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Downsizing vacuum-assisted headspace solid phase microextraction

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ABSTRACT

Recently, we proposed a new headspace solid-phase microextraction (HSSPME) procedure, termed vacuum-assisted HSSPME (Vac-HSSPME), where headspace sampling of 10 mL aqueous sample volumes took place in 500 or 1000 mL sample containers under vacuum conditions. In the present study, we downsized the extraction device to a 22 mL modified sample vial and concluded that changes in the final total pressure of the pre-evacuated vial following sample introduction were sufficiently low to allow efficient Vac-HSSPME sampling. The downsized extraction device was used to extract five low molecular weight polycyclic aromatic hydrocarbons and several experimental parameters were controlled and optimized. For those compounds whose mass transfer resistance in the thin gas-film adjacent to the gas/sample interface controls evaporation rates, reducing the total pressure during HSSPME sampling dramatically enhanced extraction kinetics in the 22 mL modified vial. Humidity was found to affect the amount of naphthalene (intermediate K_H compound) extracted by the fiber at equilibrium as well as impair extraction of all analytes at elevated sampling temperatures. All the same, the high extraction efficiency and very good sensitivity achieved at room temperature and within short sampling times comprised the most important features of Vac-HSSPME in this downsized extraction device. Analytically, the developed method was found to yield linear calibration curves with limits of detection in the low ng L^{-1} level and relative standard deviations ranging between 1.3 and 5.8%. Matrix was found not to affect extraction.

1. Introduction

More than two decades of research effort in solid-phase microextraction (SPME) resulted in wide acceptance of this sample handling technique and in growing interest of both analysts and manufacturers. The initially developed "fiber-SPME" format continues to be the most common form of the technique for sampling directly the sample matrix or the headspace above it [1,2]. Direct and headspace SPME techniques are nowadays considered mature sample preparation methods suitable for use in routine and/or automated analysis by specialists and non-specialists alike.

We recently proposed a new headspace SPME (HSSPME) sampling procedure, termed vacuum-assisted HSSPME (Vac-HSSPME), where HSSPME sampling of aqueous sample volumes commonly used in HSSPME (e.g. 10 mL) takes place in 500 or 1000 mL sample containers under vacuum conditions [3,4]. Although reduced pressure conditions during HSSPME sampling are not expected to increase the amount of analytes extracted at equilibrium, they may dramatically improve extraction kinetics compared to regular HSSPME during the non-equilibrium stage of the sampling process due to the enhancement of evaporation rates in the presence of an air-evacuated headspace. Based on the theoretical model we have formulated [3], acceleration effects on extraction rates induced by reducing the total pressure of the sample container are expected to be important when the K_H value is close or below the reported threshold values for low K_H solutes (typical values: 1.2×10^{-5} [5,6] or 1.6×10^{-4} atm m^3 mol^{-1} [7] (1 atm = 1.01×10^5 Pa)). For these compounds, mass transfer resistance in the thin gas-film adjacent to the gas/sample interface controls evaporation rates and hence, reducing the total pressure will result in a faster overall extraction process [4]. On the other hand, for intermediate K_H compounds (K_H value between the above mentioned threshold values and less than 5×10^{-3} atm m^3 mol^{-1} [5,6] (1 atm = 1.01×10^5 Pa)), Vac-HSSPME is not expected to improve extraction rates compared to regular HSSPME since mass transfer resistance located in the thin liquid-film controls evaporation rates and this process is independent of the pressure conditions in the headspace [4]. Vac-HSSPME sampling may thus be particularly advantageous for low volatile compounds since extraction rates will dramatically increase under reduced pressure leading to enhanced sensitivity within short sampling times.

Hitherto, Vac-HSSPME was investigated in large sampling vessels. Although the effect of reduced pressure conditions was found to dominate over any effect of headspace volume on the extraction kinetics of low volatility compounds [4], manipulation of the 500 and 1000 mL containers may be cumbersome to routine

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Table 1
Main physicochemical properties of the five PAHs compounds investigated here.

