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Polypyrrole-Coated Capillary Coupled to HPLC for In-Tube Solid-Phase Microextraction and Analysis of Aromatic Compounds in Aqueous Samples

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In-tube solid-phase microextraction (SPME) based on a polypyrrole (PPY)-coated capillary was investigated for the extraction of aromatic compounds from aqueous solutions. The PPY-coated capillary was coupled on-line to HPLC that was programmed with an autosampler to achieve automated in-tube SPME and HPLC analysis. Three groups of aromatics, including both polar and nonpolar compounds, were examined. The results demonstrated that the PPY coating had a higher extraction efficiency than the currently used commercial capillary coatings, especially for polycyclic aromatic compounds and polar aromatics due to the increasing $\pi-\pi$ interactions, interactions by polar functional groups, and hydrophobic interactions between the polymer and the analytes. In addition to the functional groups in the PPY coating, which contributed to the higher extraction efficiency and selectivity toward analytes, the coating's porous surface structure,which was revealed by electron microscopy experiments, provided a high surface area that allowed for high extraction efficiency. It was found that the extraction efficiency and selectivity could be tuned by changing the coating thickness. The preliminary study of the extraction mechanism indicated that analytes were extracted onto the PPY coating mainly by an adsorption mechanism. The method was used for the extraction and analysis of both polar and nonpolar aromatics in aqueous samples.

The analysis of both polar and nonpolar aromatic compounds in aqueous samples has become an important topic due to the ever-increasing environmental and health concerns as a result of the carcinogenic and mutagenic properties of these compounds. To determine these compounds at a low concentration level, it is important to use suitable sample preparation methods for the extraction and concentration of trace analytes from water samples. On-line techniques are often preferred to achieve fast analysis and automation. The traditional techniques used for extraction and concentration of aromatic compounds from water samples are solvent extraction and solid-phase extraction.[1,2] These methods require large volumes of toxic organic solvents and are often time-consuming and labor-intensive because they are mainly off-line manual techniques. Solid-phase microextraction (SPME), which has recently obtained widespread acceptance in many areas,[3−5] can overcome the problems of traditional methods by eliminating the use of organic solvents and by integrating sample extraction, concentration, and introduction into a single step. This technology is more rapid and less expensive than the traditional methods, and it can be easily automated. In-tube SPME is a relatively new version of SPME which can be easily coupled on-line with HPLC for the analysis of less volatile and/or thermally labile compounds.[6,7] This technique, using a coated open tubular capillary for SPME instead of the conventional SPME fiber, allows for convenient automation of the extraction process, which not only saves analysis time but also provides better precision relative to manual techniques. However, one of the main difficulties limiting the wide application of SPME-LC is the absence of a suitable SPME stationary phase that not only has a high extraction ability for the analytes but is also stable in solutions of various matrixes. In the development of the SPME technique, it has been a challenge to extract polar and/or ionic analytes from water samples because of the less polar properties of the commercial SPME coatings and the stronger interactions between water and polar analytes. A solution to improve the extraction ability for these analytes is to convert them to less polar, non-ionized forms by pH adjustment or derivatizations.[3,4] However, derivatizations are often complicated processes that require a great deal of time and reagent. Perhaps the best solution is to develop polar and ion-exchange coatings for direct extraction of the target species from sample matrixes. One of our objectives is to prepare such new coatings that can be used for both polar and nonpolar compounds.

The interest in conducting polymers such as polypyrrole (PPY) and its derivatives has increased rapidly in the past decades due to their potential applications as novel materials, such as ion exchangers, energy-storage materials, corrosion-resistant coatings, catalysts, and materials for separation, actuators, chemical sensors, and electronic nose.[8−22] In our previous studies, PPY and poly-

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N-phenylpyrrole (PPPY) were coated on the surface of metal wires by an electrochemical method. These coated wires were used for SPME of volatile organic compounds.[23] The two coatings showed different selectivity to various organic compounds due to their differences in structures and functional groups. Recently, PPY was successfully coated on the inner surface of a silica capillary. This PPY-coated capillary was applied for in-tube SPME of basic drugs such as β-blockers and inorganic anions such as chloride and arsenate.[24,25]

In this study, in-tube SPME based on a PPY-coated capillary was investigated for the extraction of aromatic compounds from aqueous solutions. The PPY-coated capillary was coupled on-line to HPLC to achieve automated in-tube SPME and HPLC analysis. Three groups of aromatic compounds were examined, which included a group of model compounds containing both polar and nonpolar aromatics, a group of 16 polycyclic aromatic hydrocarbons (PAHs), and a group of 6 heterocyclic amines. The results demonstrated that the PPY coating had a higher extraction efficiency than the currently used commercial capillary coatings, especially for those of polycyclic aromatic compounds and polar aromatics, due to the $\pi-\pi$ interactions, interactions from polar functional groups, and hydrophobic interactions between the polymer and analytes. In addition, the porous surface structure of the PPY coating provided a high surface area that allowed for improved extraction efficiency. It was found that, under the same extraction conditions, the extraction efficiency and selectivity could be greatly enhanced by using a thicker coating (larger surface area). The preliminary study of the extraction mechanism indicated that analytes were extracted onto the PPY coating mainly by an adsorption mechanism. The method was applied for the extraction and analysis of both polar and nonpolar aromatics in water samples.

EXPERIMENTAL SECTION

Chemicals and Reagents. Pyrrole (98%) (Aldrich, ON, Canada) was distilled before use. Ferric perchlorate ($Fe(ClO_4)_3 \cdot 6H_2O$), perchloric acid (70%), benzene, and phenol were purchased from BDH (Toronto. ON, Canada). Toluene, dimethyl phthalate (DMP), and diethyl phthalate (DEP) were obtained from Aldrich (ON, Canada). Naphthalene was obtained from Supelco (Bellefonte, PA). A standard solution (1 mg/mL) for each model aromatic compound (benzene, toluene, naphthalene, phenol, DMP, and DEP) was prepared in methanol. A prepared mixture of these model compounds in acetonitrile contained 20 μg/mL of phenol, 50 μg/mL of benzene, 50 μg/mL of toluene, 10 μg/mL of DMP, 10 μg/mL of DEP, and 10 μg/mL of naphthalene. Finally, the mixture was spiked to water for the extraction experiments. A PAHs standard containing 16 components (2000 μg/mL in CH_2Cl_2/benzene; 50:50) was obtained from Supelco (Bellefonte, PA). The solution was first diluted to 20 μg/mL with a mixture of CH_2Cl_2 and benzene (50:50), then to 2 μg/mL with acetonitrile and finally, to the low concentration with water for analysis. The six aromatic amines (which were kindly provided by Dr. H. Kataoka, University of Okayama, Japan) are 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-aminodipyrido[1,2-*a*:3′2′-*d*]imidazole (Glu-P-2), 3-amino-1,4-dimethyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-1), and 3-amino-1-methyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-2). Each amine was dissolved in methanol to make a stock solution at a concentration of 0.3 mg/mL and was used after dilution with methanol or water to the required concentration. Solvents used were of analytical−reagent or HPLC grade. Water was obtained from a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA).

