

A Study of the Behavior of Maillard Reaction Products Analyzed by Solid-Phase Microextraction–Gas Chromatography–Mass Selective Detection

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Abstract

Aqueous solutions and headspace above volatile and semivolatile compounds associated with the Maillard reaction and sugar thermal degradation are analyzed using solid-phase microextraction. The structure of the model compounds is shown to have a dramatic effect on the rate, equilibration time, and amount of compound adsorbed. Alkyl and alkoxy substitution on the ring of pyrazines, pyridines, thiazoles, and furfurals result in increases in the rates and amounts of compound adsorbed. The presence of multiple components in the aqueous solution results in a significant competition for adsorption onto the solid-phase microextraction fiber. Increases in the amount and rate of adsorption were found upon addition of NaCl.

Introduction

Solid-phase microextraction (SPME) was first developed and applied by Pawliszyn and co-workers (1,2) early in this decade at the University of Waterloo. The SPME technology involves the extraction of organic compounds from aqueous or gaseous samples onto/into a solid phase coating of a silica fiber. When a fresh SPME fiber is immersed in an aqueous sample, the organic compounds dissolved in the water begin to partition between the aqueous phase and the adsorbent phase coated on the SPME fiber. The adsorbed components can then be liberated from the adsorbent by inserting the exposed fiber now containing the adsorbed species into an elevated temperature zone such as a heated gas chromatograph injection port. Some of the parameters that have been shown to influence the efficiency of adsorption include fiber type, liquid and headspace volumes, agitation, temperature, ionic strength, exposure time, analyte type, and analyte concentration.

The SPME technique has been shown to be an inexpensive, solvent free, relatively rapid method for the extraction of organic compounds from water (3–8). Applications of this technique include the analysis of explosives, juice beverages, ground coffee, butter flavors, phenols, and BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds. Additional

applications have addressed the use of SPME in the analysis of the headspace above aqueous solutions containing dissolved organic compounds (3,9,10). The origin of aroma and flavor for many roasted products can be ascribed to the Maillard reaction sequences and to the thermal degradation of sugars (11–15). Volatile components are released into the solution and headspace surrounding the reaction as a result of these processes. These series of reactions are responsible for the production of volatile and semivolatile pyrazines, thiazoles, furans, aldehydes, and pyridines. Most of these compounds have meaningful sensory attributes. A significant portion of these relatively low molecular weight products also have appreciable solubility in water. A number of compounds associated with the Maillard reaction products have been analyzed using dynamic headspace analyses and solvent extractions (11–18). However, no report has appeared using SPME as an analytical approach for the determination of Maillard reaction type products in an aqueous matrix. This report describes the behavior of a poly(dimethylsiloxane) SPME fiber as a tool for the analysis of both aqueous solutions and the headspace above aqueous solutions of thiazoles, pyrazines, pyridines, and furfurals. The effects of exposure time, ionic strength, and analyte type will be discussed. Other critical variables were held constant.

Experimental

Standard solutions

All organic compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received. Standard aqueous solutions, at approximately 50.0 ng/ μ L, were prepared gravimetrically using deionized water. Calibration curves for each compound were generated by plotting the area counts from the total ion chromatograms of the compound(s) of interest versus concentration. Gravimetric standards in methylene chloride (Burdick and Jackson; Muskegon, MI) were used to create the calibration curve. In this manner, the amount of analyte adsorbed onto the SPME fiber could be determined.

Instrumental analysis

A Hewlett-Packard 5890 Series II Plus gas chromatograph (GC) fitted with a Hewlett-Packard 5972 mass selective detector (MSD) was used. The temperature of the injection port was 250°C. A 30-m × 0.25-mm i.d. (0.25- μ m film thickness) DB-

1701 column was used (J&W Scientific; Folsom, CA). The linear velocity was set at 40 mL/min. The GC oven was programmed from 70°C to 120°C at 5°C/min. Purified helium was the carrier gas. Splitless injections were used, and the split valve was opened after 2 min. For thermal desorption of the exposed

SPME fiber, the manual injection time was held constant at 0.2 min for all desorption procedures. No carryover was observed from exposure to exposure; that is, upon reinjection of a freshly conditioned fiber under these conditions, the amount of adsorbed analyte was below the detection limit. The detection limit for each of the analytes studied was in the range of 1–2 ng.