Compound	Molecular weight	Vapor pressure 25 °C (mmHg)[a]	K_H (atm m^3 mol^{-1})[b]	Log K_{ow}
Naphthalene (Na)	128.18	0.085	4.4×10^{-4}	3.30
Acenaphthene (Ace)	152.21	0.00215	1.84×10^{-4}	3.92
Fluorene (Fl)	166.22	0.0006	9.62×10^{-5}	4.18
Phenanthrene (Phe)	172.24	0.000121	4.23×10^{-5}	4.46
Fluoranthene (Flu)	202.26	9.22×10^{-6}	8.86×10^{-6}	5.16

[a] 1 mmHg = 133.322 Pa.
[b] 1 atm = 1.01×10^5 Pa.

users. Downsizing Vac-HSSPME will enable practical and effortless application of the method to routine analysis as well as substantially increase the automation potential of the method. This is particularly important to environmental laboratories aiming at reducing analyst time both for routine analysis and method development, faster sample throughput and greater reproducibility [2,8]. In addition, for those analytes that reach equilibrium within a reasonable amount of time, reducing the size of the sample container and accordingly the volume of the headspace will also increase the final amount of analyte extracted by the fiber as predicted by the theory [9].

The present work reports for the first time the performance of Vac-HSSPME in a 22 mL modified headspace sample vial. Five low molecular weight polycyclic aromatic hydrocarbons (PAHs) compounds were used as model compounds (Table 1). Parameters such as sample volume, agitation speed, extraction time and temperature were controlled and optimized. Comparison of the results with those obtained with regular HSSPME and our previous findings, revealed some new and important insights on the Vac-HSSPME procedure. Finally the performance of the resulting method was assessed and matrix effects upon extraction were evaluated by analyzing spiked tap water as well as effluent water sample taken from a municipal wastewater treatment plant.

2. Materials and methods

2.1. Chemicals and reagents

The five PAHs selected for investigation were all purchased from Sigma–Aldrich (Steinheim, Germany) and were each >98% in purity. A stock solution, containing 500 mg L^{-1} of each target analyte in acetonitrile (pesticide-grade; Merck, Darmstadt, Germany), was used daily for the preparation of the spiked aqueous solutions and was stored in the dark at 4 °C when not in use. Deionized water used for sample preparation was prepared on a water purification system (Barnstead EASYpure II) supplied by Thermo Scientific (Dubuque, USA). Recovery studies were carried out using tap water from the drinking water distribution network of Chania (Crete, Greece). Secondary treated wastewater effluent samples from the municipal wastewater treatment plant of Chania, serving approximately 70,000 inhabitants, were collected the day before being used and stored in glass bottles in the dark at 4 °C. HSSPME sampling of the unspiked real samples under both reduced and atmospheric conditions ensured that the samples were free of the target analytes.

2.2. Vac-HSSPME procedure

The custom-made gastight sample container used for extraction had a final volume of 22 mL and was built from a 20 mL headspace rounded bottom glass vial (O.D. 22.5 mm × H. 75.5 mm) further modified to accommodate on the top part two gastight ports: one high vacuum glass stopcock and one glass port equipped with a half-hole cylindrical Thermogreen septum (Supelco, Bellefonte, PA, USA) compatible with the needle of the SPME. For Vac-HSSPME, the modified headspace vial containing a cylindrical Teflon-coated magnetic stir bar (9 mm × 3 mm) was air-evacuated after connecting the high vacuum stopcock with the vacuum pump (7 mbar ultimate vacuum without gas ballast; Vacuubrand GmbH & Co. KG, Model MZ 2C NT, Wertheim, Germany). Upon air evacuation, the glass stopcock was closed and the vacuum pump was disconnected. Unless otherwise stated in the text, a 7 mL spiked aqueous solution was then introduced into the vial through the Thermogreen septum with the help of a 10 mL gastight syringe (SGE, Australia). The modified vial containing the sample and stir bar was then mounted on top of a stir plate (Heidolph, MR 3001K, Germany). Agitation at 1400 rpm was then applied and target analytes in the aqueous solution were left to equilibrate with the headspace for 10 min. Upon sample equilibration, the needle of the SPME fiber/holder assembly (Supelco, Bellefonte, PA, USA) was introduced into the sampling chamber by piercing the Thermogreen septum and HSSPME sampling was performed for a preset period of time (typically 30 min). Based on previous reports the 100-μm PDMS SPME fiber (Supelco, Bellefonte, PA, USA) was used for extraction [10,11]. Unless otherwise mentioned in the text, extraction was performed at 25 °C and 1400 rpm agitation speed. When microextraction sampling was completed, the PDMS fiber was retracted and the SPME device was transferred to a gas chromatographer–ion trap mass spectrometer (GC–MS-IT) for analysis. The pressure inside the modified vial was then equilibrated with atmospheric and the apparatus was emptied, washed and used for the next microextraction sampling. A schematic representation of the extraction procedure used for Vac-HSSPME is given in Fig. 1. The Thermogreen septum was replaced daily to avoid pressure loss due to septum damage. All extractions were run at least in duplicates.

