the fiber had very limited capacity and was easily overloaded.

Figure 3C shows some of the more polar analytes in the mix. The advantage of polar fibers was obvious. For the more polar BZA and aniline, the 2 polar fibers (pacrylate and CW–DVB) best extracted these analytes. Because of their relatively small size, the Carboxen-containing fiber coatings also extracted these analytes well.

Adsorbent-type fibers extracted the nonpolar DMP much better than absorbent-type fibers. Because of the aromatic ring in DMP, the pacrylate-coated fiber (though a polar absorbent-type fiber) efficiently extracted this analyte.

Figure 3D shows another group of highly polar analytes. The most-polar analytes in this group (PNA and PNP) were best extracted with the polar coated fibers CW–DVB and pacrylate. These fibers also extracted phenol and TNB well. Carboxen–PDMS efficiently extracted phenol, and DVB–Carboxen was good for extracting TNB. The PDMS–DVB coating had a high affinity for nitrogen-based analytes. This affinity also enabled the PDMS–DVB to efficiently extract PNA.

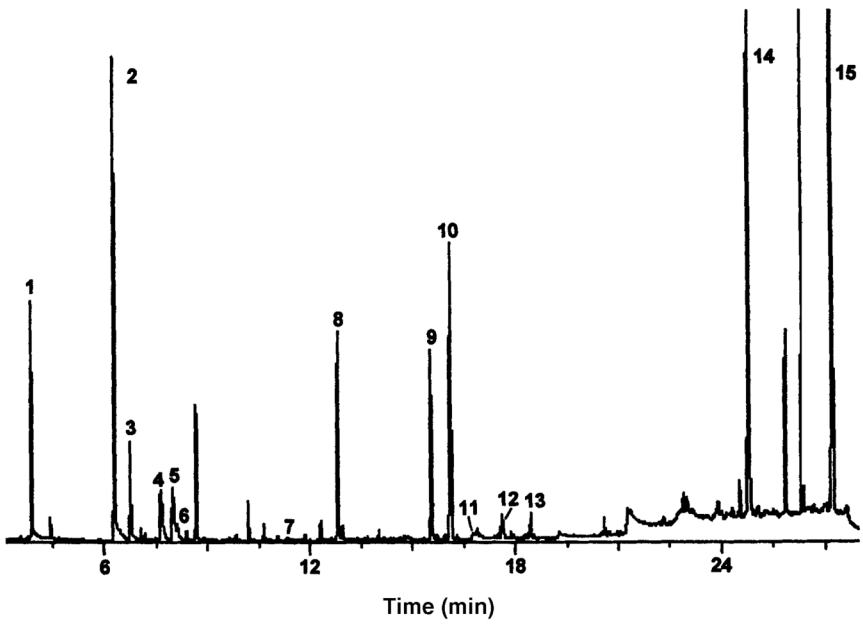

Figure 4. The extraction by immersion and analysis of analytes in stock mixture B at 75 ppb each from water at pH 7 using the pacrylate fiber. The components are as follows: 1, toluene; 2, *o*-xylene; 3, anisole; 4, BZAL; 5, aniline; 6, phenol; 7, BZA; 8, NDBA; 9, DMP; 10, ACE; 11, PNP; 12, PNA; 13, TNB; 14, chrysene; and 15, DCBP.

It appears that the polar fibers had an advantage for the extraction of semivolatile compounds. The additional affinity that pacrylate had for nonpolar aromatic analytes made it a good choice for extracting these analytes. Figure 4 shows a chromatogram of the analytes extracted with the pacrylate fiber from a solution at pH 7. Even though this was not the optimum pH for the extraction of the analytes, they were all shown in the chromatogram.