Preparation for PPY-Coated Capillary. The PPY was coated on the inner surface of a fused-silica capillary (60 cm long, 0.25 mm i.d.) by a polymerization method described previously.[24] Briefly, the coating was made by first passing the monomer solution (pyrrole in 2-propanol, 50% v:v) and then the oxidant solution (0.2 M ferric perchlorate in 0.4 M perchloric acid) through the capillary with the aid of N_2. PPY was formed by oxidative reactions when the oxidant reagent reached the monomer in the capillary. The above procedure was referred to as one PPY coating cycle, which could be repeated several times (1−4 times in this study) to increase the coating thickness. The capillary was cleaned with acetone and then dried with N_2 before it was coated. During polymerization, the color of the capillary changed gradually from yellow to black, which indicated the formation of PPY on the inner surface. The PPY-coated capillary was then washed with methanol and dried with N_2. Finally, it was coupled to the HPLC system, conditioned with the mobile phase, and checked with a blank solution before use.

Scanning Electron Microscopy (SEM). Each of the PPY-coated capillaries (with different thickness) was cut into a 1-cm long piece, coated with a gold film, and then analyzed using a HITACHI S-570 scanning electron microscope (15 kV accelerating potential).

In-Tube SPME. Because in-tube SPME and its operation procedures (including a schematic illustration) have been described in detail recently,[7,24] they will not be discussed here. To compare the extraction efficiencies of different extraction phases, a PPY-coated capillary and the following commercial capillaries (from Supelco, Bellefonte, PA) were examined under the same conditions. Omegawax 250 (0.25-μm film thickness, 0.25-mm i.d.), SPB-1 (0.25-μm film thickness, 0.25-mm i.d.), SPB-5 (0.25-μm film thickness, 0.25-mm i.d.), and a polar silica tubing (0.25-mm i.d., which was also used as the host capillary to prepare the PPY-

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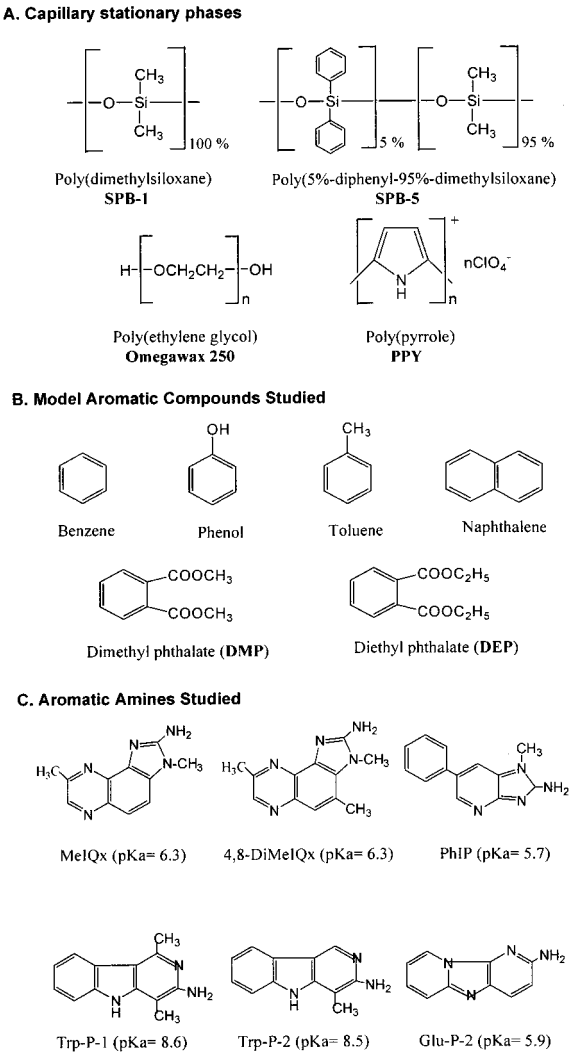

Figure 1. Structures of the capillary stationary phases and some of the compounds studied.

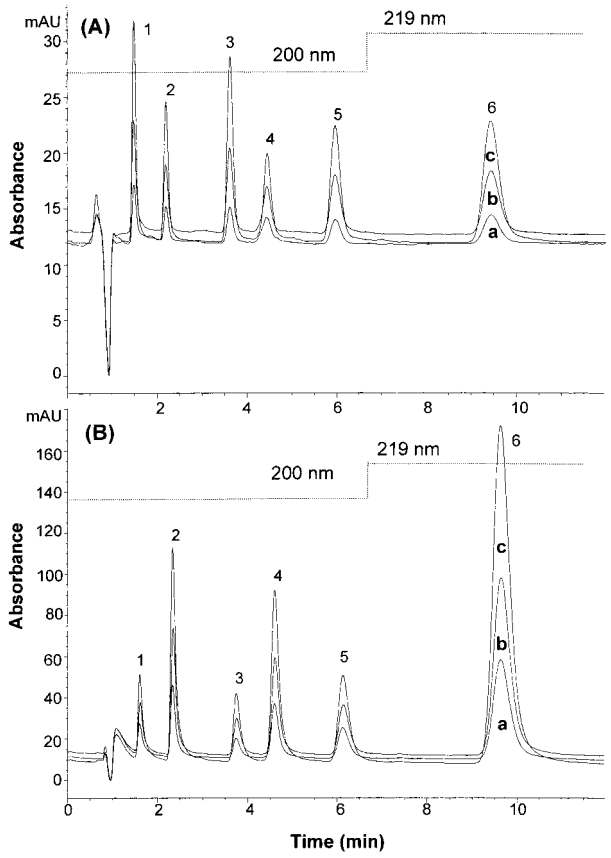

Figure 2. HPLC/UV chromatograms of the 6 model aromatic compounds by (A) standard injection (10 μL) and (B) PPY-coated capillary in-tube SPME. Peak identification and concentration: (1) phenol (a, 200 ng/mL; b, 400 ng/mL; and c, 800 ng/mL), (2) DMP (a, 100 ng/mL; b, 200 ng/mL; c, 400 ng/mL), (3) benzene (a, 500 ng/mL; b, 1000 ng/mL; c, 2000 ng/mL), (4) DEP (a, 100 ng/mL; b, 200 ng/mL; c, 400 ng/mL), (5) toluene (a, 500 ng/mL; b, 1000 ng/mL; c, 2000 ng/mL), and (6) naphthalene (a, 100 ng/mL; b, 200 ng/mL; c:, 400 ng/mL).

coated capillary). Figure 1 shows the structures of the capillary stationary phases and some of the compounds that were studied.