2.3. GC–MS-IT analysis

All analyses were carried-out on a Varian 450-GC gas chromatograph coupled with a Varian 240-MS ion-trap mass spectrometer (Varian, Walnut Creek, CA, U.S.A.) and the system was operated by Saturn GC–MS Workstation v6.9 software. Separation was carried out on a VF 5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Bruker, Netherlands. The GC oven temperature was programmed from 75 °C (held 2 min) to 150 °C at 25 °C min^{-1} and then until 240 °C at 10 °C min^{-1}. The split/splitless injector operated at 270 °C, with the purge flow closed for 5 min. Helium (>99.999% pure) was used as a carrier gas at 1.2 mL min^{-1} flow-rate. The ion trap mass spectrometer was operated in the electron impact (EI) ionization positive mode (+70 eV) using an external ionization configuration. The full scan mode was used within the mass range from 50 to 250 m/z. Manifold, ion trap, ion source and transfer line temperatures were maintained at 50, 150, 180 and 260 °C, respectively.

3. Results and discussion

During the present investigations five low molecular weight PAHs compounds were used as model compounds since they are environmentally significant and cover the range from intermediate

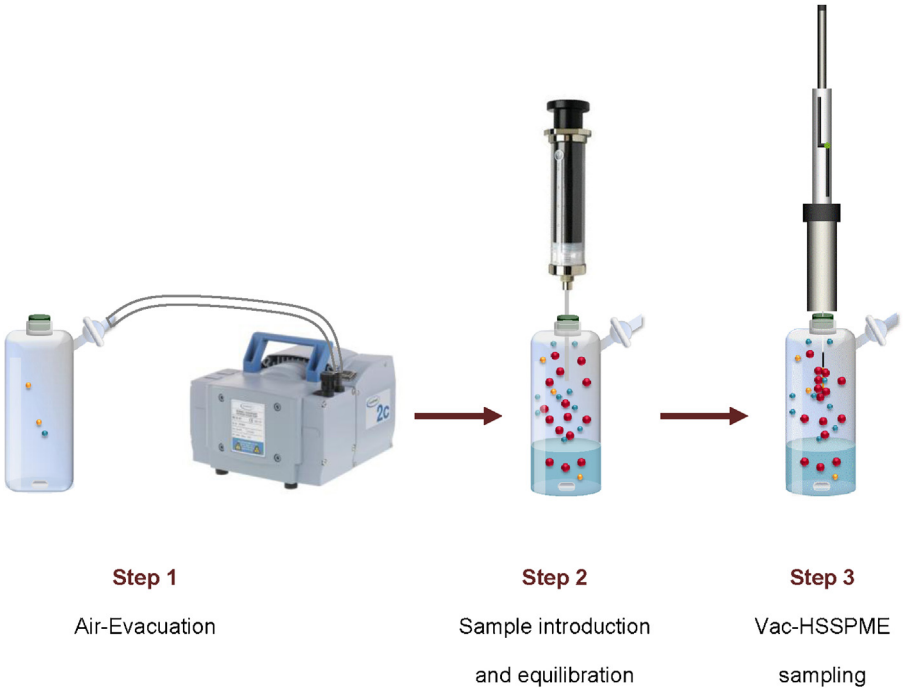

Fig. 1. Schematic representation of the extraction procedure used for Vac-HSSPME: (i) air evacuation of the modified sample vial after connecting the high vacuum stopcock with the vacuum pump, (ii) the glass stopcock was closed, the vacuum pump was disconnected and the aqueous sample was introduced through the port equipped with a septum; the aqueous solution was then left to equilibrate with the headspace for 10 min, and (iii) upon sample equilibration HSSPME sampling was performed for a preset period of time. In this simplified representation air (yellow spheres), water (blue spheres) and analyte (red spheres) molecules are also illustrated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

to low volatility compounds. Based on the K_H values of the target analytes (Table 1), naphthalene (Na) represents the case of an intermediate K_H compound, acenaphthene (Ace), fluorene (Fl) and phenanthrene (Phe) lies on the border between intermediate and low K_H compounds and fluoranthene (Flu) represents the low K_H class of compounds.