Effects of fiber polarity

Figure 5 shows the effect of fiber polarity on the recovery of the analytes. The absorbent-type fibers 100-µm PDMS and pacrylate are shown in Figure 5A. The analytes were listed by increasing polarity from left to right. The more-polar pacry-

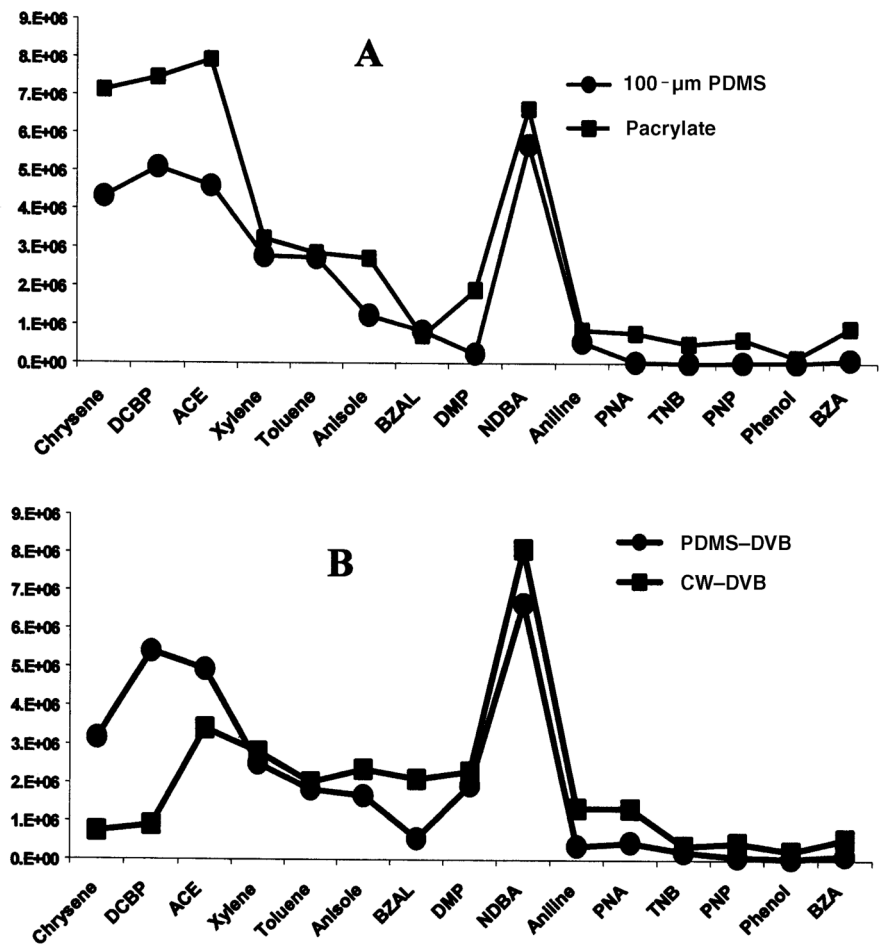

Figure 5. Analyte response using various SPME fibers versus analyte polarity. Polarity of analytes increases from left to right: the plot of responses using absorbent type fibers, the nonpolar 100-µm PDMS-coated fiber, and the polar 85-µm pacrylate-coated fiber (A); the plot of responses using adsorbent type fibers, the nonpolar PDMS–DVB fiber, and the polar Carbowax–DVB fiber (B). All of the analytes were extracted by immersion.

