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Solid-phase extraction of polycyclic aromatic hydrocarbons in surface water

Negative effect of humic acid

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Abstract

The effect of humic acid on solid-phase extraction of polycyclic aromatic hydrocarbons (PAHs) from surface water was studied. The hydrophobic PAHs show significant association with humic acid, and this was confirmed to be the cause of negative effect when conventional reversed-phase solid-phase extraction (RP-SPE) was employed to extract the analytes from aqueous samples. As an alternative, dynamic ion-exchange (DIE) SPE could simultaneously extract both the fraction of the analytes which was associated with humic acid, and that which was freely dissolved. Using the 16 US Environmental Protection Agency priority PAHs as model compounds, the recoveries of the highly hydrophobic components by DIE-SPE were 10–30% higher than those by RP-SPE for a 1000-ml water sample dissolved with Aldrich humic acid (of 4.1 mg/l dissolved organic carbon content). A similar result was also obtained for 500 ml of natural surface water although the difference in recoveries between the two methods for this sample was smaller than that for the simulated sample. For validation of the method, the artifacts in connection with DIE-SPE in extracting the fraction of analytes which was freely dissolved and that which was associated with humic acid were investigated. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants, and many of them are carcinogenic or mutagenic. The development of analytical methodologies for PAHs in various environmental samples is an important aspect of environmental analytical chemistry [1]. In this respect, on-line or

off-line solid-phase extraction (SPE) has been extensively studied for trace enrichment of PAHs in tap water and surface water [2–7]. PAHs are typical non-polar compounds and have excellent retention on a reversed-phase adsorbent such as C₁₈ bonded silica. This, however, does not mean that trace enrichment of PAHs with SPE is an easy task. As a matter of fact, PAHs have remained the compounds that are most difficult to be applied to SPE. This may be due to several physical and chemical factors. One well-known reason is that the analytes may be lost by adsorption on the walls of the vessels. This is in particular the problem of surface water, for which a

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filtration is usually needed before sample application, and this will increase the loss of the analytes.

The effect of humic acid, which will be studied in this work, is primarily a matter of interest in environmental chemistry in that the association of organic pollutants to humic acid may affect their transport and fate in the global environment. For SPE, the negative effect of humic acid has been found for pesticides and polychlorinated biphenyls (PCBs) with common C_{18} silica [8–10]. The content of humic acid in common surface water is 1–5 mg/l [11], and in some cases it was found to be as high as 10 mg/l or more [12–14]. In general, typical non-polar pollutants such as PAHs may have high potential to be associated to humic acid. As a rugged analytical method for surface water, sample preparation techniques that avoid the negative effect of humic acid are always desirable. In this respect, liquid–liquid extraction (LLE) and SPE that can isolate the organic analytes associated with humic acid have been studied [11,15]. Although those methods were demonstrated to be effective, the operations were essentially time-consuming, because digestion of the humic acid was carried out before extraction of the analytes.

In this work, a novel implementation of SPE, which we have termed dynamic ion-exchange solid-phase extraction (DIE-SPE) [16,17], is evaluated for preconcentration of PAHs in surface water to obviate the negative effect of the humic acid. The 16 US Environmental Protection Agency (EPA) priority PAHs are used as model compounds. The stationary phase for DIE-SPE is C_{18} bonded silica loaded with a long-carbon-chain surfactant such as cetyltrimethylammonium bromide (CTAB), and acts as both an ion exchanger and a reversed-phase adsorbent. The high potential of DIE-SPE to extract humic acid from water was also observed previously [16,17]. If all PAHs in surface water are simply separated into the fraction that is freely dissolved and in another one that is associated with humic acid, both states of analytes can be handled by DIE-SPE, as the former can be extracted through hydrophobic interaction while the latter will be retained along with the humic acid through ion exchange. Insofar as the analytes associated with humic acid can be effectively desorbed when the eluting solvent is percolated through the cartridge, the negative effect will be obviated.

This study is essentially a comparison of reversed-phase (RP) SPE and DIE-SPE for the preconcentration of PAHs from surface water containing humic acid. The causes and extent of the negative effect of humic acid on RP-SPE are investigated. As an implementation of a rugged method of DIE-SPE, the conditions for extraction of the fraction of the analytes which is freely dissolved and of those which are associated with humic acid are validated.