The MSD was operated in the scan mode and electron impact (70V) generated ions between m/z 33 and 250 were collected. The GC-MSD interface was set at 250°C. Positive identification of compounds was based on a comparison of GC retention times and mass spectra of authentic compounds.

General SPME procedures

The manual SPME device (part #5-7330) and poly(dimethylsiloxane) fiber (100 μ m, part #5-7300) were obtained from Supelco (Bellefonte, PA). For both liquid and headspace sampling, 10 mL of the aqueous solution containing the analyte(s) of interest at approximately 50 ng/ μ L (ppm) was placed in a 20-mL vial with a small magnetic stirring bar. The vial was immediately capped with a crimp top septum/aluminum combination. The dimensions of the septum-sealed cylindrical vial was approximately 2 cm in diameter by 6.5 cm in height. Once the solution was placed in the sealed vial, it was allowed to stand undisturbed overnight before sampling. When both liquid and headspace sampling occurred, the solution was stirred at a rate such that the vortex was approximately 1 cm deep. For liquid sampling, the entire fiber, which was fresh from thermal desorption and at room temperature, was submerged in the stirring solution for a selected time (0.5, 1.0, 2.5, 5.0, or 10 min). For headspace sampling, the fiber, which was fresh from thermal desorption, was consistently suspended 0.5 cm above the stirred liquid surface in the center of the vial and exposed for selected times. Fresh samples were used for each measurement. Upon completion of exposure, the compound-laden fiber was rapidly transferred to the GC, and a manual injection was made. All experiments were conducted at room temperature, and the same SPME fiber was used for the duration of the study. A fresh SPME fiber was

Table I. TIC Responses for Substituted Pyrazines at 50 ng/ μ L

Injection	Pyrazine	2,3-Dimethyl pyrazine	2,3,5-Trimethyl pyrazine
<i>10-min exposure</i>			
1	14,358,359	36,564,519	103,132,469
2	13,957,944	35,990,073	104,010,782
3	13,506,311	33,976,476	97,723,343
4	14,596,741	36,200,513	103,353,591
5	14,614,736	35,607,931	102,403,406
Average	14,206,818.20	35,667,902.40	102,124,718.20
SDT*	472,771.10	1,006,964.39	2,526,494.47
%RSD	3.33	2.82	2.47
<i>5-min exposure</i>			
1	13,533,996	33,496,282	92,900,615
2	14,263,104	35,632,358	95,206,588
3	13,687,488	33,778,356	95,066,083
4	13,052,312	32,530,566	92,128,409
5	14,883,878	35,380,946	98,288,992
Average	13,884,155.60	34,163,701.60	94,718,137.40
SDT	706,452.48	1,313,364.72	2,404,201.20
%RSD	5.09	3.84	2.54
<i>2.5-min exposure</i>			
1	14,561,891	32,286,302	83,844,494
2	14,965,165	32,508,484	84,784,796
3	13,542,636	29,271,074	76,958,793
4	16,409,423	32,169,639	80,817,448
5	13,947,428	30,536,427	80,861,052
Average	14,685,308.60	31,354,385.20	81,453,316.60
SDT	1,108,685.47	1,403,058.52	3,072,960.01
%RSD	7.55	4.47	3.77
<i>1-min exposure</i>			
1	9,882,619	19,792,988	47,758,169
2	9,383,519	18,583,953	44,436,012
3	10,546,387	19,353,869	46,661,823
4	10,456,960	20,978,765	52,014,198
5	9,498,553	18,926,120	44,918,751
Average	9,953,607.60	19,527,139.00	47,157,790.60
SDT	534,290.68	929,884.39	3,025,413.53
%RSD	5.37	4.76	6.42
<i>0.5-min exposure</i>			
1	8,555,329	16,369,516	39,972,910
2	8,753,508	17,135,308	41,591,500
3	8,633,394	16,273,600	39,893,488
4	8,515,097	16,706,169	40,124,157
5	8,566,014	16,677,384	40,978,641
Average	8,604,688.40	16,632,395.40	40,512,139.20
SDT	93,447.79	338,462.45	742,743.44
%RSD	1.09	2.03	1.83

*SDT = Standard deviation.

generated by exposing the fiber for 1 min to a GC injection port set at 250°C.