Separation and Detection. The HPLC system used was a model 1100 series LC coupled with a UV detector and an atmospheric pressure (AP) electrospray ionization (ESI) mass spectrometer (Agilent Technologies, Palo. Alto, CA).

For the model aromatic compounds, separation was performed using a Hypersil BDS C-18 column (5.0 cm × 2.1 mm i.d., 3-μm particle size) from Agilent Technologies at room temperature. The mobile phase consisted of acetonitrile and water (40:60) with a flow rate of 0.2 mL/min. A UV detector set to 200 nm was used for the first 7 min and was then changed to 219 nm for the rest of the run as shown in Figure 2.

For the separation of 16 PAHs, a SUPELCOSIL LC−PAH column (5 cm × 4.6 mm, 3-μm particle size) from Supelco (Bellefonte, PA) was used at ambient temperature. The mobile phase, initially CH_3CN:water, 50:50, was kept for 5 min, then the component of CH_3CN was increased linearly and reached 90% at 20 min; this ratio was held for the rest of the run. Flow rate was kept at 0.5 mL/min. UV detection was performed using a wavelength program to optimize signal intensities, as shown in Figure 3.

For separation of the 6 aromatic amines, the same C-18 column was used as for the separation of the model aromatic compounds. The mobile phase was a mixture of A (CH_3CN:CH_3OH = 1:3) and B (ammonium acetate, 100 mM), with a ratio of 50:50; flow rate was increased linearly from 0.2 to 0.5 mL/min within 20 min. ESI-MS detection: nebulizer gas, N_2 (40 psi); drying gas, N_2 (10 L/min, 350 °C); capillary voltage, 1500 V; fragmentor voltage, 90 V; ionization mode, positive; mass scan range, 100−300 amu; selected ion monitoring (SIM), m/z 214 (MeIQx), 228 (4,8-DiMeIQx), 225 (PhIP), 184 (Glu-P-2), 198 (Trp-P-2), and 212 (Trp-P-1).

Sample Preparation. Drinking water and lake water samples collected from local areas were prepared by spiking three different amounts of analytes into the sample solutions. Each (1 mL) of these spiked sample solutions was shaken thoroughly and allowed to stand for 2−5 min, then set into the autosampler and analyzed by the method developed. The results were compared to those of nonspiked samples and pure water samples, analyzed by the same method and under the same conditions, to obtain the recoveries.

Safety Considerations. The aromatic compounds that were studied are highly toxic (mutagenic and carcinogenic) and, therefore, should be handled only in a fume hood and when using

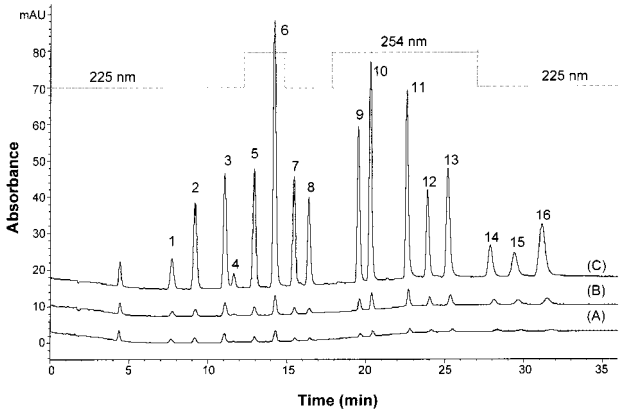

Figure 3. Separation of a PAH mixture (each 100 ng/mL) with solvent gradient by (A) standard injection (10 μL), (B) host capillary in-tube SPME, and (C) PPY-coated capillary in-tube SPME. Peak identification: 1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, benzo[*a*]anthracene; 10, chrysene; 11, benzo[*b*]fluoranthene; 12, benzo[*k*]fluoranthene; 13, benzo[*a*]pyrene; 14, dibenzo(*a*,*h*) anthracene; 15, benzo(*ghi*)perylene; and 16, indeno(1,2,3-*cd*)pyrene.

appropriate protective clothing. They should be stored in tightly sealed containers in a cool dry place.

RESULTS AND DISCUSSION

Separation and Detection Conditions. All of the three groups of aromatic compounds could be separated under the conditions listed in the Experimental Section. UV detections were optimized by selecting appropriate wavelengths at which most of the compounds had better signal intensities, as shown in Figures 2 and 3. Under these wavelengths, various sample matrixes (buffer solutions) did not show significant effects on the UV detections (data not shown). For the group of amine compounds, a mass detector was used due to their weak UV signals. For each amine, mass spectra under positive ion mode were initially analyzed by liquid injection. Each amine gave a simple spectrum in the mass range m/z 100−300, with the $[M + 1]^+$ ion as the base ion. These base ions were selected for the analyte quantification. Optimization of the mass detection conditions included capillary voltage, fragmentor voltage, nebulizer pressure, drying gas flow rate, and temperature. The results are summarized in the Experimental Section.

Optimization of In-Tube SPME Conditions. Several parameters were optimized to achieve the best extraction efficiency for in-tube SPME. These parameters include capillary length, extraction time profile (the number of draw/eject cycles for each extraction, the sample volume and flow rate for each draw/eject cycle), sample matrix and pH, and desorption conditions. A detailed theoretical description and discussion of these parameters was reported previously.[6]

Extraction Time Profile and Capillary Length. In the extraction process, a 30-μL sample was drawn from the sample vial into the capillary at a flow rate of 100 μL/min. The same volume of sample was then ejected back into the same sample vial. The two steps together are referred to as one draw/eject cycle, which can be repeated through programming of the autosampler software. As shown in Table 1, the amounts of analytes extracted increase greatly when the number of cycles increases from 0 to 15. After this number of draw/eject cycles, the amounts of analytes extracted continue to increase but with smaller slopes. However, a further increase in the extraction cycles increases the analysis time, which is not desirable for the routine analysis. The sample volume used for each draw/eject cycle can also be optimized. It was found that the larger the volume, the higher the amount of analytes extracted. However, the maximum volume that could be used in each step was limited by the inner capillary volume (30 μL). The flow rate in each draw/eject step is also an important factor. The extraction efficiency was higher with a higher flow rate as a result of improved agitation.[6] However, very high flow rates affected the precision due to the formation of air bubbles at the edges of the capillary. A capillary 60 cm long was the optimum for in-tube SPME. Below this level, extraction efficiency was reduced, and above this level, peak broadening was observed.