2. Experimental

2.1. Reagents and chemicals

All organic solvents were HPLC- or pesticide-grade from Fisher Scientific (Fair Lawn, NJ, USA) and J.T. Baker (Phillipsburg, NJ, USA). Milli-Q (Millipore, Milford, MA, USA) water was used as reagent water. CTAB (analytical-reagent grade), was purchased from Tokyo Kasei (Tokyo, Japan). Solid CTAB was dissolved in water for a solution of 0.025 M with the aid of sonication. The PAH standards were obtained from Supelco (Bellefonte, PA, USA) as a reagent kit which contains all the 16 EPA priority PAHs. Individual stock solutions at concentrations of 100 $\mu\text{g}/\text{ml}$ each were prepared in dichloromethane–acetone (50:50). The working standard solution was prepared by mixing each of the PAH stock solutions and diluting with dichloromethane–acetone (50:50) for a final concentration of 2 $\mu\text{g}/\text{ml}$. *p*-Terphenyl (analytical-reagent grade) from Tokyo Kasei was used as internal standard (I.S.) in gas chromatography–mass spectrometry (GC–MS) analysis. The standard solution for this compound was prepared in benzene at a concentration of 8 $\mu\text{g}/\text{ml}$. All the standard solutions were stored in the dark at 4°C to prevent photolysis of the compounds. Aldrich (Milwaukee, WI, USA) humic acid (sodium salt) was used for production of artificial aqueous samples by directly dissolving in tap water. The samples were filtered through a 0.45- μm PTFE filter membrane before being spiked with the PAHs and extracted. For SPE, Bakerbond and Ultra-Sep C_{18} cartridges were obtained from J.T. Baker and Lida (Kenosha, WI, USA), respectively. Both products have

500 mg C₁₈ bonded silica in 6-ml barrels. The Bakerbond cartridges are of high carbon content of 18.2%, and the Ultra-Sep cartridges are of low carbon content of 5.6%. The tap water was from our laboratory. The surface water was sampled from a pond in a local park. The surface water was filtered through a 0.45- μ m PTFE membrane before being spiked with the PAHs and applied to the cartridges. The dissolved organic carbon (DOC) of the sample was measured to be 9.1 mg/l with a Shimadzu (Tokyo, Japan) TOC-5000 total organic carbon analyzer.

2.2. SPE

SPE was carried out in the positive pressure mode and nitrogen was used as the source of pressure. Before sample application, the cartridge was cleaned with 5 ml of benzene and air-dried for 1 min, and then sequentially conditioned with 5 ml of methanol and 5 ml of water. For DIE-SPE, the cartridge was further conditioned with 5 ml (or indicated below) of 0.025 M CTAB solution. The flow-rate of the sample through the cartridge was controlled at 10 ml/min by adjusting the nitrogen pressure. After all the sample had percolated through the cartridge, nitrogen was allowed to continue to flow through the apparatus to dry the wall of the separating funnel, and the cartridge was dismantled from the apparatus and centrifuged at 2500 rpm for 5 min to remove most of the residential water in the adsorbent bed. After that, the cartridge was dried with an air stream for 10 min; 200 μ l of acetone was added and the cartridge was dried for another 10 min. An aliquot of 5 ml of benzene was used to rinse the separating funnel and sequentially elute the PAHs from the cartridge. Another aliquot of 5 ml of benzene was used to rinse the separating funnel for the second time and the solution was mixed with the eluate from the cartridge. After 50 μ l of I.S. was added, the eluate was mixed with a vortex-mixer and concentrated at 45°C with a nitrogen stream to ~0.5 ml. The sample was then transferred to a 1.5-ml sample vial and adjusted to 1.0 ml with benzene before GC–MS analysis. (Note: benzene is toxic and harmful to health, operations involving use of the solvent should be conducted in a fume hood).

