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Micro-Solid Phase Extraction of Polycyclic Aromatic Hydrocarbons in Water using either C_{18} or Molecularly Imprinted Polymer Membranes: Analytical Merits and Limitations

(Pengekstrakan Fasa Mikro Pepejal bagi Hidrokarbon Aromatik Polisiklik dalam Air Menggunakan Sama Ada Membran C_{18} atau Polimer Molekul Teraan: Kebaikan dan Kelemahan Analisis)

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ABSTRACT

Sample pre-treatment is often the bottleneck in an analytical process. Due to the drawbacks of conventional sample pre-treatment methods, microextraction utilizing lower amounts of adsorbents and organic solvents are therefore favoured. A micro-solid phase extraction (μ-SPE) technique coupled with gas chromatography-flame ionization detection (GC-FID) was successfully developed for the analysis of selected polycyclic aromatic hydrocarbons (PAHs), namely phenanthrene, fluoranthene, and pyrene, in environmental water. In this study, μ-SPE techniques using C_{18} and molecularly imprinted polymer (MIP) membranes were optimized, validated, and applied to the analysis of selected PAHs in environmental water samples. The analytical merits were compared, and the two methods were evaluated in terms of linearity, repeatability, and relative recovery. Under the optimal extraction conditions, both μ-SPE techniques using either C_{18} or MIP membranes as the adsorbents offered comparable ultratrace analysis of the selected PAHs in the range of 0.003 to 0.01 μg L^{-1}. The extraction strength of C_{18} membranes was superior to that of MIP membranes for the extraction of low molecular weights PAHs from water in the presence of humic acid as a matrix factor. The C_{18} membranes overcome the non-covalence interaction between PAHs and humic acid and thus achieve better recovery.

Keywords: C_{18}; humic acid; micro-solid phase extraction; MIP; polycyclic aromatic hydrocarbons

ABSTRAK

Pra-rawatan sampel selalu menjadi halangan dalam satu proses analisis. Disebabkan kelemahan yang timbul dalam kaedah pra-rawatan sampel yang konvensional, mikro pengekstrakan yang menggunakan amaun penjerap dan pelarut organik yang lebih rendah adalah lebih disukai. Satu teknik pengekstrakan fasa mikro pepejal (μ-SPE) bergabungan kromatografi gas-pengesanan pengionan nyala (GC-FID) telah berjaya dibangunkan untuk analisis hidrokarbon aromatik polisiklik (PAHs) terpilih, iaitu fenantrena, fluorantena dan pirena, dalam air alam sekitar. Dalam kajian ini, teknik μ-SPE menggunakan C_{18} dan polimer molekul teraan telah dioptimum, divalidasi dan diaplikasi dalam analisis PAHs terpilih dalam sampel air alam sekitar. Kebaikan analitikal dibandingkan dan kedua-dua teknik dinilai daripada segi kelinearan, kebolehulangan dan perolehan semula secara relatif. Di bawah keadaan pengekstrakan yang optimum, kedua-dua teknik μ-SPE yang menggunakan sama ada membran C_{18} atau MIP sebagai penjerap menawarkan analisis ultra-surih PAHs terpilih yang setanding dalam lingkungan 0.003 hingga 0.01 μg L^{-1}. Kekuatan mengekstrak membran C_{18} adalah terunggul jika dibandingkan dengan membran MIP khususnya dalam mengektrak PAHs berjisim molekul rendah daripada air dengan kehadiran asik humik sebagai satu faktor matriks. Membran C_{18} mengatasi interaksi bukan kovalen yang wujud antara PAHs dan asik humik dan seterusnya mencapai perolehan semula yang lebih baik.

Kata kunci: Asid humik; C_{18}; hidrokarbon aromatik polisiklik; MIP; pengekstrakan fasa mikro pepejal

INTRODUCTION

Sample pre-treament is always a decisive step in a sensitive and selective analysis. Sample pre-treatment is applied to concentrate target analytes and exclude matrix effects, thereby reducing instrument maintenance and operation costs. Liquid-liquid extraction (LLE) is the most used sample pre-treatment technique because it provides exhautive and simple extraction. Solid phase

extraction (SPE) has received considerable attention as a sample pre-treatment technique, and it has emerged as an alternative to LLE because the conventional LLE may result in secondary pollution due to its high consumption of organic solvents. SPE provides selective multiresidue analysis with improved reproducibility because it uses various types of adsorbents. Miniaturized sample pre-treatment techniques, such as solvent microextraction and solvent extraction in a microdrop, were first demonstrated in 1996 (Jeannot & Cantwell 1996; Liu & Dasgupta 1996), and since then they have emerged as alternatives to both LLE and SPE. Microextraction techniques are eco-friendly, as they require only small amounts of organic solvents, chemicals, and adsorbents, which reduces secondary pollution.