Five replicate manual injections were made for each sample to obtain the precision of the technique. The data in Table I shows that the precision was acceptable, with an average percent relative standard deviation of less than 5%. This value is generally acceptable with trace organic analyses and in concert with previous published findings (4).

Salt-containing solutions were made with reagent-grade NaCl.

Results and Discussion

SPME headspace and liquid sampling were examined for 16 aroma and flavor compounds closely associated with the Maillard reaction chemistries and sugar thermal degradation processes (Table II). The amount of each compound adsorbed by the poly(dimethylsiloxane) SPME fiber (in nanograms) from a relatively dilute aqueous solution (50 ppm each component) is listed. The values shown in Table II were taken from the 5-min exposure time experiment.

These aroma and flavor compounds rarely exist as single components in heat-treated food products due to the nature of the products from the Maillard reaction chemistries, the sugar thermal degradation processes, and the naturally occurring presence of inorganic anions and cations. The Maillard and sugar thermal degradation reactions yield, among others, varying types of low molecular weight aldehydes, substituted pyrazines, and substituted pyridines. Thus, the adsorbed values listed in Table II were determined from solutions containing all of the compounds in a specific class; for example, the values for the pyrazines were obtained from an aqueous solution containing all of the pyrazines. The behavior of the SPME extraction of aqueous solutions containing only one component will be addressed later.

SPME using the poly(dimethylsiloxane) fiber exhibits dramatic selectivity (Table II, Figure 1). Note that the amount of compound adsorbed from an aqueous sample increased with the degree of alkyl substitution even though the concentration of all compounds was the same (50 ppm). For example, the amount of 2,3,5-trimethyl-pyrazine adsorbed at 5 min was 130.04 ng, and the amount of methylpyrazine adsorbed at the equivalent time was much less (22.99 ng). Likewise, the amount of pyridine adsorbed, 55.98 ng, was approximately a factor of 10 less than that found for 5-ethyl-2-methylpyridine, 717.49 ng. This adsorption profile was characteristic for all of the compound classes examined. In other words, as the alkyl substitution increased, more of the compound was adsorbed. This phenomenon was the same for both the headspace and liquid sampling procedures. It was significant to

note that, in a number of cases, the amount of material adsorbed seemed to be similar in the liquid and headspace procedures; for example, 135 ng of 2-methoxy-pyrazine was adsorbed at 5 min in both the headspace and liquid samples.

It was noteworthy to find that the presence of relatively polar alkoxy substituents such as methoxy and ethoxy actually did not hinder the adsorption characteristics of the molecule relative to other alkyl substituents. This was clearly evident in the behavior of 2-methoxypyridine and 2-ethoxythiazole (Table II). In a somewhat similar fashion, the presence of the

Table II. Nanograms Adsorbed for Liquid and Headspace SPME Sampling*

Compound type	Nanograms adsorbed	
	Liquid	Headspace
<i>Pyrazines</i>		
2-Methylpyrazine	22.99	21.16
2,3-Dimethylpyrazine	59.21	36.02
2,3,5-Trimethylpyrazine	130.04	58.05
2-Methoxypyrazine	135.04	135.93
2-Methoxy-3-methylpyrazine	352.97	359.85
2-Acetylpyrazine	18.29	15.01
<i>Pyridines</i>		
Pyridine	55.98	101.56
4-Methylpyridine	147.49	179.55
4-Ethylpyridine	408.93	359.96
5-Ethyl-2-Methylpyridine	717.49	547.84
<i>Furans</i>		
Furfural	21.09	13.23
5-Methylfurfural	44.22	35.45
<i>Thiazoles</i>		
4-Methylthiazole	64.90	90.19
4,5-Dimethylthiazole	200.27	230.87
2-Ethoxythiazole	355.45	451.38
2,4,5-Trimethylthiazole	575.97	504.97

* 5-min exposure.