Sample matrix and pH. The effects of the sample matrix and pH on the extraction were examined using several buffer solutions with pH 3.0−10.0. For the 6 model compounds and the group of PAHs studied, no significant effects on the extraction efficiency were found under the matrixes and pHs tested, although the extraction ability increased slightly when a salt (NaCl) was added to the solution. However, sample pH showed great effects on the extraction of aromatic amines due to their basic properties. Similarly to a previous study[7] on using an Omegawax capillary for extraction of the same type of compounds, the extraction efficiency of PPY coating to these amines increased with increasing sample pH. Tris-buffer at pH 8.5 was used for the amines in this study.

Desorption of Analytes from the Capillary. Most of the analytes studied could be desorbed from the extraction capillaries with mobile phases by simply switching the six-port valve to the inject position.[7,24] However, some analytes, such as the aromatic amines studied in this work, had stronger interactions with the extraction phase and, thus, could not be desorbed easily by the mobile phase. Therefore, a 30 μL portion of methanol was drawn into the capillary to assist desorption of these analytes before switching the valve to the inject position.

Extraction Efficiency and Selectivity. In SPME, extraction efficiency and selectivity of the coatings to the analytes depend on the interactions between the analytes and the stationary phases, which include hydrogen bonding, acid−base, $\pi-\pi$, dipole−dipole, dipole−induced-dipole, and dispersion (hydrophobic interaction) forces. Ideally, the extraction ability of a coating should be evaluated by the distribution coefficient K of the analyte between the coating and sample matrix. Selectivity should be judged by the selectivity factor (α) defined as $\alpha_{ji} = K_j/K_i$, where K_j and K_i are the distribution coefficients of compounds j and i between the same coating and sample matrix. However, for some compounds or coatings, K values are not available or difficult to measure accurately. Therefore, the extraction efficiency is often evaluated by the amount of analyte extracted by the coating which, for an absorption-based SPME coating, can be expressed as[3]

$$n_A = K_A V_f V_s C_A^o/(K_A V_f + V_s) \quad (1)$$

where n_A is the amount of analyte A extracted by the coating at equilibrium, V_s and V_f are the volumes of the sample solution and coating, respectively, C_A^o is the initial concentration of the analyte in the sample, and K_A is the distribution coefficient. For porous

Table 1. Effects of the Number of Extraction Cycles on the Extraction Efficiency of PPY Coating to PAH Compounds

	detector response[a]	amount of analytes extracted (ng) or extraction yield (%)[b]					selectivity[c]
PAH compounds	F	5 cycles	10 cycles	15 cycles	20 cycles	25 cycles	$\alpha_{A/1}$
naphthalene	0.058	3.0	4.5	5.9	6.7	7.5	1.0
acenaphthylene	0.046	3.9	5.9	7.6	8.8	9.9	1.3
acenaphthene	0.027	3.9	5.5	6.7	8.1	8.9	1.2
fluorene	0.213	5.7	6.8	8.1	9.2	10.1	1.3
phenanthrene	0.050	5.7	8.6	11.1	13.2	14.6	2.0
anthracene	0.024	6.2	9.3	12.8	14.0	15.4	2.1
fluoranthene	0.090	7.9	12.7	17.5	21.0	23.6	3.1
pyrene	0.115	8.2	13.2	18.0	21.9	24.7	3.3
benzo[*a*]anthracene	0.082	8.3	14.4	20.7	25.7	30.0	4.0
chrysene	0.052	7.2	12.9	18.7	23.0	26.9	3.6
benzo[*b*]fluoranthene	0.072	8.7	15.4	24.3	30.7	34.7	4.6
benzo[*k*]fluoranthene	0.107	5.5	10.3	15.7	19.3	22.7	3.0
benzo[*a*]pyrene	0.079	6.1	12.3	18.7	23.6	26.2	3.5
dibenz(*a.h*)anthracene	0.098	3.2	6.9	10.7	13.4	14.7	2.0
benzo(*ghi*)perylene	0.099	3.2	7.2	10.8	13.6	15.6	2.1
Indeno(1,2,3-*cd*)pyrene	0.088	5.5	11.4	16.0	20.0	23.9	3.2

[a] F was obtained by injecting 10 μL of 1 μg/mL solution (for each PAH, 10 ng was injected, see eq 3). [b] A 1-mL sample (100 ng/mL for each PAH) was analyzed by in-tube SPME, and the amount of analyte extracted (n_A) was calculated by eq 3; the extraction yield (%) $= n_A \times 100/M$, where M is the total amount of each analyte in the 1 mL solution. Because the concentration of each analyte is 100 ng/mL, M =100 ng. [c] Selectivity factors were calculated only for the 25 extraction cycles on the basis of n_A relative to n_1 (naphthalene).

coatings that extract analytes by adsorption (we assume PPY is this kind of coating), the equation that took into account the active extraction sites on the porous surface can be expressed as follows[26]

$$n_A = K_A V_f V_s C_A^{\circ} (C_{f\,max} - C_{fA}^{\infty})/[V_s + (K_A V_f (C_{f\,max} - C_{fA}^{\infty})] \quad (2)$$

where $C_{f\,max}$ is the maximum concentration of active sites on the coating, and C_{fA}^{∞} is the equilibrium concentration of the analyte on the coating. The other terms in eq 2 have the same meanings as in eq 1, with the exception of the distribution coefficient K. In eq 2, K is defined as the adsorption equilibrium constant, but it is the partition coefficient in eq 1.

Although the two expressions are different in some terms, the extraction efficiencies of different coatings for the same sample can be compared by the amount of analytes extracted (or extraction yield) under the same conditions, especially when the coatings have the same thickness or volume. For practical purposes, even when the data on thickness for some coatings are unknown, the comparison of the amount of analyte extracted by different coatings can still provide useful guidance for the coating selection.

When the initial concentrations of all of the analytes in a sample are the same, the selectivity of a coating to different compounds in the sample can be evaluated by the selectivity factor defined as $\alpha_{ji} = K_j/K_i = n_j/n_i$.