Adsorbents applied in a sample pre-treatment technique have a significant effect on the selectivity and sensitivity of a method. An ideal adsorbent offers efficient and rapid adsorption, which indirectly reduces the extraction time and minimizes the operation costs. Silica-based C_{18} is among the most popular adsorbents, and it applied extensively in extraction techniques. C_{18} extracts a wide range of analytes with good recovery via hydrophobic interaction. Lab-made C_{18} was first synthesized using the sol-gel process and then applied as an adsorbent in SPE to pre-concentrate fluoxetine and its metabolite, norfluoxetine, from human plasma samples. Lab-made C_{18} offered comparable analyte recoveries but produced cleaner samples as compared to commercial C_{18} adsorbents (Domingues Nazario et al. 2014). Meseguer Lloret et al. (2002) successfully pre-concentrated and derivatised aliphatic amines from water samples using C_{18} SPE cartridges, and the method showed satisfactory ultra-trace detection limits ranging from 2 to 340 μg L^{-1} and greatly reduced the total analysis time to less than 25 min. In another study, magnetic Fe_3O_4-C_{18} composite nanoparticles and common C_{18} materials were applied for the enrichment of organophosphorous pesticides. The performances were comparable, but the extraction using magnetic Fe_3O_4-C_{18} composite nanoparticles in a beaker was easier and speeded up the cleanup as compared to C_{18}-based SPE (Shen et al. 2007). C_{18} micro-solid phase extraction (μ-SPE) tips were successfully applied to recover > 95% of posaconazole from the plasma matrix (Shen et al. 2006), which proved that C_{18} adsorbent can be widely applied in different extraction formats and matrices.

Molecularly imprinted polymer (MIP) is a tailor-made polymer processed using molecular imprinting techniques, and it consists of cavities with affinity to a target molecule. It is a selective adsorbent due to its specific molecular recognition cavities, and the extraction is based mainly on the 'lock and key' model. MIP is a very promising adsorbent when dealing with complex biological and environmental matrices (Brambilla et al. 2001; Chapuis et al. 2006; Deng et al. 2012; Madikizela et al. 2018; Zhang et al. 2011; Zhao et al. 2014). Study results have shown the potential of MIP as a selectivity tool to extract target analytes and exclude interferences from complex matrices.

Conventional C_{18} SPE cartridges and MIP SPE cartridges have been compared for their selectivity in retaining bisphenol A from milk samples (Alexiadou et al. 2008; Maragou et al. 2006). The absolute recovery achieved by the MIP SPE cartridge (85%) was superior to that obtained by the C18 SPE cartridge (56%). The limits of quantification and detection achieved by MIP SPE were 0.2 and 0.5 ng g^{-1}, respectively, which were much better than those offered by C_{18} SPE. These findings showed that MIP provided better recovery and selective recognition of bisphenol A from complex milk samples, thereby further improving the detection limit. However, comparison of MIP and C_{18} adsorbents using online SPE for the clean-up of zearalenone in beer samples showed almost equal analytical performance. The researchers concluded that the MIP applied behaved more like a reversed-phase sorbent for the extraction of zearalenone, which might explain the lack of significant difference in the selectivity between the two techniques (Lhotská et al. 2018). Martin et al. (2004) demonstrated that C_{18} SPE was superior to MIP SPE for the extraction of β-blocker from plasma. They found that MIP SPE offered poorer accuracy at low concentration due to the leaching of low-level template impurities.

The goal of this study was to compare the sensitivity and selectivity of MIP and C_{18} for the extraction of selected polycyclic aromatic hydrocarbons (PAHs) from water spiked with humic acid. C_{18} was chosen as the adsorbent in this study because the reversed-phase C_{18} has strong affinity for the non-polar PAHs that were selected as model compounds in this project. We propose that the universal C_{18} remains an excellent adsorbent for the retention of a broad spectrum of organic pollutants despite the development of MIP, which is known as a specific structural recognition adsorbent.

Materials and Methods

Reagents and chemicals

Acetonitrile (ACN) and methanol (MeOH) of HPLC grade, isopropanol (IPA), tetrahydrofuran (THF), hexane, chloroform, ethanol (EtOH), and sodium chloride (NaCl) were obtained from Merck, (Darmstadt, Germany). Bondesil - C_{18} (average particle size, 52 μm) was supplied by Agilent Technologies (La Jolla, CA, USA). The MIP for PAHs, called RENSA PAH MIP 4003 (average particle size, 58 μm), was obtained from Biotage, MIP Technologies

(Sweden). Humic acid (practical grade) was purchased from MP Biomedicals (Illkirch, France). Cellulose triacetate (CTA) and PAHs (phenanthrene (PHE), pyrene (PYR), and fluoranthene (FLA) were purchased from Sigma Aldrich (St. Louis, MO, USA). The double-distilled deionized water was purified using a Barnstead Nano ultrapure water system (Barnstead, NH, USA).

PREPARATION OF STANDARD SOLUTIONS AND SAMPLES

The standard stock solutions of PAHs (500 mg L^{-1}) were prepared individually. Approximately 0.005 g of FLA was weighed and added to a 10 mL volumetric flask and diluted to volume with MeOH, whereas 0.005 g of both PHE and PYR were weighed and added to 10 mL of volumetric flasks and diluted to volume with ACN. The standard stock solutions were stored at 0 °C in darkness when not in use. The serial working mixture standard solutions were diluted from the standard stock solutions using MeOH. The working standard solutions were prepared weekly and stored at 0 °C in darkness when not in use.