2.3. GC–MS analysis

A Shimadzu QP-5000 GC–MS system equipped with a Model AOC-17 autosampler was used for PAH analysis. Chromatographic separation of the 16 PAHs and the internal standard was accomplished with a DB-5 (J & W, Folsom, CA, USA) fused-silica capillary column (30 m \times 0.32 mm I.D., 0.25 μ m film thickness). Helium was the carrier gas at a flow-rate of 2.0 ml/min, and an inlet split ratio 1:20 was used. Sample injection was in the splitless mode with an injection volume of 2 μ l and an injection time of 2 min. The GC oven temperature programme was as follows: initial temperature 50°C held for 2 min and then ramped at 15°C/min to 220°C followed by another ramp of 5°C/min to 300°C, held for 1 min. The temperatures of the injection port and the interface with the MS system were set at 300°C. For selected ion monitoring (SIM), the voltage of the detector was 1.5 kV, and for each compound, one ion was chosen for quantification while two other ions were for identification. The retention times and quantitative ions for the analytes and I.S. are listed in Table 1. Linear calibration curves could be obtained for all the PAH components across a 50-fold concentration range; typically, $R^2 > 0.999$. The instrumental detection limit was measured to be from 2 (for the two-ring and three-ring PAHs) to 10 pg/injection (for five-ring and six-ring PAHs).

3. Results and discussion

3.1. Method establishment and comparison

PAHs are typical non-polar compounds; eluting the analytes from a C₁₈ cartridge is usually carried out with a non-polar solvent. It was found that the potential of organic solvent in eluting the PAHs was not affected, whether the cartridge was conditioned with CTAB (DIE-SPE) or not (RP-SPE). Three non-polar solvents, hexane, dichloromethane and benzene were tested as the eluent. Hexane was found to be inefficient in eluting some of the five- and six-ring PAH components. Both dichloromethane and benzene could completely elute the PAH analytes from the C₁₈ cartridges. However, dichloromethane also eluted a small portion of the humic substance

Table 1
Retention times and quantitative ions for GC–MS (SIM) analysis of PAHs

Compound	Retention time (min)	Quantitative ion (<i>m/z</i>)
Naphthalene	7.24	128
Acenaphthylene	9.92	152
Acenaphthene	10.24	153
Fluorene	11.10	166
Phenanthrene	12.71	178
Anthracene	12.79	178
Fluoranthene	14.96	202
Pyrene	15.44	202
<i>p</i> -Terphenyl ^a	16.05	230
Benz[<i>a</i>]anthracene	18.88	228
Chrysene	19.00	228
Benzo[<i>b</i>]fluoranthene	22.65	252
Benzo[<i>k</i>]fluoranthene	22.73	252
Benzo[<i>a</i>]pyrene	23.74	252
Indeno[1,2,3- <i>cd</i>]pyrene	27.61	276
Dibenz[<i>a,h</i>]anthracene	27.76	278
Benzo[<i>ghi</i>]perylene	28.40	276

^a Internal standard.

extracted to the cartridge, as evidenced by the characteristic color of the eluate after it was concentrated to a small volume. In comparison, benzene is the most suitable solvent in terms of selectivity and efficiency. The ability of benzene to elute the portion of PAH analytes that are associated with the humic acid has been substantiated by the following two experimental facts. First, a sample of 1000 ml tap water and a sample of 1000 ml tap water with Aldrich humic acid dissolved were spiked with the PAHs and subjected to DIE-SPE under identical conditions. No obvious lowering of the recoveries of the PAHs was found for the sample with humic acid content (see Table 2). Second, the PAHs on the cartridge for the sample of humic acid content were eluted with an aliquot of 5 ml benzene. Another aliquot of 5 ml benzene was used to elute the same cartridge; no obvious amount of PAHs was detected in this eluate.

Recoveries of the 16 PAHs from a sample of 1000 ml tap water with RP-SPE and from samples of 1000-ml tap water with Aldrich humic acid dissolved with RP-SPE and DIE-SPE are given in Table 2. The DOC of the latter sample was 4.1 mg/l. As can be seen, all the 16 PAHs could be quantitatively extracted from the sample of 1000 ml tap water with RP-SPE. However, obvious lowering of the recoveries was found when the same method was used

for the sample with Aldrich humic acid dissolved. In comparison, the recoveries of the highly insoluble PAH components, i.e., those with >3 rings, were lowered to a greater extent. Additionally, this negative effect could be obviated when the DIE-SPE was used.