It is often difficult and time-consuming to calculate K because some terms are hard to measure accurately, such as V_f, $C_{f\,max}$, and C_{fA}^{∞}. Therefore, for practical purposes, one of the advantages of using the amount of analytes extracted, n_A, to evaluate the extraction efficiency and selectivity (by $\alpha_{ji} = n_j/n_i$) is that n_A can be easily obtained from experimental measurements with the following expression

$$n_A = FA = (m/A_d)A \quad (3)$$

where n_A is the amount (mass) of analyte extracted by SPME, F is the detector response factor which can be calculated by comparing the amount of analyte (m) injected to the area counts (A_d) obtained by liquid injection, A is the response obtained by SPME.

Effects of PPY Coating Thickness and Surface Property on Extraction. Previous in-tube SPME studies did not consider the effect of coating thickness, an important parameter, on extraction properties due to the expensive cost of testing commercial coatings of different thicknesses and the low availability of some commercial coatings of different thicknesses. Because the thickness of the PPY coating can be controlled easily by changing the number of PPY coating cycles (see Experimental Section), this provides the opportunity to systematically study the effect of coating thickness on in-tube SPME. It can be predicted from eq 1 and 2 that the amount of analytes extracted will increase when the coating thickness and, thus, the volume of the coating, V_f, increases. Thus, the extraction efficiency and selectivity can be manipulated by controlling the coating thickness. This expectation was proved by experimental results in this work. For example, the extraction efficiency of the PPY coating to the group of model compounds studied increased gradually with an increase in PPY thickness (the number of PPY coating cycles), as shown in Table 2. Meanwhile, the coating's selectivity to polar aromatic compounds such as DMP and DEP and to polycyclic aromatics such as naphthalene was also increased relative to benzene and toluene. For a porous coating, in which the active surface area controls its extraction ability, it is more important to consider the total surface area than the coating volume. This is because the surface area of a porous coating is larger than that of a nonporous coating even though it has the same or even smaller coating volume relative to a nonporous coating due to the high porosity of the

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Table 2. Effect of PPY Coating Thickness on the Extraction for the Model Aromatic Compounds

		amount of analyte extracted (ng)[c]					extraction yield (%)[d]					selectivity factor ($\alpha_{A/benzene}$)[e]				
compound[a]	F[b]	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY
phenol	0.059	3.6	4.9	5.7	6.6	7.2	1.8	2.5	2.9	3.3	3.6	0.8	1.1	1.1	1.1	1.2
DMP	0.048	3.0	5.3	8.7	11.6	13.9	3.0	5.3	8.7	11.6	13.9	1.4	2.4	3.4	3.9	4.5
benzene	0.138	10.9	11.3	12.7	15.0	15.5	2.2	2.3	2.5	3.0	3.1	1.0	1.0	1.0	1.0	1.0
DEP	0.039	2.6	6.1	9.2	11.9	14.4	2.6	6.1	9.2	11.9	14.4	1.2	2.7	3.6	4.0	4.4
toluene	0.124	10.8	13.9	19.3	26.5	32.2	2.2	2.8	3.9	5.3	6.4	1.0	1.2	1.5	1.8	2.1
naphthalene	0.013	2.6	5.8	9.3	13.4	18.8	2.6	5.8	9.3	13.4	18.8	1.2	2.6	3.7	4.5	6.0

[a] Compound concentrations in the sample: phenol, 200 ng/mL; DMP, 100 ng/mL; benzene, 500 ng/mL; DEP, 100 ng/mL; toluene, 500 ng/mL; and naphthalene, 100 ng/mL. [b] A 10-μL sample was directly injected to obtain F (detector response factor for each analyte; see eq 3). [c] A 1-mL sample was analyzed by in-tube SPME, and the amount of analyte extracted (n_A) was calculated by eq 3. [d] The extraction yields (%) are the percentages of extracted amounts of the analytes per initial amounts of the analytes in a 1-mL sample solution. [e] Selectivity factors were calculated by comparing the extraction yield of an analyte relative to that of benzene. The thickness of the PPY coating increases from 0-PPY (coating cycle, without coating) to 4-PPY (coating cycles). 15 extraction cycles were used for in-tube SPME.

porous coating.[27−29] If a porous coating layer can be treated as an idealized bed consisting of uniform spherical microparticles, the total surface area can be expressed as

$$S_t = \pi L[(D/2 + a)^2 - (D/2)^2]d\rho s = a\pi L(D + a)d\rho s \qquad (4)$$

where S_t is the total surface of the porous coating, a is the coating thickness, L is the length of the capillary, D is the inner diameter of the coated capillary (after coating), d is the density of the PPY particles, ρ is the porosity of the porous coating, and s is the specific surface area. Therefore, when the coating thickness and polymer particle density increases, the coating surface area and, hence, the extraction efficiency will increase. A SEM study of the PPY-coated inner surfaces confirmed this prediction, as shown in Figure 4. The increase in the extraction selectivity with an increase of the coating thickness is due to the enhancement of specific interactions ($\pi-\pi$ and polar functional groups) relative to nonspecific interactions (hydrophobic). The porous inner-surface characteristics of PPY-coated capillaries with 1−3 coating cycles can be seen clearly when comparing them to the surface of a noncoated host capillary. The SEM image on the inner surface of a 4-cycle PPY coating is not given in Figure 4 because it does not show a significant difference when compared to that of a 3-cycle coating. The estimated thickness of a 4-cycle PPY coating is less than 0.5 μm, according to the SEM study and the calculation that is based on the density[9] and mass of PPY. To our knowledge, this is the first systematic SEM study of the PPY-coated capillary inner surface with differing thicknesses.

As expected, the extraction efficiency for the group of PAHs and amine compounds was also increased when the coating thickness was increased (data not shown). Actually, the extraction ability of a thick PPY coating toward aromatic amines was so strong that these compounds could not be desorbed easily by the mobile phase. To overcome this problem, a thin PPY-coated capillary (2 coating cycles) had to be used for amines rather than a thicker coating (4 coating cycles), which was used for other analytes. However, even a thin PPY coating could extract more analytes from the sample than other coatings, as shown in the next section. The enhanced extraction efficiency of thinner porous SPME coatings was also demonstrated in the recent studies of porous SPME coatings[27,28] and sol−gel SPME coatings.[29]

Comparisons of PPY Coating with Commercial Coatings. Previous studies showed that Omegawax was the best capillary for in-tube SPME of polar compounds among the commercial GC capillaries tested,[6,7] including SPB-1 and SPB-5 (see Figure 1). In this work, a PPY-coated capillary, a host silica capillary (without PPY coating), and the three commercial capillaries described above were evaluated for their extraction ability. Three groups of aromatic compounds were selected that represented several functionalities. The first group, the so-called model compounds, included both nonpolar (benzene, toluene, and naphthalene) and polar (phenol, DMP and DEP) compounds containing one or two π rings. The second group contained 16 polycyclic hydrocarbons (PAHs). The third group consisted of 6 aromatic amines representing a wide range of basic heterocyclic aromatics.