The environmental water samples (sea water, pond water, river water, and tap water) were obtained from selected locations in Terengganu, Kelantan, and Pahang, Malaysia. The samples were filtered through *Whatman ® Grade 1 filter paper purchased from* Sigma Aldrich to exclude the larger suspended particles prior to the extraction. The samples were kept in a freezer at -20 °C until analysis.

PREPARATION OF C_{18} AND MIP MEMBRANES

Approximately 0.04 g of CTA was weighed in a vial, and 2 mL of chloroform were added to the same vial. The solution was capped and left for at least 5 h at room temperature to allow for complete dissolution. Approximately 0.02 g of C_{18} was weighed in a glass Petri dish (inner diameter (I.D.) 47 mm). Next, 2 mL of the CTA solution were poured into the glass Petri dish containing the C_{18}. Then the resulting solution was sonicated for 2 min to ensure dispersal of C_{18} in the polymer matrix (Figure 1). The solution was then left in the fume hood for 3 h to gradually evaporate. The C_{18} membrane was peeled off the glass Petri dish by immersing it in deionised water. The film was then air dried and punched into small circular pieces (I.D. 5 mm). The procedures were then repeated using 0.005 g of MIP instead of C_{18}.

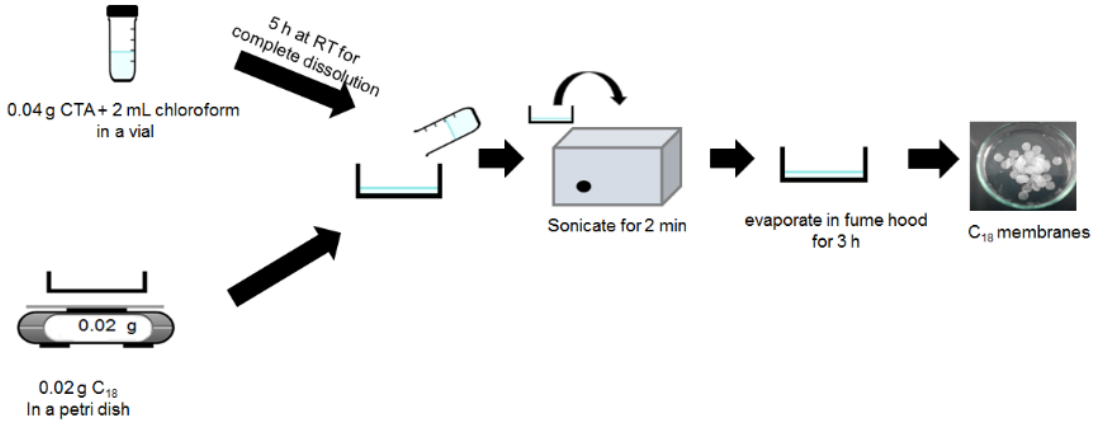

FIGURE 1. Schematic of C_{18} membranes preparation procedure

CHARACTERIZATION OF MEMBRANES

Minimal characterization was performed mainly to physically observe the homogeneity of the adsorbents that were immobilized within the CTA. The samples were observed under a DFC 450C microscope (Leica Microsystems Wetzlar, Germany) and evaluated using a Brunauer, Emmett, and Teller (BET) Quantachrome instrument (GmbH).

µ-SPE USING C_{18} AND MIP MEMBRANES AS ADSORBENTS

The sample solution (20 mL) was pipetted into a 25 mL sample vial. The C_{18} membranes (six pieces) were dipped into MeOH for 30 s to activate the C_{18}. The membranes were then tumbled into the sample solution. The vial was capped loosely and sonicated (Elmasonic, USA) for 25 min. The C_{18} membranes were then removed using forceps and transferred into a safe-lock centrifuge tube

containing 150 µL of IPA. Next, the tube was sonicated for 5 min to desorb the PAHs from the membranes. Finally, the PAHs in the IPA extract were quantitated using GC-FID. The procedures were then repeated for seven pieces of MIP, but with ultrasonication (extraction) for 30 min and desorption of the selected PAHs using 100 µL of IPA.

CHROMATOGRAPHIC CONDITIONS

All analyses were performed using a Shimadzu gas chromatography device (Kyoto, Japan) coupled with flame ionization detection (GC-FID). The separation was carried out on a BPX-5 column (30.0 m × 0.25 mm × 0.25 µm film thickness). Helium gas was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector and detector temperatures were fixed at 250 and 270 °C, respectively. The oven temperature was programed at 150 °C for 3 min, then increased to 250 °C at 10 °C/min and held for 6 min. All injections were performed in the splitless mode with 1 µL injection volume. The chromatographic data were processed using FID-Shimadzu GC Solution software.

OPTIMIZATION AND VALIDATION OF µ-SPE

The µ-SPE technique was optimized by modifying the amounts of adsorbents and membranes, ultrasonication time, extraction time, mechanical agitation speed, type of desorption solvent, desorption time, desorption volume, and salt addition in order to improve the amount of analytes extracted. Each variable was tested in triplicate, and one-way ANOVA was performed to determine whether there were any significant differences among the variables. The mean values were tested using Tukey's HSD at 95% confidence level.