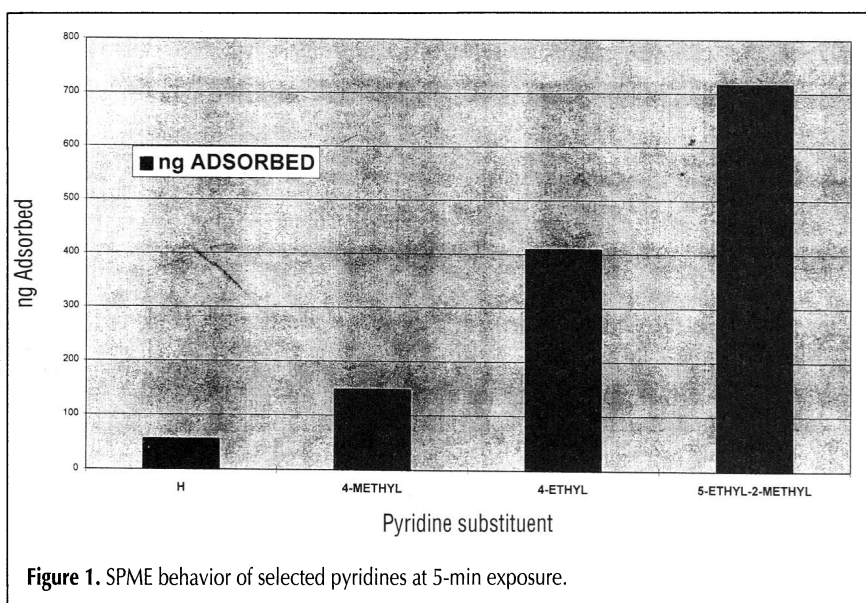


Figure 1. SPME behavior of selected pyridines at 5-min exposure.

2-methoxy substituent increased the adsorption characteristics of an alkyl substituted pyrazine (i.e., 2-methoxy-3-methylpyrazine versus 2-methylpyrazine). In contrast, the presence of a carbonyl group in 2-acetylpyrazine, furfural, and 5-methylfurfural precipitated a dramatic drop in adsorptive characteristics. The adsorption characteristics of these three compounds were found to be the poorest of the 16 examined. The addition of an alkyl substituent on a relatively

polar molecule like furfural significantly increased its adsorption characteristics (furfural, 21.09 ng; 5-methylfurfural, 44.22 ng).

It would seem that the differences in adsorption characteristics of these compounds can be related in a large part to differences in the affinity of the specific compound for the relatively nonpolar poly(dimethylsiloxane) phase. This increasing affinity as a function of alkyl substituents has been demonstrated in studies with the BTEX series of compounds. For example, it was shown, under a well defined SPME headspace extraction study, that the amount of ethylbenzene extracted by a poly(dimethylsiloxane) fiber was approximately 10 times that of benzene even though their concentrations in the aqueous solution were the same (9). This affinity is due, in a large part, to the non-polar nature of the poly(dimethylsiloxane) phase (8); in other words, "like dissolves like". This observation, relative to the characteristics of the more polar alkoxy substituent on substituted pyrazines (Table II), does not seem to follow this trend.

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Effect of salinity

The "salting out" effect is widely used to increase the effectiveness of an organic solvent to extract organic compounds dissolved in water. It has been clearly demonstrated (3,4) that NaCl enhances the performance of SPME fibers in the extraction of flavor and phenol compounds. A similar trend was found in the examination of these Maillard reaction products (Table III). Note that the amount of 2-methylpyrazine adsorbed from the NaCl-containing aqueous solution was 35.52 ng and that the amount captured from the deionized aqueous solution at identical starting concentrations was much lower at 22.99 ng. A similar pattern was found with the headspace experiments (Table III). Increases in the effectiveness of the SPME fiber due to the presence of NaCl was observed for all compounds examined.

Adsorption rate studies

In order to demonstrate the time required for all analytes to equilibrate with the fiber, the fiber was exposed to fresh solutions or headspaces for selected times. The selected times that are chosen should be long enough for a constant amount of analyte to be removed from the solution or headspace. For aqueous samples agitated by sonication, this time can be less than or equal to 60 s (2). For samples agitated by magnetic stirring, the equilibrium time is established by diffusion from a thin layer to the fiber and can be

Table III. TIC Nanograms Adsorbed for Liquid and Headspace SPME Sampling*

Compound type	No salt		Salt	
	Liquid	Headspace	Liquid	Headspace
2-Methylpyrazine	22.99	21.16	35.52	41.18
2,3-Dimethylpyrazine	59.21	36.02	81.29	75.48
2,3,5-Trimethylpyrazine	130.04	58.05	291.99	166.64

* 5-min exposure.