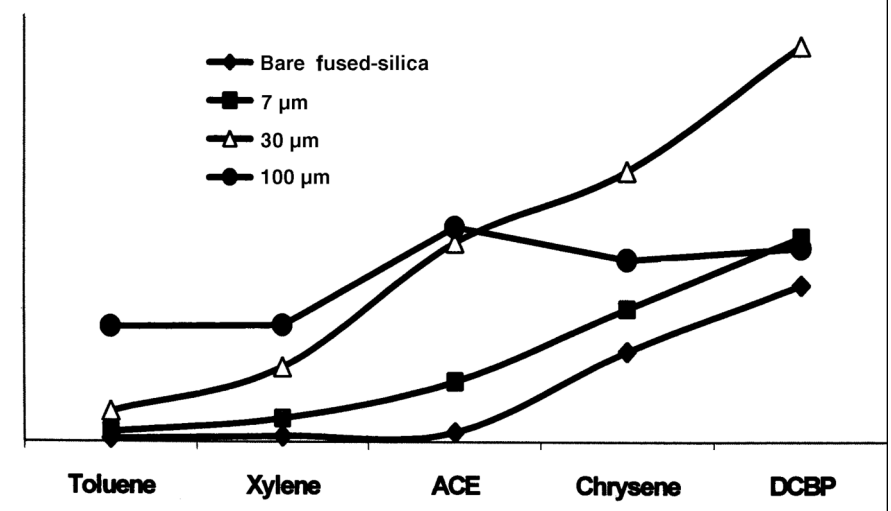

Figure 6. Comparison of analyte response using PDMS-coated fibers and bare fused-silica fiber versus analyte size. Analyte size increases from left to right. The analytes were extracted by immersion.

late-coated fiber extracted the more-polar analytes from 1 to 2 orders of magnitude greater than the 100-µm PDMS fiber. The advantage of using a polar fiber to extract these polar analytes was significant. This is in sharp contrast with the effects of polarity for smaller analytes (< 90 amu). For those analytes, there was no significant advantage in using a polar fiber (1). The graph shows that the pacrylate-coated fiber also extracted the more-nonpolar analytes better than the PDMS fiber because of the affinity of pacrylate for aromatics.

Figure 5B compares the analyte response and polarity obtained by extraction with 2 adsorbent-type fibers. The less-polar PDMS–DVB extracted the less-polar analytes (shown on the left side) more efficiently than the polar CW–DVB fiber. But as the polarity of the analytes increased, the response of the analytes with PDMS–DVB decreased with respect to CW–DVB. The advantage of using the more-polar CW–DVB-coated fiber for the extraction of polar analytes was significant. The improvement in response ranged from 2- to 10-fold.

Unlike the 100-µm PDMS fiber shown in Figure 5A, the PDMS–DVB fiber was capable of extracting the polar analytes at levels that were easily detected. This made the advantage of the CW–DVB fiber over the PDMS–DVB fiber less than the pacrylate fiber over the PDMS fiber. However, when comparing the CW–DVB fiber with the pacrylate-coated fiber for the extraction of polar analytes, the responses were nearly the same.

Effects of analyte size on fiber coating

Figure 6 shows the effects of analyte size and fiber coating thickness using 3 PDMS fibers and bare fused-silica. The molecular weights increase from left to right. The smaller analytes were extracted best with the thicker coating, as expected. The results show that the response was directly proportional to the thickness of the fiber coating. The 100-µm PDMS fiber extracted these analytes very well with respect to the others. However, the advantage of the thick film diminished as the size of the analytes increased. The larger analytes such as chrysene and DCBP did not migrate quickly into the thick phase coating. For the response to increase, the extraction time would need to increase.

The bare fused-silica fiber and the 7-µm PDMS-coated fiber did not extract the

smaller analytes as well as expected. It should be noted that the bare fused-silica line in the graph and the 7-µm PDMS line ran parallel with each other. The parallel lines indicated that the mechanism for retaining the analytes on the bare fused-silica fiber might be the same as the mechanism for the 7-µm PDMS fiber. One might conclude that the 7-µm PDMS fiber extracted by both adsorbing and absorbing the analytes. The thin coating of the 7-µm fiber did not impede the analytes from interacting with the fused-silica core, but the coating appeared to help retain more of the analytes.

The 30-µm PDMS-coated fiber was an excellent choice for extracting a wide molecular weight range of nonpolar analytes. This coating thickness allowed a sufficient amount of the lower molecular weight analytes to be extracted while efficiently extracting large molecular weight analytes such as DCBP in a reasonable amount of time. Unlike the 100-µm PDMS fiber, the 30-µm PDMS fiber was able to extract more of each analyte as the molecular weights and distribution constants increased.