The method was then assessed for linearity, relative recovery, limit of detection (LOD), limit of quantification (LOQ), and precision before sample anaysis. The LOD and LOQ were calculated based on signal-to-noise ratios of 3:1 and 10:1, respectively. The extraction strength of the C_{18} and MIP membranes towards PAHs was also assessed using humic acid as a matrix factor.

RESULTS AND DISCUSSION

CHARACTERIZATION OF C_{18} AND MIP MEMBRANES

Morphological characterization of C_{18} and MIP membranes was conducted using electronic microscopy and pore volume estimation *via* BET. Figure 2(a) shows images of CTA, C_{18}, and MIP membranes prepared for the µ-SPE analysis, and Figure 2(b) shows the membranes viewed under the Leica microscope. The images show that both C_{18} and MIP were homogeneously distributed within the CTA. Homogeneity of the adsorbent is important to ensure consistency in extraction repeatability. The certificate of analysis provided by the supplier stated the particles sizes of C_{18} and MIP were in the range of 47 to 60 µm and 32 to 100 µm, respectively. The distribution of the particle sizes of MIP was in a wider range, which was clearly observed under the Leica microscope.

The pore volumes of the CTA, C_{18}, and MIP membranes measured with BET were 0.175, 0.162, and 0.163 cm^3g^{-1}, respectively. The results indicated that both C_{18} (0.02 g) and MIP (0.005 g) occupied certain parts of the CTA membrane during impregnation. The pore volume in the CTA membrane allowed for adsorbent immobilization within the pores to function as an adsorbent membrane in the microextraction application. The distribution of the particle size of MIP was wider, which explains why slightly lesser amounts of MIP were required to occupy the pore volume of the CTA.The thickness of 10 membranes was measured using a digimatic vernier caliper. The thickness averages of the C_{18} and MIP membranes were 0.03 and 0.01 mm, respectively.

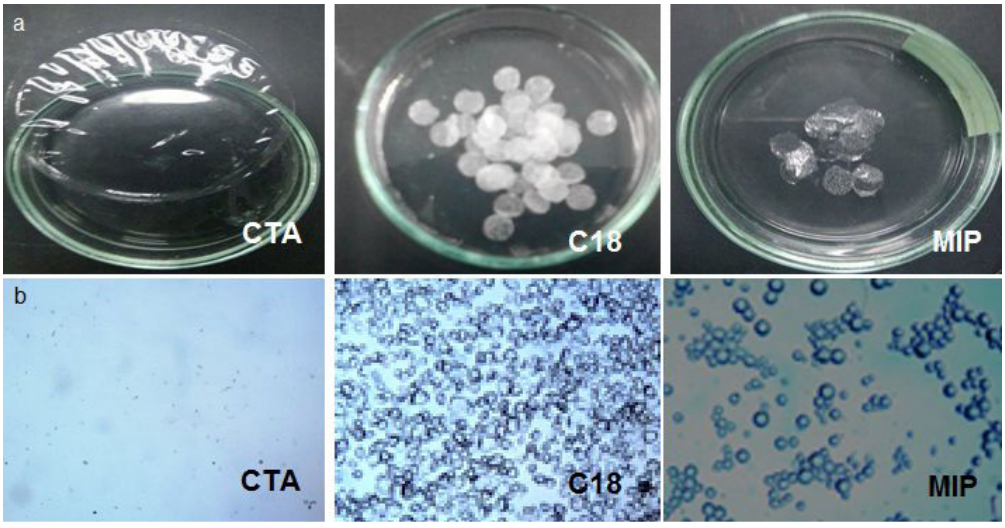

FIGURE 2. Images of CTA, C_{18}, and MIP membranes prepared (a) and viewed under a Leica microscope (b)

OPTIMIZATION OF THE μ-SPE TECHNIQUE USING C_{18} MEMBRANES AS ADSORBENTS

The following parameters were investigated for their effect of enrichment of selected PAHs (PHE, FLA, and PYR) from water samples: Amount of membrane, extraction time, agitation speed, type of desorption solvent, desorption time, volume of desorption solvent, and salt addition. Deionized water was spiked with each PAH at 500 μg L^{-1} for the optimization study, and triplicate extractions were carried out for each variable.

AMOUNT OF C_{18}

The concentration of CTA was fixed at 2% (w/v) because concentrations lower than 2% (w/v) resulted in membranes that were easily torn and not feasible for routine handling and membranes prepared with a concentration higher than 2% (w/v) were thicker and yielded lower peak areas during the random screening test.

C_{18} in amounts ranging from 0.01 to 0.04 g were immobilized in the 2% (w/v) CTA to form C_{18} membranes. The membranes were loose and not firm at C_{18} amounts of 0.03 and 0.04 g. Ultimately, 0.02 g of C_{18} was chosen as the optimal amount to produce the membranes for the extraction of selected PAHs because the peak areas for these PAHs were higher than those achieved when 0.01 g of C_{18} was used.