3.1. Effect of extraction time

Fig. 2 shows the extraction time profiles obtained in the 22 mL sample container under vacuum and atmospheric pressure conditions. As seen, Vac-HSSPME dramatically improved extraction kinetics compared to regular HSSPME for almost all compounds investigated here. This is emphatically visible in the case of Ace and Fl, where Vac-HSSPME extraction time profiles clearly showed the two-stage nature of the HSSPME process (~20 min equilibration time). On the contrary, regular HSSPME of Ace and Fl was still far from equilibrium even after sampling the headspace for 60 min (Fig. 2). For these two compounds, reducing the total pressure of the system resulted in an indubitable transition from slow to fast equilibration. A 20 min Vac-HSSPME equilibration time was also recorded for Fl in the 500 and 1000 mL sample containers (Na, Fl and Flu were the three model compounds included in our previous investigations) [4], demonstrating once again that Vac-HSSPME extraction kinetics are independent of the headspace volume since evaporation rates dramatically increase under reduced pressure conditions and the sample responds much faster to the concentration drops in the headspace. For Phe, equilibrium was not attained even after sampling the headspace for 60 min under reduced pressure conditions (Fig. 2). Nevertheless, the positive effect of reduced pressure sampling conditions remained important throughout the sampling times tested as evidenced by the Vac-HSSPME/HSSPME peak area ratios obtained at each sampling time point (e.g. 12 and 8 at 30 and 60 min, respectively). Similar conclusions can be reached for Flu, the most hydrophobic and least volatile compound investigated here. With a K_H value well below the reported threshold values for low K_H compounds, gas-phase resistance controlled evaporation rate and HSSPME sampling under reduced pressure conditions dramatically enhanced extraction kinetics when compared to regular HSSPME. As expected [4], for Flu equilibrium was not attained under both pressure conditions after sampling the headspace for 60 min (Fig. 2). Nevertheless, the positive effect of reduced pressure sampling conditions remained markedly important and even after sampling the headspace for 60 min the amount of Flu extracted with Vac-HSSPME was ~33 times larger compared to that with regular HSSPME.

Overall, with the exception of Na, Vac-HSSPME sampling was found to be noticeably beneficial for extraction. For Na, the compound with the highest K_H value investigated here, an unexpected ~30% decrease (on average) in the amount extracted at equilibrium was recorded with Vac-HSSPME compared to regular HSSPME. This observation is not in agreement with the thermodynamic theory, which predicts that at equilibrium Vac-HSSPME should behave similarly to regular HSSPME [3]. It is also inconsistent with our previous experimental findings in the large sample containers where equilibrium concentrations were found to be essentially the same under both pressure conditions [4]. In general, absolute humidity defined as the ratio of the mass of water vapor to the mass of dry air in a given volume of the mixture is expected to increase when decreasing the total pressure of the system at a constant temperature. This should result in an enhancement in water molecule collisions with the fiber during Vac-HSSPME leading to changes in analyte mass uptake due to water sorption on hydrophilic impurity sites on the surface of and within the PDMS material [12–15]. Changes in the fiber's characteristics are expected to be more pronounced when sampling in the 22 mL vial compared to the large sample containers since stirring efficiency (primarily in the liquid sample and secondarily