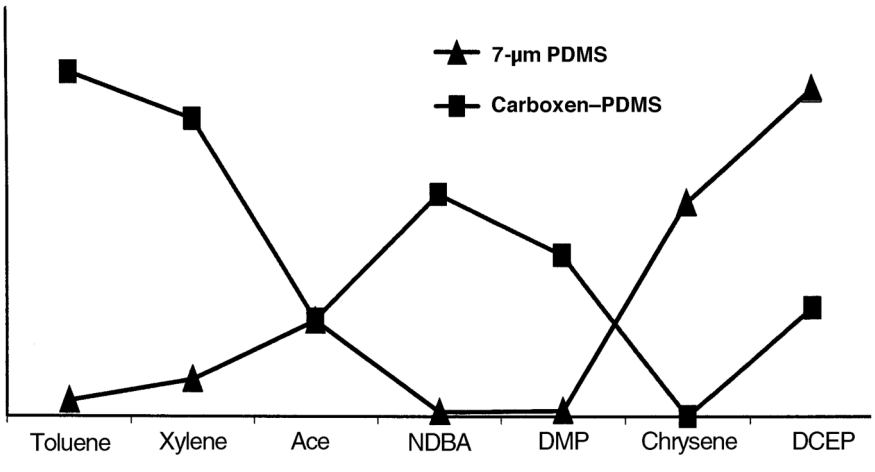

Figure 7. Comparison of analyte response using Carboxen–PDMS and 7-µm PDMS-coated fibers versus analyte size. Analyte size increases from left to right. The analytes were extracted by immersion.

Figure 7 shows the analyte response relative to its molecular weight with the Carboxen–PDMS fiber and the 7-µm PDMS fiber. These fibers were compared because the Carboxen–PDMS fiber was the strongest fiber, whereas the 7-µm PDMS was the weakest coated fiber in terms of retention of analytes.

For the Carboxen-coated fiber, the amount of analyte extracted or detected decreased as the molecular weight increased. If one ignores the PAHs that were not efficiently desorbed from the fiber, there was a fairly linear decrease in response as the size of the analytes increased.

Conversely, for the 7-µm PDMS fiber, the opposite was true. The amount of analyte detected increased as the size of the analytes increased. If one overlooks the more-polar analytes DMP and NDBA that are not well extracted by this fiber, the increase in response was proportional to the molecular weight of the analyte.