AMOUNT OF C_{18} MEMBRANE

The optimal amount of C_{18} membrane was determined by testing five to eight pieces of the circular membranes. Figure 3(a) shows that the peak areas of the extracted PAHs increased as the number of membrane pieces increased; this occurred because more membranes provided more adsorption sites for the extraction of PAHs. No significant difference ($p > 0.05$) in the extraction efficiency was detected between six and seven pieces, and the peak areas remained unchanged at eight pieces, except the value for PHE decreased. Therefore, the six pieces were chosen for use in the subsequent experiments.

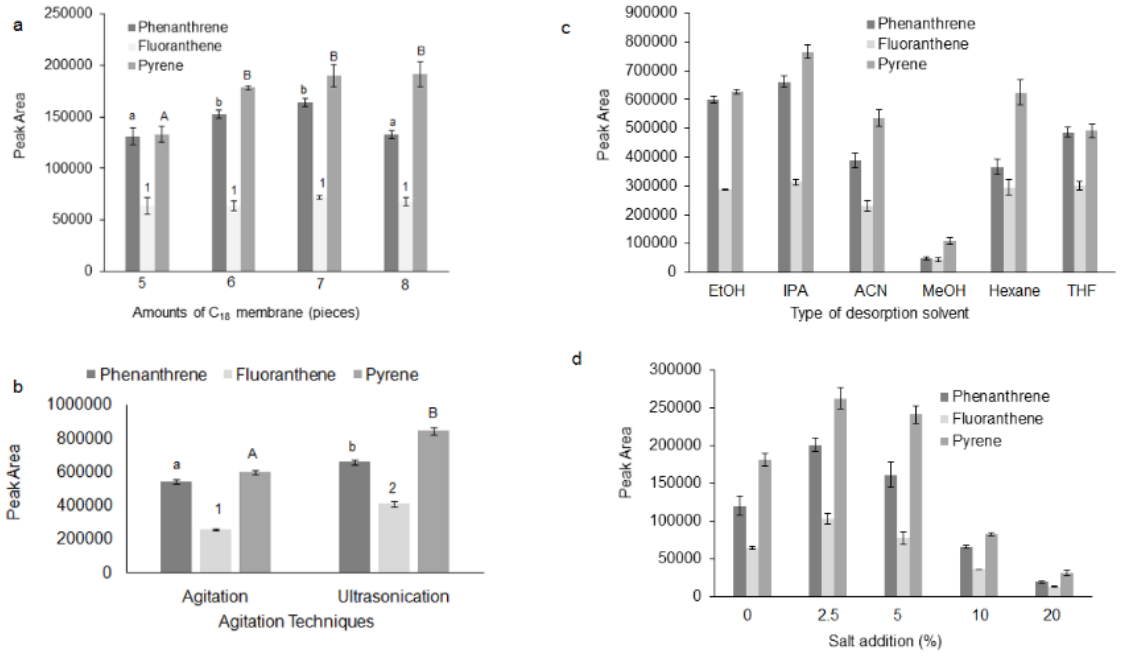

FIGURE 3. Effect of amounts of C_{18} membrane (a), agitation techniques (b), type of desorption solvent (c), and salt addition (d) on μ-SPE of selected PAHs from spiked deionized water samples using C_{18} membranes as the extraction tool. Error bars represent the standard deviations. Different letters and numeric numbers that are directly above the error bars indicate significant differences according to ANOVA-Tukey's HSD test at $p < 0.05$

EXTRACTION TIME OR ULTRASONICATION TIME

In this study, extraction times ranging from 5 to 30 min were examined as the equilibrium time for the non-exhaustive extraction process. The peak areas of the targeted analytes increased when the extraction time increased from 5 to 25 min but then declined at 30 min. The peak areas did not plateau after reaching equilibrium at 25 min, but they did indicate a decline. This trend was also found in previous studies (Naing et al. 2016; See et al. 2010), which reported that the drop was due to back extraction of the analytes from the membranes into the sample solution. Based on these results, 25 min was used as the extraction time in the following experiments.

AGITATION SPEED AND TIME

Mechanical agitation is an important factor that promotes the mass transfer of analytes from the sample solution onto the adsorbent. Mechanical agitation was applied to the sample solution by stirring the sample solution in the range of 200 to 1000 rpm using a stir bar to replace the ultrasonication technique. The peak areas of the extracted PAHs were optimal at agitation speed of 600 rpm. Stronger vortex flow created at speeds above 600 rpm reduced the contact surface area between the analytes and the membranes, thereby decreasing the extraction efficiencies. Based on these results, 600 rpm was chosen for use in the subsequent experiments.

Agitation time ranging from 10 to 35 min was examined to determine the equilibrium time required for the analytes to be adsorbed on the C_{18} membranes. The extraction equilibrium was achieved at 30 min when the sample solution was agitated at 600 rpm. A decline in all peak areas was observed at 35 min. Therefore, agitation time was fixed at 30 min because all peak areas were optimal at this time.