3.2. Cause of negative effect of humic acid for RP-SPE

The potential causes for the negative effect of humic acid with RP-SPE were evaluated with three tandem-cartridge systems with 1000 ml tap water and 1000 ml tap water with Aldrich humic acid dissolved as test samples. Systems 1 and 2 were constructed with two reversed-phase cartridges in series and were applied with the above two samples, respectively. System 3 was constructed with a reversed-phase and a dynamic ion-exchange cartridge in that sequence and applied with the sample of humic acid content. No significant amount of the PAHs was measured on the bottom cartridge of system 1. The recoveries of the PAHs on the bottom cartridges of the systems 2 and 3 are listed in Table 3.

Tap water contains minimal humic acid, and the portion of PAHs that is associated with the humic acid in this sample is negligible. As found, all the

Table 2

Comparison of percentage recoveries of DIE-SPE and RP-SPE of PAHs from 1000 ml of tap water and 1000 ml of tap water dissolved with Aldrich humic acid

Compound	Recovery (%)		
	RP-SPE (sample 1 ^a)	RP-SPE (sample 2 ^b)	DIE-SPE (sample 2 ^b)
Naphthalene	93.3	89.5	92.3
Acenaphthylene	81.4	91.2	95.0
Acenaphthene	86.5	94.0	98.0
Fluorene	89.5	94.8	98.1
Phenanthrene	96.0	95.6	104.6
Anthracene	93.8	95.7	99.6
Fluoranthene	94.5	85.8	97.8
Pyrene	94.5	83.8	97.0
Benz[<i>a</i>]anthracene	91.9	74.9	95.3
Chrysene	92.9	78.4	96.6
Benzo[<i>b</i>]fluoranthene	89.7	62.4	93.2
Benzo[<i>k</i>]fluoranthene	90.6	72.5	93.6
Benzo[<i>a</i>]pyrene	89.5	66.8	92.7
Indeno[1,2,3- <i>cd</i>]pyrene	86.0	67.9	94.8
Dibenz[<i>a,h</i>]anthracene	89.7	78.1	93.8
Benzo[<i>ghi</i>]perylene	88.1	71.1	94.4

^a Sample 1, 1000 ml of tap water, spiking level, 0.1 ng/ml.

^b Sample 2, 1000 ml of tap water dissolved with Aldrich humic acid, DOC 4.1 mg/l, spiking level, 0.1 ng/ml. For DIE-SPE, the cartridge was conditioned with 5 ml of 0.025 M CTAB.

Table 3

Percentage recoveries of PAHs on the bottom cartridges of two tandem-cartridge systems for 1000 ml of tap water dissolved with Aldrich humic acid

Compound	Recovery (%)	
	System 2 ^a	System 3 ^a
Naphthalene	NA	NA
Acenaphthylene	ND	0.3
Acenaphthene	ND	0.2
Fluorene	ND	0.4
Phenanthrene	ND	1.4
Anthracene	ND	1.6
Fluoranthene	2.4	8.2
Pyrene	3.3	11.7
Benz[<i>a</i>]anthracene	5.3	19.4
Chrysene	4.4	16.2
Benzo[<i>b</i>]fluoranthene	6.0	34.1
Benzo[<i>k</i>]fluoranthene	5.3	26.7
Benzo[<i>a</i>]pyrene	5.1	28.7
Indeno[1,2,3- <i>cd</i>]pyrene	3.6	26.5
Dibenz[<i>a,h</i>]anthracene	2.6	15.4
Benzo[<i>ghi</i>]perylene	3.7	21.1

^a System 2: two cartridges in RP-SPE mode. System 3: two cartridges in RP-SPE and DIE-SPE mode. Sample, 1000 ml of tap water dissolved with humic acid, DOC 4.1 mg/l, PAHs spiking level, 0.1 ng/ml. NA, Not available. ND, Not detected.

analytes in this sample could be satisfactorily extracted with the 500 mg Bakerbond C₁₈ cartridge. In comparison, the varying recoveries of the PAHs on the bottom cartridges of systems 2 and 3 can be considered to correspond to part or all of the negative effect of the humic acid for RP-SPE. The percentage of the passthrough was 10–30% for PAHs with >3 rings. An inspection of the recovery data in Table 3 reveals that DIE-SPE gave much higher recoveries for the portion of PAHs that was associated with the humic acid than RP-SPE. This result is consistent with the efficiency of RP-SPE and DIE-SPE cartridges in extracting humic acid from water, as evidenced by the gathering of the colored humic substance on the white adsorbent bed of the C₁₈ cartridge during sample application. As a matter of fact, the humic acid could be exhaustively extracted after the sample was percolated through the DIE-SPE cartridge, and accordingly, all the PAHs associated with the humic acid were efficiently extracted. This fact is also supported by the data in Table 2, since quantitative extraction of all the PAHs from the 1000 ml tap water of humic acid content was obtained with DIE-SPE.