For the model compounds, the extraction efficiency and selectivity of PPY can be clearly observed from Figure 2 and Table 3. In these experiments, larger amounts of phenol, benzene, and toluene relative to DMP, DEP, and naphthalene were used to increase their responses. For liquid injection, as shown in Figure 2A, peaks 1, 3, and 5 are higher than peaks 2, 4, and 6 due to their larger concentrations. However, the opposite trends were obtained when the same samples were analyzed by the PPY in-tube SPME, as shown in Figure 2B. The above opposite trends and the selectivity factors listed in Table 3 illustrate that PPY had better selectivity toward the polar compounds DMP and DEP and the two-π-ring naphthalene. These results can be explained by considering the structures of PPY and the analytes. Because PPY contains a conjugated π structure, it will extract aromatics by $\pi-\pi$ interactions as well as by hydrophobic interactions, and these interactions will increase accordingly with increasing numbers of aromatic rings, such as for naphthalene and polycyclic aromatics (PAHs). The results shown in Figure 2 and Table 3 agree well with the above expectation. The high selectivity of PPY for polar aromatics such as DMP and DEP is possibly due to the additional interactions between the polar components of the polymer and the analytes. However, PPY did not show a high extraction ability for phenol, which is probably due to the weak interaction between the PPY and the undissociated acidic molecules (because PPY is a weak acid itself).[12] The effect of the PPY coating on the extraction is also easily observed when comparing the results of a PPY-coated capillary with those of the noncoated host capillary,

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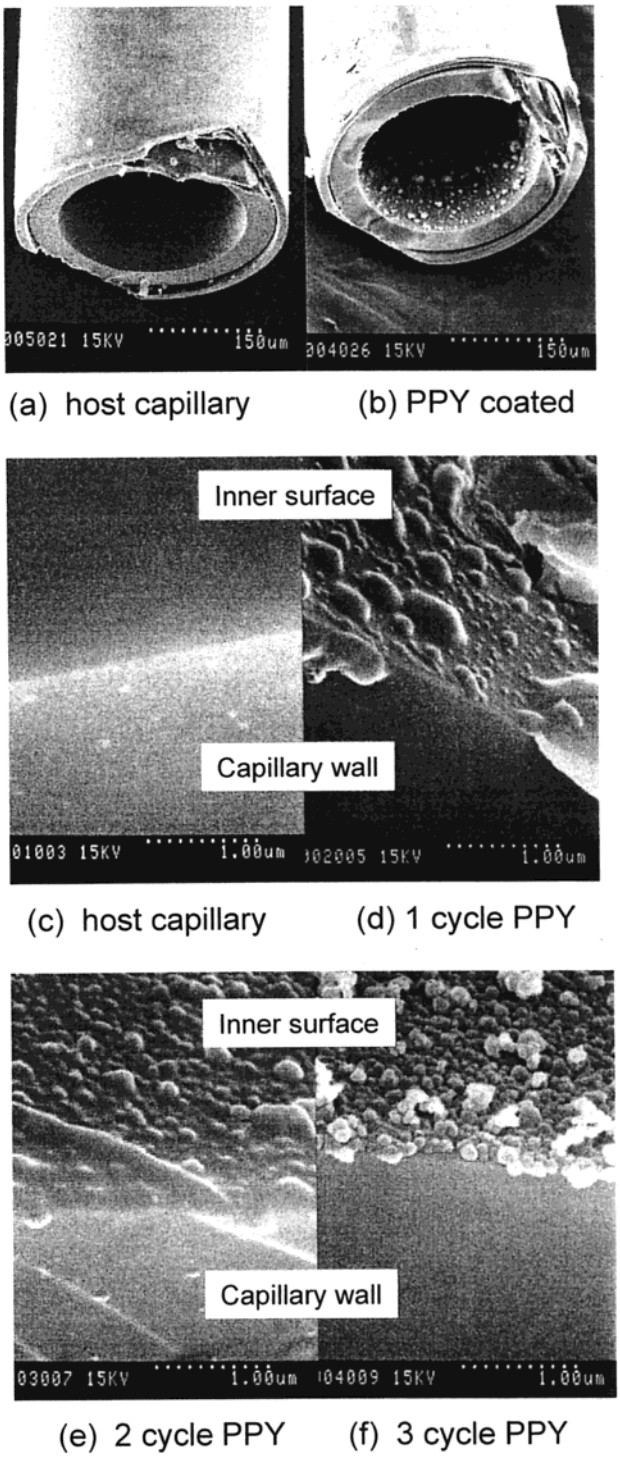

Figure 4. Scanning electron micrographs of the PPY-coated capillaries and the host silica capillary: (a) cross-sectional view of the host capillary; (b) cross-sectional view of the PPY-coated capillary (4-cycle coating); (c−g) the enlarged inner surface views for the host capillary (c), and for the PPY-coated capillaries with 1 PPY coating cycle (d), 2 PPY coating cycles (e), and 3 PPY coating cycles (f).

as listed in Table 3. These results are consistent with those that were obtained by Wallace and co-workers[11,12] in their studies on using PPY-coated stationary phases for HPLC separation of similar compounds.

Compared to other coatings tested under the same conditions, as shown in Table 3, PPY demonstrated the best extraction efficiency for most of the compounds that were studied. SPB-1 and SPB-5 contain nonpolar coatings, and they showed better selectivity to nonpolar compounds such as benzene, toluene, and naphthalene. However, compared with SPB-1, the extraction ability of SPB-5 to nonpolar aromatics was significantly increased due to the $\pi-\pi$ interactions that were introduced by the phenyl group (5%) in the polymer. Omegawax did not show good extraction efficiency for the compounds studied, but it did show a better extraction selectivity to DMP, DEP, and naphthalene relative to benzene and toluene due to its polar property.