COMPARISON BETWEEN ULTRASONICATION AND AGITATION TECHNIQUES FOR μ-SPE

Both ultrasonication and agitation were investigated and compared for the efficiency to accelerate the mass transfer of analytes from the sample solution onto the membranes. The extraction efficiencies for both techniques were compared using each of the respective optimum conditions for speed and extraction time. The peak areas (Figure 3(b)) for all of the targeted analytes were significantly higher when the ultrasonication technique was applied as compared to the agitation technique. Ultrasonication converts electrical energy into physical vibration, which causes intense convection in the sample solution, thereby leading to microturbulence for more efficient contact between the analytes and membranes (Naveena et al. 2015). This resulted in a 5 min shorter extraction time required to achieve equilibrium. Therefore, the ultrasonication technique was applied in the subsequent analysis.

TYPE OF DESORPTION SOLVENT, DESORPTION VOLUME, AND DESORPTION TIME

The solubility of analytes and the polarity of the solvent are important to determine the best type of desorption solvent, as PAHs are relatively hydrophobic and have strong interaction with the C_{18}. The organic solvents EtOH, IPA, ACN, MeOH, hexane, and THF were evaluated for use as the desorption solvent. Figure 3(c) shows that the mid-polar IPA was the most suitable desorption solvent, as it was less polar and was capable of completely desorbing the targeted PAHs.

The effect of desorption volume was tested in the range of 100 to 250 μL. The peak areas for all PAHs increased from 100 to 150 μL but decreased thereafter. The results indicated that 150 μL was sufficient for the immersion of the membranes and desorption of the selected PAHs from the membranes. Increasing the volume beyond 150 μL resulted in a dilution effect that did not favour analyte enrichment.

The membranes were subjected to ultrasonication to desorb the PAHs from the membranes after extraction, and desorption times ranging from 2 to 15 min were tested. The peak areas for all PAHs were optimal at 5 min. Peak areas decreased when desorption time was prolonged to 10 and 15 min. This occurred because extended desorption time leads to re-adsorption of the analytes onto the membranes and thus a drop in extraction efficiency was observed (Ge & Lee 2011). Therefore, 5 min was employed as the desorption time in subsequent experiments.

SALT ADDITION

Salt (NaCl) addition ranging from 0 to 20% (weight/volume) was investigated to study the effect of ionic strength of the sample on the extraction process. Increasing ionic strength by adding salt to the sample solution was reported to increase analyte sorption and is known as the salting out effect (Turner 2003). A slight salting out effect was observed when 2.5% of NaCl was added to the sample solution (Figure 3(d)) because traces of the selected PAHs with low to mid molecular weights were soluble in water. The selected PAHs in this study were semi-polar compounds; a slight increase in salinity therefore enhanced the extractability of PAHs onto the membranes.

OPTIMIZATION OF THE μ-SPE TECHNIQUE USING MIP MEMBRANES AS ADSORBENTS

The RENSA PAH MIP 4003 was chosen as an adsorbent in this study with the goal to achieve higher selectivity

for PAHs regardless of the existence of interferences in the environmental water samples. The concentration of CTA was fixed at 2% (w/v) because this concentration produced a workable membrane for routine analysis.

MIP at 0.001 and 0.005 g, respectively, was immobilized in two different dishes of 2% (w/v) of CTA to form MIP membranes. MIP at 0.001 g produced a very thin and almost transparent membrane, whereas MIP at 0.005 g produced a slightly filmy and thicker membrane. MIP at 0.005 g almost saturated the 2% (w/v) CTA membrane due to the light mass of MIP. The peak areas of the extracted PAHs were higher in the 0.005 g of MIP membranes than in the 0.001 g of MIP membranes, illustrating a direct efficiency increase from 0.001 to 0.005 g of MIP for the selected PAHs. Therefore, 0.005 g of MIP was used for further analysis.

Two to nine pieces of MIP membrane were investigated to determine the opitmal amount of MIP membrane for the µ-SPE process. Figure 4(a) shows that seven pieces resulted in the highest peak area of PAHs, and there was no significant increase in peak areas when more than seven pieces were applied. Therefore, this number was used for subsequent experiments.

Other optimum extraction conditions for this technique were similar to those identified for C_{18} membranes, except that the ultrasonication extraction required 30 min (Figure 4(b)) for both PHE and FLA, and 100 µL of IPA (Figure 4(c)) was sufficient to completely immerse the thinner MIP membranes. A slight prolonged extraction time for MIP-µ-SPE may indicate a slow interaction between the MIP membranes and the target analytes (Maier et al. 2004).