Moreover, from comparison of the recovery data

of the PAHs with system 3, it can be concluded that the negative effect of the humic acid in RP-SPE could be attributed to the association of PAHs with humic acid. This can be inversely proved from the retention mechanism of reversed-phase chromatography. Compared to other PAH components, the two- and three-ring PAHs are less hydrophobic and show lower retention ability on the C_{18} bonded silica. If saturation of the adsorbent occurred, the less hydrophobic PAH compounds should be more affected and have higher recoveries on the bottom cartridges of systems 2 and 3. This is in contrast to the data in Table 3.

For the SPE mechanism, it is noteworthy that insufficient retention of the organic analytes on the reversed-phase cartridge as a consequence of association with the humic acid is different from the breakthrough of the analytes due to chromatographic displacement or saturation of the solid phase. In the case of the breakthrough, the organic analytes eluted from the first cartridge can be sequentially extracted with a backup cartridge of the same type and the recoveries of the organic analytes on the two cartridges are comparable, or the extraction is quantitative if no breakthrough appears for the backup cartridge. This is indicated by an example in which a low carbon content Ultra-Sep C_{18} cartridge (5.7%) instead of the high carbon content Bakerbond cartridge (18.2%) was used for extraction of the PAHs from a sample of 1000 ml tap water. Under the conditions, naphthalene, acenaphthylene, acenaphthene and fluorene had apparent breakthroughs. When a second Ultra-Sep cartridge was used as the backup, the analytes that were eluted from the first cartridge were readily extracted by the second one.

The null recoveries of the PAHs on the bottom cartridge of system 1 illustrate that when freely dissolved in water, the non-polar analytes like PAHs can be exhaustively extracted (100%) by the C_{18} cartridge. It is worthwhile to make this fact clearer because it may be of practical significance. In this respect, reversed-phase separation with C_{18} bonded silica has been widely used for measuring the partition coefficients of association of organic pollutants to humic acid [18]. A prerequisite for the method is that all the organic analytes eluted out of the reversed-phase cartridge are those that are associated with the humic acid. This is substantiated by the

above experimental facts. Additionally, on the basis of the above findings, we also established a method that has been used to study the colloid formation of trace PAHs in water, which is the topic of fundamental importance to the study of the environmental effects of non-polar pollutants, such as PAHs.

3.3. Artifacts on recoveries of PAHs with DIE-SPE

In the practice of DIE-SPE, loading the long-carbon-chain ionic surfactant onto the C_{18} cartridge bed is an essential step prior to sample application. When DIE-SPE is used to extract the non-ionogenic compounds like PAHs, an obvious question is: will the efficiency of the solid phase in adsorbing the freely dissolved analytes be affected by the presence of the surfactant as the limitation of adsorption capacity of the cartridge? The so-called solid-phase saturation effect by CTAB was found previously for preconcentration of acidic herbicides with DIE-SPE under circumstances of low pH and large amounts of CTAB being used to condition the cartridge [17]. The effect of CTAB on extraction of the freely dissolved PAHs was studied here with aqueous samples of different pH and with varying amounts of CTAB used to condition the cartridges. Mechanistically, the retention of the freely dissolved PAHs on the CTAB-loaded C_{18} cartridge can be thought to be a competition between the organic analytes and the CTAB for the reversed-phase adsorption sites of the solid-phase. PAHs have a strong retention on the reversed-phase material, and it was found in this work that only the recoveries of some of the less hydrophobic two- or three-ring PAHs were affected under a circumstance of simultaneous low sample pH and large amount of CTAB used to condition the cartridge. A comparison of the recoveries of the 16 PAHs from 1000 ml tap water of pH 3.0 with RP-SPE and DIE-SPE is shown in Table 4, where the cartridge for DIE-SPE was conditioned with 10 ml of 0.025 M CTAB. It was found previously [17] that the solid-phase saturation effect of CTAB was related to the sample pH and could be explained by the change of the electrostatic interaction between CTAB and the ionized residual silanols of the bonded silica with pH of the sample. This explanation is also applicable to extraction of the PAHs.