To examine the compatibility of the in-tube SPME with solvent gradient conditions, a 16-PAH mixture was analyzed by both liquid injection and in-tube SPME methods. As shown in Figure 3, the retention times of PAHs by liquid injection agree well with those obtained by SPME, which illustrates that SPME sampling does not affect the retention of the analytes under solvent gradient conditions. Compared with other coatings studied, PPY again showed the highest extraction efficiency for the PAHs studied (Table 4). In addition, the extraction efficiency increased with the increase in molecular size due to the increased $\pi-\pi$ and hydrophobic interactions. However, for PAHs larger than benzo[*b*]fluoranthene, the hydrophobic interactions became dominant, and a slight decrease in extraction efficiency was observed. These trends were also found in a previous study on porous coating SPME of PAHs.[28] Due to the high extraction ability of PPY, the in-tube SPME-HPLC method could be applied to detect a low concentration of PAHs (up to 0.5 ng/mL), which was not detectable with the liquid injection method (detection limit, *DL* = 10 ng/mL). The extraction yield for naphthalene was smaller in Table 4 relative to that in Table 3, possibly due to the sample matrix effect, which will be discussed later.

Six aromatic amines that are target mutagens or carcinogens were also examined. A previous study showed that Omegawax was better in extraction of these compounds than SPB-1, SPB-5, and a nonpolar precolumn.[7] However, as illustrated in Table 5, even a thin PPY coating (two coating cycles) showed a higher extraction efficiency for these compounds than did Omegawax and other coatings tested. The high extraction ability of PPY to these compounds is due to the increasing interactions of polar functional groups (such as hydrogen bonding, base−acid, and dipole−dipole) between polymer and analytes. Table 5 did not include the results obtained by SPB-1 and SPB-5 because they were discussed previously.[7]

Extraction Mechanism for PPY Coating SPME. Porous coatings extract analytes mainly by adsorption processes. The theory of analyte extraction by porous SPME coatings was developed recently on the basis of Langmuir adsorption isotherm.[26] According to this theory, the number of effective surface sites where adsorption can take place is limited. When all such sites are occupied, no more analyte can be extracted. This suggests that analyte extraction is a competitive process in which a molecule with a higher affinity for the surface can replace a molecule with a lower affinity. In other words, the amount of analyte A extracted (n_A) from a sample mixture must be lower than that (n_A) obtained from a sample containing only analyte A.

The large difference in extraction yields (%) for naphthalene obtained by PPY from a sample containing 6 model compounds (Table 3) and a sample having 16 PAHs (Table 4) indicates that the PPY extraction is mainly based on an adsorption mechanism, which was further confirmed by the results shown in Figure 5. At high concentration ranges (Figure 5), the slopes of the

Table 3. Comparison of the Extraction Properties for the Model Compounds by Different Capillary Coatings

		analyte extracted (ng)[c]					extraction yield (%)[d]					selectivity factor ($\alpha_{A/benzene}$)[e]				
compd[a]	F[b]	host	SPB-1	SPB-5	Omeg	PPY	host	SPB-1	SPB-5	Omeg	PPY	host	SPB-1	SPB-5	Omeg	PPY
phenol	0.059	3.6	3.9	1.0	3.9	7.2	1.8	1.9	0.5	2.0	3.6	0.8	0.9	0.1	0.9	1.2
DMP	0.048	3.0	1.9	0.8	3.6	13.9	3.0	1.9	0.8	3.6	13.9	1.4	0.9	0.2	1.6	4.5
benzene	0.138	10.9	10.3	18.1	11.1	15.5	2.2	2.1	3.6	2.2	3.1	1.0	1.0	1.0	1.0	1.0
DEP	0.039	2.6	1.7	1.5	3.5	13.8	2.6	1.7	1.5	3.5	13.8	1.2	0.8	0.4	1.6	4.4
toluene	0.124	10.8	10.0	32.7	11.6	32.2	2.2	2.0	6.5	2.3	6.4	1.0	1.0	1.8	1.0	2.1
naphthalene	0.013	2.6	3.8	10.3	9.4	18.8	2.6	3.8	10.3	9.4	18.8	1.2	1.8	2.8	4.2	6.0

[a] Compound concentrations and other conditions (including the notes *b*, *c*, *d*, and *e*) are the same as in Table 2. A 4-PPY-cycle coating was used.

Table 4. Comparison of the Extraction Efficiencies for the PAHs by Different Capillary Coatings

	detector response[b]	amount of analyte extracted (ng)[c] or extraction yield (%)[d]				
PAH compd[a]	F	host	Omeg	SPB-1	SPB-5	PPY
naphthalene	0.058	0.9	1.7	1.9	3.0	5.9
acenaphthylene	0.046	0.6	1.8	2.5	3.7	7.6
acenaphthene	0.027	0.7	1.8	4.1	4.6	6.7
fluorene	0.213	1.2	2.1	3.8	6.3	8.1
phenanthrene	0.050	0.8	2.5	4.4	5.4	11.1
anthracene	0.024	0.8	2.6	4.9	7.3	11.8
fluoranthene	0.090	1.0	3.2	5.8	10.3	17.5
pyrene	0.115	1.1	3.1	6.1	12.0	18.0
benz[*a*]anthracene	0.082	1.5	4.8	6.1	9.9	20.7
chrysene	0.052	1.4	4.4	5.2	9.2	18.7
benzo[*b*]fluoranthene	0.072	1.9	6.3	6.4	8.6	23.3
benzo[*k*]fluoranthene	0.107	1.5	5.8	5.7	8.4	15.7
benzo[*a*]pyrene	0.079	1.8	6.0	6.0	8.8	18.7
dibenz(*a.h*)anthracene	0.098	1.4	4.9	2.9	6.1	10.7
benzo(*ghi*)perylene	0.099	1.6	5.2	3.1	6.7	10.8
Indeno(1,2,3-*cd*)pyrene	0.088	1.9	7.3	6.3	8.8	16.0

[a] Compound concentrations and the experimental conditions (and the notes *b*, *c*, and *d*) are the same as in Table 1 except that 15 extraction cycles were performed for in-tube SPME here.