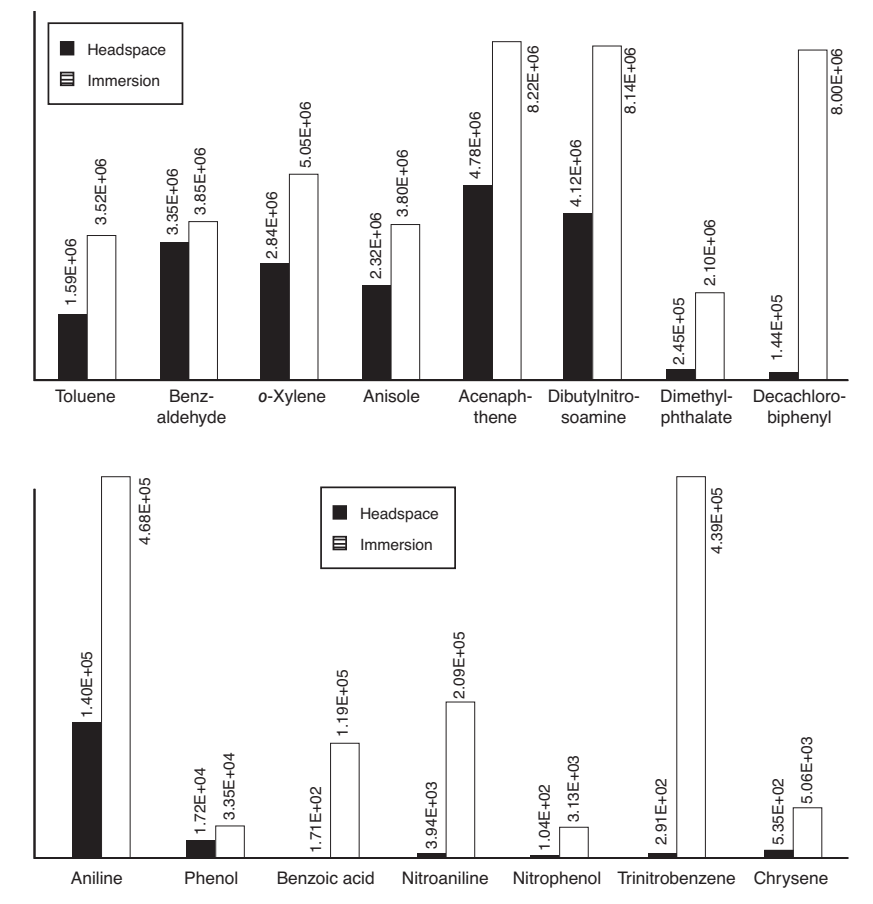

Figure 8. Comparison of SPME extraction techniques using the DVB–Carboxen–PDMS fiber. The graphs compare the area responses for each analyte obtained by both extraction techniques described in the Experimental section.

Comparison of extraction techniques

SPME enables the analyst to extract samples from water by either immersing the fiber in the water or placing the fiber in the headspace above the water. The choice of extraction technique often depends on the sample media and the analytes. The extraction of volatile analytes can be accomplished by either technique; however, the extraction of semivolatile analytes becomes more complex because of the varying vapor pressures of the analytes.

For the evaluation of the analytes in this study, the comparison of the headspace mode versus the immersion mode was made with one SPME fiber. Heated headspace at 65°C was selected to help volatilize

some of the analytes in the mix. The results of the comparison are shown in Figure 8.

The results show that for these analytes, immersion was superior to heated headspace. For the smaller nonpolar analytes, the headspace responses were 40–85% of the responses for immersion. It was anticipated that analyte responses for headspace and immersion extractions would be similar. The lower responses could be because of the fact that too much heat was applied to the sample. This might cause some of the more volatile analytes to be desorbed off the fiber during extraction.

For the larger nonpolar analytes such as chrysene and DCBP, the headspace response was better than originally anticipated. These analytes have boiling points in excess of 300°C, but with the sample at only 65°C, these analytes could be extracted. The response for heated headspace was approximately 3–10% of the respective responses by immersion. Llamport et al.(30) have demonstrated that PCBs could be extracted using heated headspace SPME. Other relatively nonpolar analytes such as DMP, NDBA, and ACE yielded responses for heated headspace that were approximately 50% of the response for immersing the fiber.

For the polar analytes, the advantage of immersion was more pronounced. Many of these analytes had poor vapor pressures and were soluble in water. For the smaller polar analytes such as phenol and aniline, the vapor pressures were sufficiently high enough that the response by heated headspace was 25–50% of the response obtained by immersion of the fiber. The more polar analytes were more soluble and generally had lower vapor pressures. The heated headspace responses ranged from 0.6% to 3% of the response for immersion. In some cases, the solubility in water and not the vapor pressure of the analyte was the cause for poor recovery. Jenkins and co-workers at the Army Cold Region Lab (31,32) showed that nitroaromatic explosives in the parts-per-trillion range in soils could be extracted using headspace SPME. However, the recovery of the analytes decreased when the soil was wet.

Even though the response for some of the analytes was poor using heated headspace, all of the analytes at a concentration of 75 ppb could be detected. For high-level screening of semivolatile analytes from a variety of substrates, it appeared that SPME would be a suitable extraction method.

Conclusion

The variety of semivolatile analytes made the process of optimizing the extraction conditions difficult. Understanding the variables (such as sample preparation, physical characteristics of the analytes, extraction mechanisms, and chemistry of the fibers) made the task less difficult.

The pH of the sample should be considered and adjusted when necessary for optimum extraction efficiency. The results indicated that acidic analytes were best extracted from acidified water samples and basic analytes were best extracted from basic solutions. However, the extraction of some analytes that were not obviously basic or acidic were also affected by different pH levels. The stability of an analyte at various pH levels must also be monitored to assure proper and reproducible extractions.