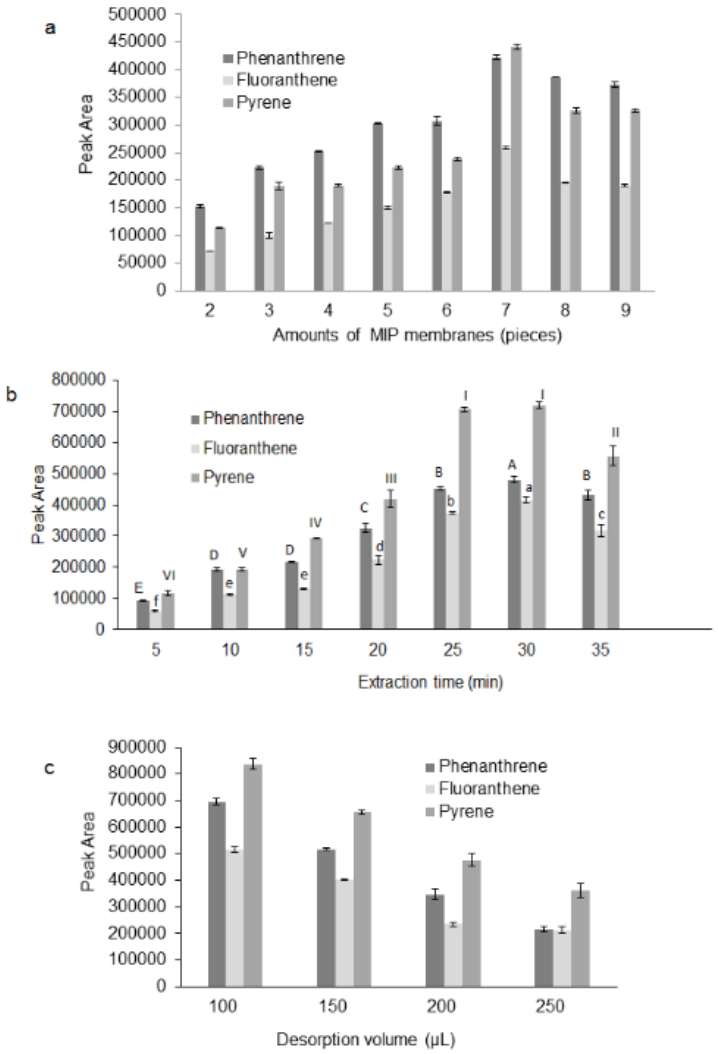

FIGURE 4. Effect of amounts of MIP membrane (a), extraction or ultrasonication time (b), and desorption volume (c) on µ-SPE of selected PAHs from spiked deionized water samples using MIP membranes as the extraction tool. Error bars represent the standard deviations. Different letters and Roman numerals that are directly above the error bars indicate significant differences according to ANOVA-Tukey's HSD test at $p < 0.05$

VALIDATION OF THE M-SPE TECHNIQUES

Table 1 summarizes the regression data and LODs for both μ-SPE techniques. Excellent linearity in the range of 0.01 to 1000 μg L^{-1} for FLA and PYR and 0.05 to 1000 μg L^{-1} for PHE was established, with correlation coefficients ≥ 0.9960 using both C_{18} and MIP membranes as adsorbents. The amount of MIP used to prepare membranes with similar dimensions was four times lower than that for C_{18}, although both adsorbents had rather similar average particle sizes (MIP = 58 μm, C_{18} = 52 μm). This indicated that MIP membranes had a greater ability to measure the selected PAHs than C_{18} in the similar range. Both μ-SPE techniques were comparable in offering ultratrace LODs ranging from 0.003 to 0.01 μg L^{-1} and 0.003 to 0.008 μg L^{-1} for C_{18} and MIP membranes, respectively, as adsorbent tools. The sensitivity of the μ-SPE using MIP membranes was slightly improved compared to that of the C_{18} membranes, except for FLA. In summary, the validation results obtained for both techniques were rather similar, except that MIP had greater capacity and sensitivity.

HUMIC ACID AS A MATRIX FACTOR AND RESULTS OF THE RELATIVE RECOVERY STUDY

Humic acid was applied as one of the matrix factors to assess the extraction efficiency of the μ-SPE techniques using C_{18} and MIP membranes as adsorbents. Table 2 shows that the hydrophobic PAHs had a significant association with humic acid, which resulted in a decline in relative recovery with increased amounts of humic acid. The presence of humic materials in water is known to modify the strength of PAH adsorption onto adsorbents (Conte et al. 2001). However, the selectivity of the C_{18} membranes was superior to that of the MIP membranes, which were synthesized based on specific structural recognition. This was illustrated by the fact that the extraction of selected PAHs was less affected by 50 mg L^{-1} of humic acid when C_{18} membranes were applied as the adsorbents. The addition of humic acid into the deionized water samples greatly decreased the extraction of selected PAHs by MIP membranes; addition of 20 mg L^{-1} of humic acid decreased the relative recovery of both FLA and PYR to < 80%. Humic acid may form a macromolecular structure with PAHs bound together by site complexation (Gauthier et al. 1986) and additive combination of partitioning and adsorption (Laor & Rebhun 2002). This resulted in low efficiency in the extraction of PAHs even though the MIP is known to be a structural recognition adsorbent. On the other hand, the extraction of PAHs by C_{18} membranes via hydrophobic interactions overcame the non-covalent interaction between PAHs and humic acid, thus achieving better recovery. Therefore, the C_{18} membrane is a more powerful adsorbent tool compared to the MIP membrane for the extraction of PAHs from environmental water, especially lake water containing 0.5 to 40 mg L^{-1} of humic substances (Thurman 1986).