Table 5. Comparison of the Extraction Efficiencies for the Aromatic Amines by Different Capillary Coatings

		detector response [a]	amount of analytes extracted (ng)[b]			extraction yield (%)[c]		
compd	m/z (M + 1)	F ($\times 10^{-6}$)	host	Omeg	PPY	host	Omeg	PPY
MeIQx	214	1.71	1.3	3.3	18.3	1.3	3.3	18.3
4,8-DiMeIQx	228	1.97	1.3	4.5	18.3	1.3	4.5	18.3
PhIP	225	1.27	1.1	5.0	15.9	1.1	5.0	15.9
Glu-P-2	184	4.33	0.9	9.4	15.7	0.9	9.4	15.7
Trp-P-2	198	1.44	1.4	10.7	23.1	1.4	10.7	23.1
Trp-P-1	212	2.48	1.6	14.5	28.9	1.6	14.5	28.9

[a] Detector response factors (F) were obtained by liquid injection of 10 μL of 1 μg/mL solution (for each amine, 10 ng was injected; see eq 3). [b] A 1-mL sample containing 100 ng/mL of each analyte was analyzed by in-tube SPME; the amount of analyte extracted (n_A) was calculated by eq 3. [c] The extraction yields (%) are the percentages of extracted amount of the analytes per initial amounts of the analytes in a 1-mL sample solution. 15 extraction cycles were performed by in-tube SPME.

calibration curves obtained from the sample mixtures are smaller than those obtained from the samples containing only a single analyte due to the larger competing effects on extraction in sample mixtures. However, these phenomena were hardly observed at low concentration ranges (<400 ng/mL) where the linear calibration curves have almost the same slopes. This is because the number of unoccupied active surface sites on the coating is relatively larger in diluted solutions and thus, there are fewer competitions (replacements) in the extraction process. Because the amount of analyte extracted from a sample can be significantly affected by sample matrix composition, as shown above, appropriate calibration methods must be used in quantitative analysis for adsorption-based coatings. In addition, diluted solutions should always be used to reduce competing effects on extractions.

It must be pointed out, however, that conducting polymers such as PPY are complicated systems. Further studies are needed for better understanding of the interaction mechanism between the analytes and the polymers. The adsorption of organic vapors on PPY films was studied recently.[30] A mechanism considering both adsorption and absorption was also proposed.[31,32]

Precision, Limit of Detection, and Linearity. The precision of the method varies between 1.8 and 7.2% RSD ($n = 7$), depending on the compounds and concentrations studied. Due to the higher extraction efficiency of the PPY coating, lower detection limits ($S/N = 3$) can be achieved for most of the analytes when

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Table 6. Linear Regression Data and Detection Limits (DL) for the Model Compounds Studied

compd	linear range (ng/mL)	linear regression equation[a]	correlation (R^2)	DL (ng/mL)
phenol	20 ~ 1000	$y = 0.3964x + 19.275$	0.9984	2.0
DMP	2 ~ 500	$y = 2.3258x + 15.103$	0.9967	0.6
benzene	40 ~ 2500	$y = 0.1960x - 2.5944$	0.9983	10.0
DEP	2 ~ 500	$y = 2.7897x + 25.055$	0.9978	0.4
toluene	20 ~ 2500	$y = 0.3866x + 13.788$	0.9955	5.0
naphthalene	1 ~ 500	$y = 11.889x + 7.9823$	0.9994	0.1

[a] Obtained from calibration curves by plotting the peak area counts of each compound against corresponding concentrations; number of data points, 11 points (3 repeats for each point).

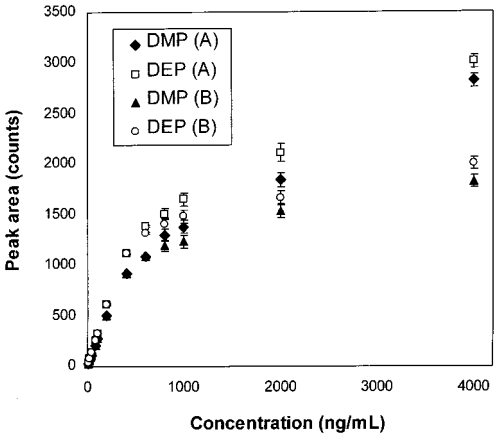

Figure 5. Calibration curves for the DMP and DEP that was obtained under the conditions of (A) sample solutions contain only DMP or DEP, and (B) sample solutions containing all six model compounds, with increased concentrations as shown in Table 6.

compared to commercial coatings. Under current experimental conditions, the detection limits are at low ng/mL levels for most of the compounds studied. Calibration curves (peak area counts against analyte concentrations) are linear at least within 2 orders of magnitude. For example, for the model compounds, good linear relationships were obtained in the listed concentration ranges, as shown in Table 6. The in-tube SPME method can generally enhance sensitivity more than 10 times for most of the compounds studied relative to liquid injection method (10-μL injection). The extraction efficiency can be further increased by using a relatively larger volume of sample, and by increasing the extraction time.

Stability of In-Tube PPY Coating. The stability of PPY coating for in-tube SPME is comparable to or better than commercial coatings tested because PPY is stable in most of the mobile phases that are used for HPLC. No significant changes in its extraction performance were observed after hundreds of extractions over several months during this study. More importantly, PPY is stable over a pH range of 1.5−10.0. This quality of a coating provides an advantage for manipulation of the extraction efficiency and selectivity on the basis of the acid−base property of the analytes, especially for basic, acidic, ionic compounds and amphiprotic species.

Analysis of Water Samples. Tap water, lake water, and deioned water samples spiked with analytes of three different concentrations (50, 100, and 200 ng/mL, respectively, for the model compounds; 10, 50, and 100 ng/mL for both PAHs and aromatic amines) were analyzed. The results were compared to those of nonspiked water samples. The recoveries of the analytes from sample matrixes compared with a pure water matrix were between 89.6 and 96.4%. No analytes were found in drinking water and lake water samples. However, a small peak at the retention time of DEP was detected from a deionized water sample. More importantly, the intensity of this peak increased significantly for a deionized water sample that was stored in a plastic bottle for about 3 months. This result suggests that this compound might be leaching from the plastic bottle. Further studies to identify the peak and the sources of the compound are necessary, although a similar result was also found by other researchers.[33]

CONCLUSIONS

In this study, a PPY-coated capillary has shown higher extraction ability toward polycyclic aromatic compounds than toward mononuclear aromatics due to the increased $\pi-\pi$ and hydrophobic interactions between the polymer and the analytes. The extraction efficiency of PPY to polar aromatics is higher than that to nonpolar ones because of the additional interactions between polar components of the polymer and the analytes. These results are consistent with the expectations from the structure of PPY and its interactions with analytes. On the other hand, these results also indicate that SPME can be a simple and useful method for studying the properties of materials such as polypyrrole by using compounds having known properties.

The higher extraction efficiency of PPY compared to commercial coatings that was demonstrated in this study highlights the importance of developing new coating materials for SPME to extend its application areas. Due to the multifunctional properties of PPY, the method can be extended to other groups of analytes with little modification.

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