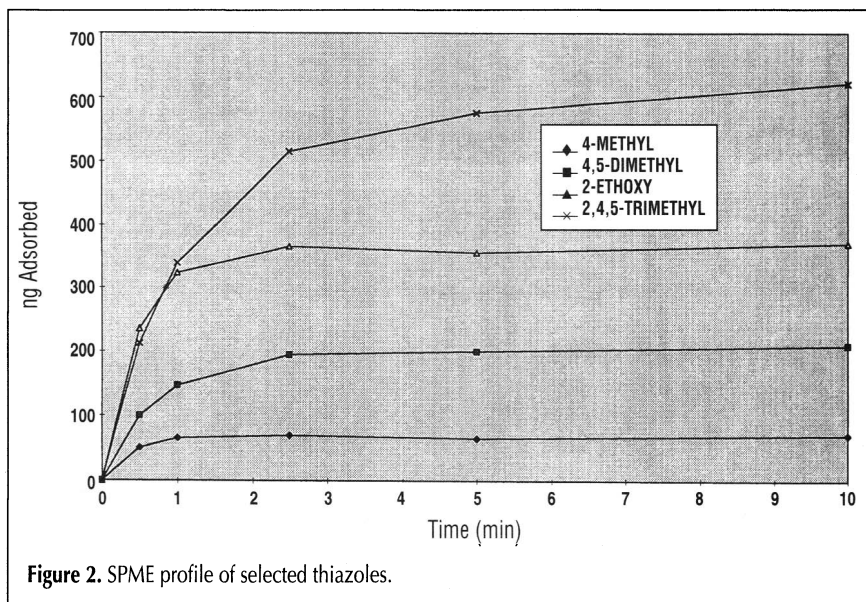


Figure 2. SPME profile of selected thiazoles.

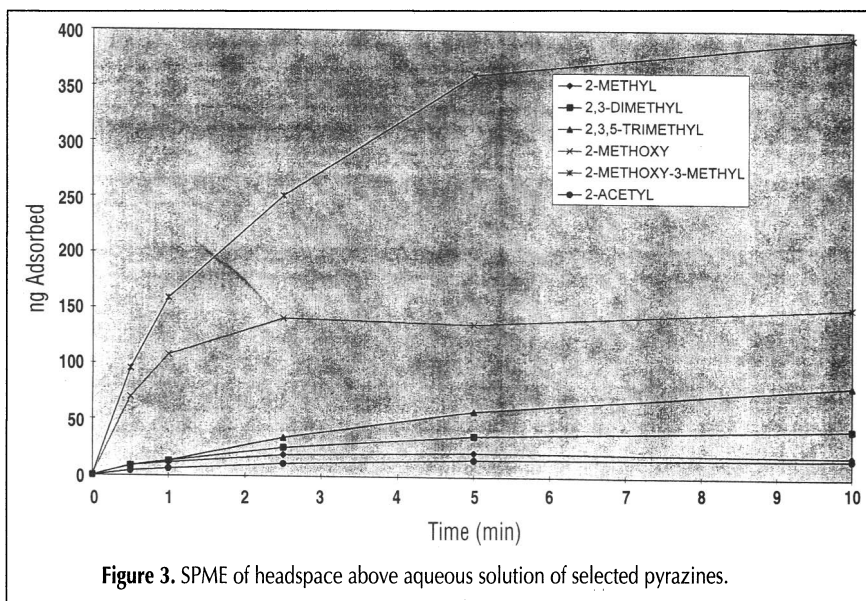


Figure 3. SPME of headspace above aqueous solution of selected pyrazines.

much longer than 60 s. For example, in an SPME study of the BTEX compounds that used a poly(dimethylsiloxane) fiber, it was found that the required equilibrium time of benzene was very short (approximately 30 s), compared with the time required for *o*-xylene (400 s). Studies with these Mailard and sugar thermal degradation reaction products have shown that the equilibrium time is sensitive to the number and/or type of substituents attached to the ring. For example, an adsorption–time profile for a series of thiazoles dissolved in an aqueous solution at approximately 50 ppm illustrates this point (Figure 2). Within 1 min, equilibrium was established for 4-methylthiazole. In contrast, at least 2.5 min was required before the 4,5-dimethyl and 2-ethoxy derivatives reached equilibrium. Figure 2 also shows that even after 10-min exposure, equilibrium had yet to be established for the 2,4,5-trimethylthiazole. The examination of the aqueous solutions of the other compounds (Table II) confirmed this trend. In other words, the time required to establish equilibrium between the fiber and the compounds dissolved in the aqueous solution was sensitive to the structure of the compounds.