The local environmental water samples (sea, river, tap, and pond water) were spiked with each of the PAHs at concentrations of 5 and 50 μg L^{-1}, respectively. Blank samples were analyzed to ensure that the blank concentration was deducted for the relative recovery study. Table 3 summarizes the excellent relative recoveries obtained in the range of 80.3 to 116.5%, with good repeatabilities of ≤ 9.7% for μ-SPE techniques using C_{18} or MIP membranes as adsorbent tools. The matrix effect was insignificant when the extraction of selected PAHs was perfomed using either μ-SPE technique. This result showed that both adsorbent membranes were comparable in terms of accuracy in the analysis of selected PAHs from the selected water samples that contained humic substances at concentrations < 4 mg L^{-1} (Thurman 1986). Therefore, matrix-match calibration was not required in analysing the samples.

TABLE 1. Validation data of μ-SPE for the analysis of selected PAHs in spiked deionized water

PAHs	Linearity range, μg L^{-1}		Correlation coefficients, r		Limits of detection, μg L^{-1}	
	C_{18} membranes	MIP membranes	C_{18} membranes	MIP membranes	C_{18} membranes	MIP membranes
PHE	0.05-1000	0.05-1000	0.9979	0.9992	0.009	0.008
FLA	0.05-1000	0.01-1000	0.9965	0.9990	0.01	0.003
PYR	0.01-1000	0.01-1000	0.9960	0.9998	0.003	0.003

TABLE 2. Relative recovery of 500 µg L^{-1} of each selected PAHs from deionized water spiked with humic acid ranging 0 to 500 mg L^{-1}

Concentration of humic acid, mg L^{-1}	PAHs	Average relative recovery ± RSD, % (n=3)	
		C_{18} membranes	MIP membranes
0	PHE	105.5 ± 7.6	104.7 ± 2.7
	FLA	101.3 ± 1.5	100.1 ± 3.3
	PYR	112.5 ± 1.8	93.8 ± 2.5
10	PHE	109.0 ± 0.6	98.7 ± 0.3
	FLA	104.3 ± 0.6	96.3 ± 0.4
	PYR	112.2 ± 1.0	89.1 ± 0.8
20	PHE	95.1 ± 1.3	95.2 ± 1.5
	FLA	88.6 ± 2.3	79.6 ± 3.8
	PYR	84.4 ± 2.0	73.8 ± 5.2
50	PHE	94.1 ± 3.0	86.5 ± 1.4
	FLA	87.4 ± 1.2	75.4 ± 3.3
	PYR	77.8 ± 2.5	73.9 ± 0.8
100	PHE	84.7 ± 3.4	82.3 ± 1.2
	FLA	73.0 ± 7.0	60.1 ± 3.3
	PYR	64.9 ± 8.6	53.9 ± 2.9
200	PHE	74.6 ± 2.5	61.4 ± 0.7
	FLA	71.6 ± 1.2	38.0 ±3 .6
	PYR	60.0 ± 3.3	31.0 ± 2.3

TABLE 3. Relative recovery study for the extraction of selected PAHs in environmental water using µ-SPE techniques

Samples	PAHs	Average relative recovery ± RSD, % (n=3)			
		Spiked at 5.0 µg L^{-1}		Spiked at 50 µg L^{-1}	
		C_{18} membranes	MIP membranes	C_{18} membranes	MIP membranes
River water	PHE	111.9 ± 9.7	84.1 ± 5.4	83.3 ± 2.7	92.5 ± 1.0
	FLA	109.4 ± 7.4	95.6 ± 6.6	92.9 ± 6.7	113.5 ± 6.4
	PYR	116.5 ± 6.7	81.3 ± 2.6	91.2 ± 7.3	109.8 ± 7.0
Sea water	PHE	91.9 ± 4.3	83.0 ± 3.1	81.5 ± 0.3	100.2 ± 3.5
	FLA	83.1 ± 4.9	84.5 ± 0.5	81.2 ± 1.1	91.4 ± 2.8
	PYR	87.4 ± 7.2	83.2 ± 1.5	88.8 ± 3.3	80.3 ± 0.9
Pond water	PHE	93.7 ± 2.6	99.4 ± 9.0	95.0 ± 2.4	92.8 ± 3.8
	FLA	108.7 ± 7.1	98.2 ± 7.2	89.5 ± 2.1	92.1 ± 1.2
	PYR	82.6 ± 1.6	95.3 ± 6.9	100.4 ± 3.6	92.7 ± 1.8
Tap water	PHE	86.8 ± 3.3	83.0 ± 2.3	82.9 ± 3.3	96.6 ± 6.5
	FLA	89.5 ± 3.2	85.8 ± 6.8	100.2 ± 6.4	90.7 ± 2.1
	PYR	80.5 ± 2.1	90.0 ± 5.0	83.1 ± 1.0	83.1 ± 4.5

Conclusion

This study showed that the μ-SPE technique using either C_{18} or MIP membranes as adsorbent tools was suitable for ultratrace analysis of selected PAHs in environmental water samples. However, MIP, which is characterised by specific structural recognition, was poorer at recovering the hydrophobic PAHs from water samples with high humic acid content. The universal C_{18} remains an excellent adsorbent that overcomes the interaction between PAHs and humic acid in environmental water via its hydrophobic interactions.

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