Table 4
Comparison of percentage recoveries of DIE-SPE and RP-SPE of PAHs from an acidic water sample

Compound	Recovery ^a (%)	
	RP-SPE	DIE-SPE ^b
Naphthalene	87.5	43.5
Acenaphthylene	85.6	64.9
Acenaphthene	91.7	73.0
Fluorene	90.1	76.3
Phenanthrene	94.0	89.0
Anthracene	87.1	85.9
Fluoranthene	94.8	93.3
Pyrene	95.0	93.6
Benz[<i>a</i>]anthracene	91.6	93.9
Chrysene	93.7	93.2
Benzo[<i>b</i>]fluoranthene	90.3	94.3
Benzo[<i>k</i>]fluoranthene	92.3	94.1
Benzo[<i>a</i>]pyrene	83.4	90.7
Indeno[1,2,3- <i>cd</i>]pyrene	86.9	93.2
Dibenz[<i>a,h</i>]anthracene	91.7	94.0
Benzo[<i>ghi</i>]perylene	89.3	93.4

^a A 1000-ml volume of tap water, pH 3.0, PAH spiking level: 0.10 ng/ml.

^b The cartridge was conditioned with 10 ml of 0.025 M CTAB solution.

Therefore control of sample pH is an effective approach to avoid the lowering of recoveries of the PAHs as the solid-phase saturation effect of CTAB. In fact, when the sample pH was higher than 4.0, no significant solid-phase saturation effect was observed for all the PAH components even when 10 ml of 0.025 M CTAB solution was used to condition the cartridge. In any case, the solid-phase saturation effect by CTAB is unlikely to appear in real environmental analysis for PAHs or the similar non-polar pollutants, because the pH of environmental water samples is seldom <5.0 [19].

On the other hand, the effectiveness of the DIE-SPE cartridge in extracting PAHs that are associated with the humic acid is dependent on its capacity to adsorb the humic acid from water, and this is related to the mass of adsorbent as well as to the amount of CTAB used to condition the cartridge. The adsorption capacity for humic acid was approximately assessed by percolating an aqueous sample of known humic acid concentration through the DIE-SPE cartridge. Accompanying the sample flowing through the cartridge, the frontier of the focused amber-

colored band of the humic acid would move downwards along the adsorbent bed. When it reached the lower frit, the humic acid began to elute out of the cartridge and the effluent appeared to have the characteristic color of the humic acid. The adsorption capacity of the DIE-SPE cartridge for the Aldrich humic acid was estimated to be more than 10 mg of DOC when the cartridge was conditioned with 5 ml of 0.025 M CTAB solution. However, for real environmental waters, the adsorption capacity for humic acid was obviously lower. This seems to be attributable to the fact that the natural aquatic humic acid is more hydrophilic and has a lower breakthrough volume on the DIE-SPE cartridge, or the fact that the aquatic humic acid has a smaller molecular mass and higher specific charge on their molecules. In the latter case, the same amount of the humic acid trapped will occupy more ion-exchange sites on the surface of the DIE-SPE cartridge. For practical work, increasing appropriately the amount of CTAB to condition the cartridge can improve the capacity of the DIE-SPE in adsorbing humic acid.

3.4. Real application

The established DIE-SPE method was applied to isolate the PAHs from a sample of 500 ml of pond water. Before extraction, the sample was filtered and spiked with the PAH mixture with the procedure described in the Experimental section and 20 mg/l of NaN₃ was added to prevent bacterial growth and decomposition of the analytes. For comparison, the same sample was concurrently extracted by RP-SPE and DIE-SPE, and for the two procedures the extraction was conducted in half an hour, and 2 days after the PAH analytes were spiked. The recovery data obtained are tabulated in Table 5. As can be seen, with RP-SPE the recoveries of five- and six-ring PAHs are obviously lower than those obtained with DIE-SPE and the difference appeared to increase when the spiked sample was left for 2 days. The results illustrate that the negative effect of humic acid also appeared for the surface water when conventional RP-SPE was used to isolate the PAHs. On the other hand, the quantitative recoveries obtained with DIE-SPE for all the PAHs illustrate that the negative effect of the humic acid could be obviated with this procedure. Moreover, the in-