The dramatic effect on equilibrium time due to structure was demonstrated in SPME headspace experiments above the aqueous solutions as well. This is illustrated in Figure 3 for a series of alkyl and alkoxy substituted pyrazines. Note that for 2-acetylpyrazine and 2-methylpyrazine, equilibrium is established at 2.5 min. Dissimilar behavior was found with the other derivative; for example, 2-methoxy-3-methylpyrazine had not yet reached equilibrium after 10 min.

An adsorption–time profile of methylpyrazine reveals the effect of salt on the rate of adsorption (Figure 4). It was evident that, after 2.5 min, equilibrium had been established in the solutions containing salt. In addition, it was obvious that the rate of adsorption and total amount adsorbed increased with increasing salt concentration. A similar trend was found in the headspace experiments with methylpyrazine (Figure 5).

Sample matrix effects

The sample matrix has been shown to have a dramatic effect on the SPME extraction of phenols from an aqueous solution employing a poly(acrylate) fiber (3).

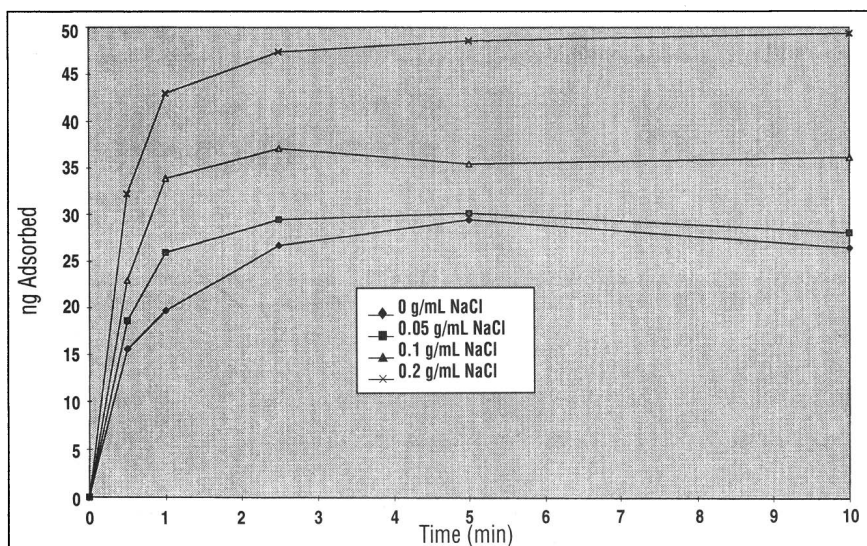


Figure 4. SPME behavior of methylpyrazine as a function of salt concentration in an aqueous solution.