When looking at the physical characteristics of the analytes being extracted, two things should be considered prior to the selection of an SPME fiber: (a) the polarity and functionality and (b) the molecular weight and shape of the analyte. For semivolatile analytes, polarity with respect to fiber type was critical. The more-polar analytes were best extracted by polar fibers such as pacrylate and CW–DVB. The less-polar analytes were extracted by both polar and nonpolar fibers and in some cases were extracted better with the pacrylate fiber. The functional group that increases polarity was also important. Amines were well extracted by the PDMS–DVB fiber, whereas CW–DVB and pacrylate were better for other polar functional groups.

The size and shape of the analytes were also important. Smaller analytes (< 200 amu) were more efficiently extracted by adsorbent-type fibers. The pores offered the advantage of better retaining the analytes compared with absorbent-type fibers. Larger analytes (> 200 amu) or highly planar analytes such as PAHs were better extracted by absorbent-type fibers than adsorbent fibers. Layering the DVB over Carboxen expanded the molecular weight range for improved recovery when compared with the Carboxen fiber; however, large PAHs still were not efficiently released during desorption. The bare fused-silica fiber was capable of extracting nonpolar analytes but not reproducibly, and it had limited capacity.

When comparing extraction techniques, immersion of the fiber in the water sample was superior to heated headspace sampling. However, the nonpolar analytes (even those with high molecular weights and boiling points) could be extracted using heated headspace. The recovery using heated headspace was 3–85% of the recovery obtained by immersion. The recovery of the polar semivolatile analytes using heated headspace was 0.6–50% that of the recovery by immersion.

References

1. R.E. Shirey. Optimization of extraction conditions for low-molecular-weight analytes using solid-phase microextraction. *J. Chromatogr. Sci.* **38:** 109–116 (2000).
2. R.-K. Smith. *Handbook of Environmental Analysis.* Genium, Schenectady, New York, NY, 1993, p 86.
3. D. Potter and J. Pawliszyn. Rapid determination of polyaromatic hydrocarbons and polychlorinated biphenyls in water using solid-phase microextraction. *Environ. Sci. Technol.* **28**: 298–302 (1994).
4. R.E. Shirey, V. Mani, and J. Savrock. Solid-phase microextraction—a solventless sample preparation method for organic compounds in water. *Am. Environ. Lab.* **April:** 43–45 (1994).
5. Z. Zhang and J. Pawliszyn. Headspace solid-phase microextraction. *Anal. Chem.* **64:** 1960–66 (1993).
6. Z. Zhang and J. Pawliszyn. Analysis of organic compounds in environmental samples using headspace solid-phase microextraction. *J. High Res. Chromatogr.* **16:** 689–92 (1993).
7. J. Hong and S. Huang. Determination of semi-volatile compounds nitrobenzene, isophorone, 2,4-dinitrotoluene and 2,6-dinitrotoluene in water using SPME with polydimethylsiloxane-coated fibre. *J. Chromatogr. A* **678:** 313–16 (1994).
8. M. Durrach, A. Chujitan, and G. Plett. Trace explosives signatures from World War II unexploded undersea ordinance. *Environ. Sci. Technol.* **32:** 1354–58 (1998).
9. S. Barshick and W. Griest. Trace analysis of explosives in seawater