Table 5

Comparison of percentage recoveries of DIE-SPE and RP-SPE of PAHs from 500 ml of surface water stored for different periods of time after spiking

Compound	Recovery (% , mean+RSD, %) ^a			
	RP-SPE (0 days)	DIE-SPE (0 days)	RP-SPE (2 days)	DIE-SPE (2 days)
Naphthalene	80.7 (19.9)	85.2 (20.4)	75.0 (9.2)	78.5 (7.6)
Acenaphthylene	92.2 (2.9)	92.6 (5.0)	83.5 (3.1)	79.5 (6.5)
Acenaphthene	95.1 (3.3)	94.4 (4.8)	88.5 (1.0)	86.5 (7.1)
Fluorene	95.4 (3.0)	94.6 (4.8)	90.3 (2.2)	88.5 (5.5)
Phenanthrene	100.9 (1.5)	101.6 (3.3)	89.1 (5.4)	94.9 (4.3)
Anthracene	94.0 (1.6)	94.3 (4.0)	89.8 (2.2)	90.6 (2.6)
Fluoranthene	93.8 (2.5)	92.8 (1.6)	90.1 (3.4)	95.7 (2.0)
Pyrene	96.8 (5.8)	95.6 (2.6)	92.8 (4.0)	97.8 (0.2)
Benzo[<i>a</i>]anthracene	93.6 (1.8)	98.2 (2.2)	94.0 (2.6)	98.1 (1.2)
Chrysene	90.6 (1.9)	92.3 (2.5)	94.0 (3.2)	95.9 (0.9)
Benzo[<i>b</i>]fluoranthene	88.9 (4.6)	97.3 (2.3)	87.0 (1.3)	95.9 (0.7)
Benzo[<i>k</i>]fluoranthene	86.4 (1.7)	93.8 (2.8)	85.8 (2.7)	93.5 (1.5)
Benzo[<i>a</i>]pyrene	87.9 (1.8)	97.8 (1.7)	82.5 (0.8)	92.9 (1.2)
Indeno[1,2,3- <i>cd</i>]pyrene	77.9 (3.7)	95.9 (2.6)	70.2 (3.0)	91.6 (1.7)
Dibenz[<i>a,h</i>]anthracene	82.4 (2.5)	97.5 (2.7)	82.3 (0.4)	96.4 (2.2)
Benzo[<i>ghi</i>]perylene	81.0 (2.0)	95.6 (3.4)	79.1 (1.2)	96.0 (2.1)

^a PAH spiking level: 0.1 ng/ml. Average of four replicate analyses.

creased negative effect of humic acid with time for the RP-SPE reveals that the association of the PAHs with the humic acid includes a slow process; this supports the findings of Driscoll et al. [15] for organochlorine pesticides. In fact, as found in our experiment [18], the time needed for the PAHs to reach association equilibrium with the humic acid can be more than 5 days and the fractions of the PAHs associated with the Aldrich humic acid after equilibrium is nearly two- to fivefold higher compared to those measured at the moment the analytes were spiked. This observation brings up the problem relating to trace enrichment for analysis of PAHs in aqueous samples of high humic acid content when extraction efficiency is studied. For those organic pollutants that show significant association with the humic acid, a particular period of equilibrium time is necessary to ensure that the fortified sample is representative of the real sample. This is because in real surface water the organic pollutants have enough time to reach the association equilibrium with humic acid, and the equilibrium process is speeded up by biological decomposition considering that the organic pollutants associated with the humic acid

generally have lower bioavailability than the freely dissolved components [20].

4. Conclusion

In conclusion, for preconcentration of trace PAHs from surface waters, the negative effect of humic acid was shown by RP-SPE. The problem could, however, be obviated with DIE-SPE, indicating the superiority of DIE-SPE for this type of work. The effectiveness of DIE-SPE in extracting organic analytes that are associated with humic acid may also find other applications. In our laboratory, DIE-SPE and RP-SPE have been combined in a tandem-cartridge system to measure the partition coefficients of association of PAHs to humic acid [18].

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