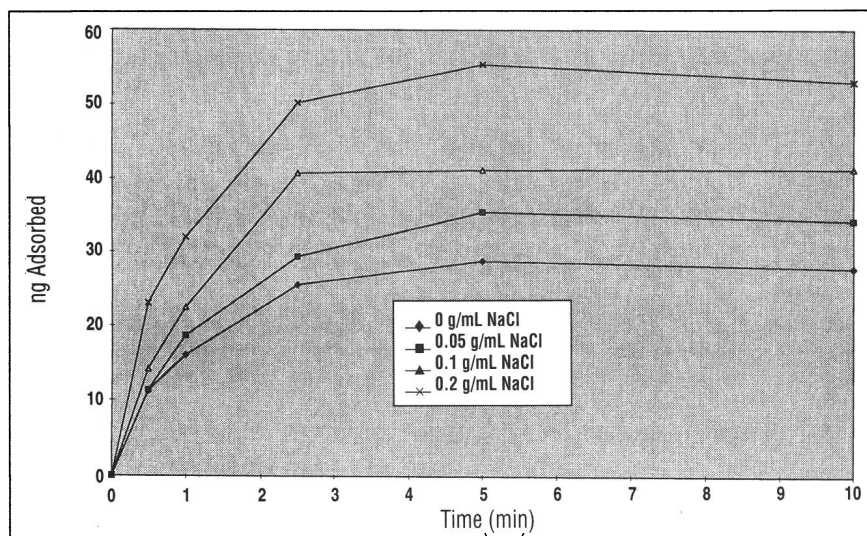


Figure 5. SPME behavior of methylpyrazine as a function of salt concentration: headspace measurements.

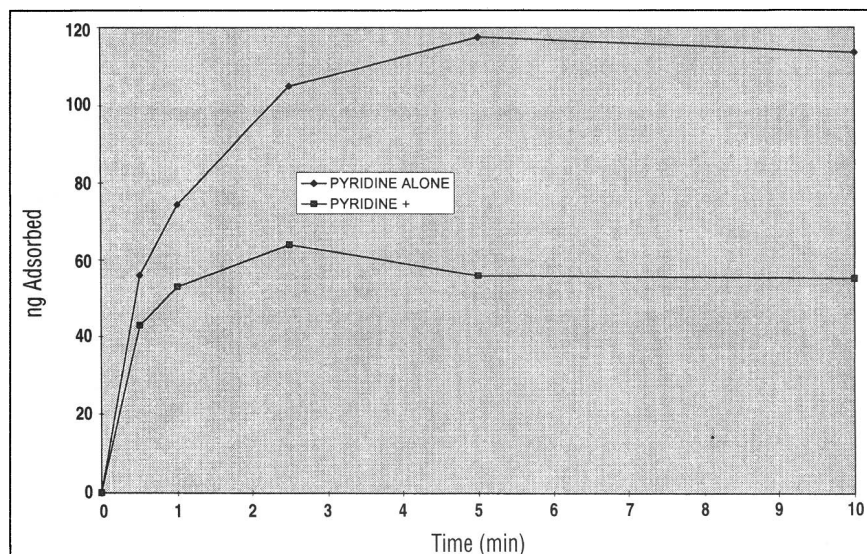


Figure 6. SPME behavior of pyridine solutions.

In this study, pH levels and salt concentrations significantly affected the sensitivity of the method designed to determine the phenols in a sewage matrix. In addition, a dramatic compound substituent effect was demonstrated. The pleasant aroma from roasting natural products such as coffee, peanuts, and tea has been identified as a very complex mixture of volatile organic compounds and can be easily classified as a complex sample matrix (16–18). Thus, it seemed that the presence of multiple components, inorganic and organic, in the aqueous extract of a roasted natural product such as coffee might result in significant competition between each component for adsorption on the SPME fiber. To obtain an impression of the magnitude of this sample matrix effect, an SPME experiment was conducted on an aqueous solution of pyridine with and without other alkyl-substituted pyridines. It was readily apparent that the presence of additional alkyl-substituted pyridines altered the adsorption–time profile of the pyridine (Figure 6). Although minimal change was found in the rates of adsorption, the amount of pyridine adsorbed on the poly(dimethylsiloxane) fiber was reduced significantly by the presence of additional pyridines. Thus, a significant impact on quantities of analytes adsorbed in SPME studies employing poly(dimethylsiloxane) coated fibers with natural product aqueous solutions should be anticipated.

Conclusion

Solid-phase microextraction (SPME) has been shown to be an effective tool for the analysis of representative volatile and semivolatiles associated with the Maillard reaction and sugar thermal degradation. The rate and amount of adsorption were observed to be a function of the structure of the model compounds. Increases in rates, equilibrium times, and amounts were found to be related to the degree of alkyl and alkoxy substitution on the basic ring structure. A pronounced sample matrix effect was observed. Intense competition for adsorption on the SPME poly(dimethylsiloxane) fiber was documented for a series of pyridine compounds wherein compounds were found to be adsorbed to a much greater extent when they were the only organic components in the aqueous solution versus when they were in the presence of additional organic species. The addition of NaCl to solutions of the model compounds resulted in significant increases in the rate and amount of compound adsorbed.

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