using SPME and GC ion trap mass spectrometry. *Anal. Chem.* **70:** 3015–20 (1998).
10. L. Muller, E. Fattore, and E. Benfenati. Determination of aromatic amines by SPME GC–MS in water samples. *J. Chromatogr. A* **791:** 221–30 (1998).
11. K. Buchholz and J. Pawliszyn. Determination of phenols by SPME and GC analysis. *Environ. Sci. Technol.* **27:** 2844–48 (1993).
12. P. Bartak and L. Cap. Determination of phenols by solid-phase microextraction. *J. Chromatogr. A* **767:** 171–75 (1997).
13. B. Schafer and W. Engewald. Enrichment of nitrophenols from water by means of SPME. *Fresenius J. Anal. Chem.* **352:** 535–36 (1995).
14. L. Conte, R. Botolomeazzi, S. Moret, A. Sibaini, and G. Marcazzan. Application of SPME in determination of phenol in honey. *Riv. Sci. Aliment.* **26(314):** 97–102 (1997).
15. T.J. Clark and J. Bunch. Quantitative determination of phenols in mainstream smoke with SPME–GC selected ion-monitoring MS. *J. Chromatogr. Sci.* **34:** 272–75 (1996).
16. N. Sen, S. Seaman, and B. Page. Rapid semi-quantitative estimation of *N*-nitrosodibutylamine and *N*-nitrosodibenzylamine in smoked hams by SPME followed by GC thermal energy analysis. *J. Chromatogr. A* **788:** 131–40 (1997).
17. E. Ibanez and R. Bernard. SPME of pyrazines in model reaction systems. *J. Sci. Food Agric.* **72:** 91–95 (1996).
18. W.M. Coleman, III. A study of the behavior of polar and nonpolar solid-phase microextraction fibres for the extraction of Malliard reaction products. *J. Chromatogr. Sci.* **35:** 245–58 (1997).
19. W.M. Coleman, III and B.M. Lawrence. Examination of the enantiomeric distribution of certain monoterpene hydrocarbons in selected essential oils by automated solid-phase microextraction–chiral gas chromatography–mass selective detection. *J. Chromatogr. Sci.* **38:** 95–99 (2000).
20. J. Ali. Solid phase microextraction for quantitative analysis in nonequilibrium situations. *Anal. Chem.* **69:** 1230–36 (1997).
21. *Applications of Solid Phase Microextraction*. J. Pawliszyn, Ed. Royal Society of Chemistry, Cambridge, U.K., 1999, pp 1–645.
22. *Solid Phase Microextraction—A Practical Guide*. S.S. Wercinski, Ed. Marcel Dekker, New York, NY, 1999, pp 1–241.
23. Jerry March. *Advance Organic Chemistry, Reactions, Mechanisms and Structures*, 3rd ed. Wiley-Interscience, New York, NY, 1985, pp 577–78.
24. A. Saraullo, P.A. Martos, and J. Pawliszyn. Water analysis by solid-phase microextraction based on physical chemical properties of the coating. *Anal. Chem.* **69:** 1992–98 (1997).
25. R.E. Shirey. *Solid Phase Microextraction—A Practical Guide*. S.S. Wercinski, Ed., Marcel Dekker, New York, NY, 1999, pp 83–94.
26. R.E. Shirey, V. Mani, and R.F. Mindrup. Dual-layered solid-phase microextraction (SPME) fibers to expand analyte range and detection limits. Pittsburgh Conference Presentation. Supelco publication T499038, 1999, pp 1–14.
27. T.F. Jenkins and M.E. Walsh. Development of field screening methods for TNT, 2,4-DNT and RDX in soil. *Talanta* **39:** 419–28 (1992).
28. K. Buchholz and J. Pawliszyn. Optimization of solid phase microextraction (SPME) conditions for phenol analysis. *Anal. Chem.* **66:** 160–67 (1994).
29. T.M. Huret, Eastman Chemical, personal communication, 1999.
30. M. Llompart, M. Fingas, and K. Li. SPME and headspace SPME for the determination of PCBs in water samples. *Anal. Chem.* **70:** 2510–15 (1998).
31. T.F. Jenkins, D.C. Leggett, P.H. Miyares, M.E. Walsh, T.A. Ranney, and V. George. Chemical signatures on TNT-filled land mines. *Talanta,* in press.
32. T.F. Jenkins, D.C. Leggett, and T.A. Ranney. Vapor signatures from military explosives: part 1. Vapor transport from buried military-grade TNT. *CRREL Special Report,* pp 99–121.

Manuscript accepted May 17, 2000.