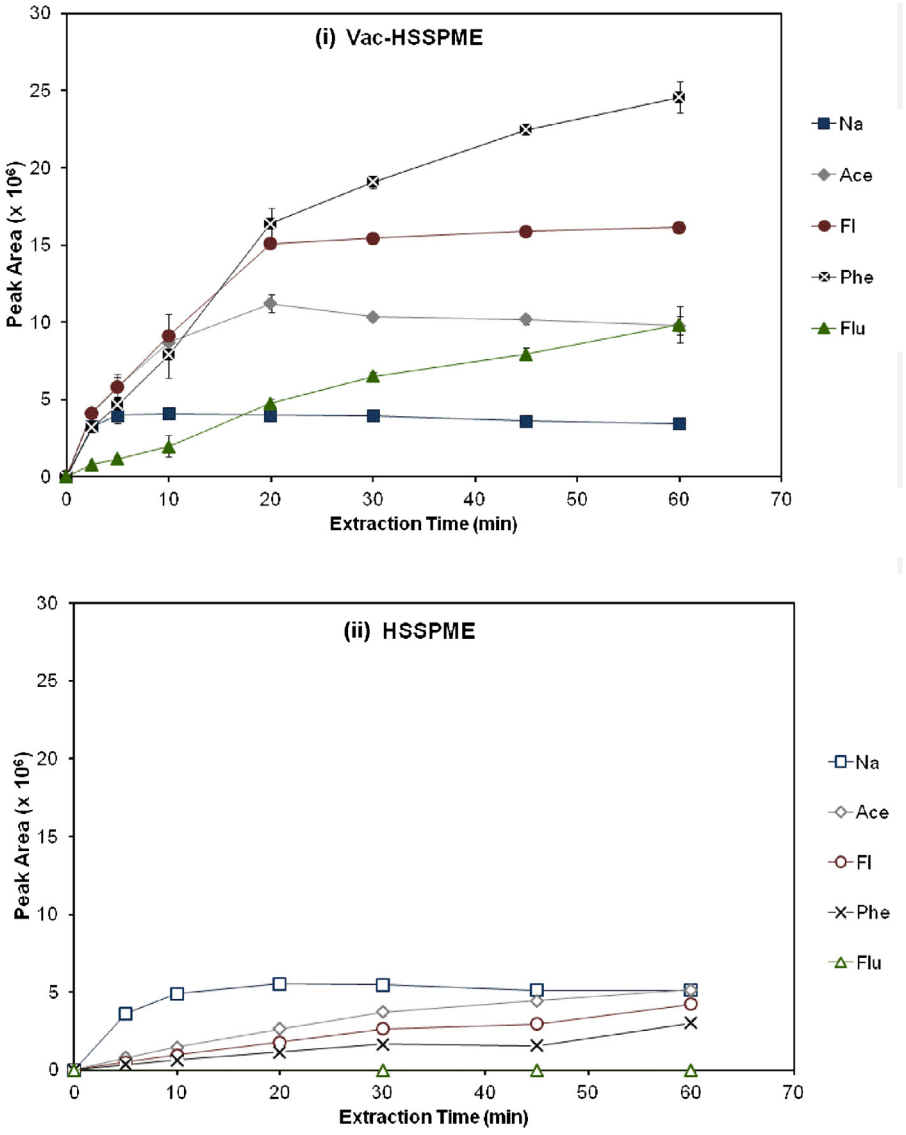

Fig. 2. Extraction time profiles for all target PAHs obtained in the 22 mL modified sample vial (i) under reduced (Vac-HSSPME; filled symbols) and (ii) atmospheric (HSSPME; open symbols) pressure conditions. Other experimental conditions: 7 mL aqueous sample spiked at 5 μg L^{-1}; 1400 rpm agitation speed; 25 °C sampling temperature. Some error bars are too small to be visible as compared with the physical size of the symbol.

in the headspace) is increased in the 22 mL vial [11] and the tip of the SPME fiber is located much closer to the surface of the liquid phase, thus allowing more efficient water molecule collisions with the fiber. Reducing the sorbent efficiency will result in a sorbent coating that may not behave as a zero sink for all analytes [16] and in this context PDMS has been reported not to be a perfect zero sink for naphthalene [17]. Furthermore, the water vapor in the headspace should be close to saturation during Vac-HSSPME at 25 °C since reducing the total pressure of the system is also expected to reduce the boiling point of water [18]. Hence, it is also possible that water condensation on the inner wall of the container may have affected the amount of Na in the gas phase available for extraction [19] leading to a decrease in the response of the instrument when sampling under vacuum conditions. Such water condensation is expected to be more prominent in smaller volume sampling containers due to the larger surface area to volume ratio. Substantial water condensation on the sheath of the SPME fiber was excluded here given that variations in the retention times of the target analytes were not recorded [13,20]. Based on the above discussion, a 30 min sampling time was chosen for all subsequent experiments.

3.2. Effect of sample volume

In Vac-HSSPME, samples are introduced into an air-evacuated vial and sample equilibration with the gas phase is allowed for a preset amount of time, ultimately leading to a gas phase that consists primarily of water vapor and a very small amount of analytes and residual air. The final total pressure in the headspace (P) is then given by,

$$P = \Sigma P_i + P_w + P_{vac} \quad (1)$$

where ΣP_i are the sum of the analytes' partial pressures, P_w is the partial pressure of water and P_{vac} is the final pressure after most of the air has been removed from the sampling chamber and the aqueous sample has been introduced. The value of P_{vac} is directly related to the pressure attained upon air-evacuation of the sampling vessel, P_{evac}, through the ideal gas law

$$P_{vac} = P_{evac}\left(1 + \frac{V_s}{V_g}\right) \quad (2)$$

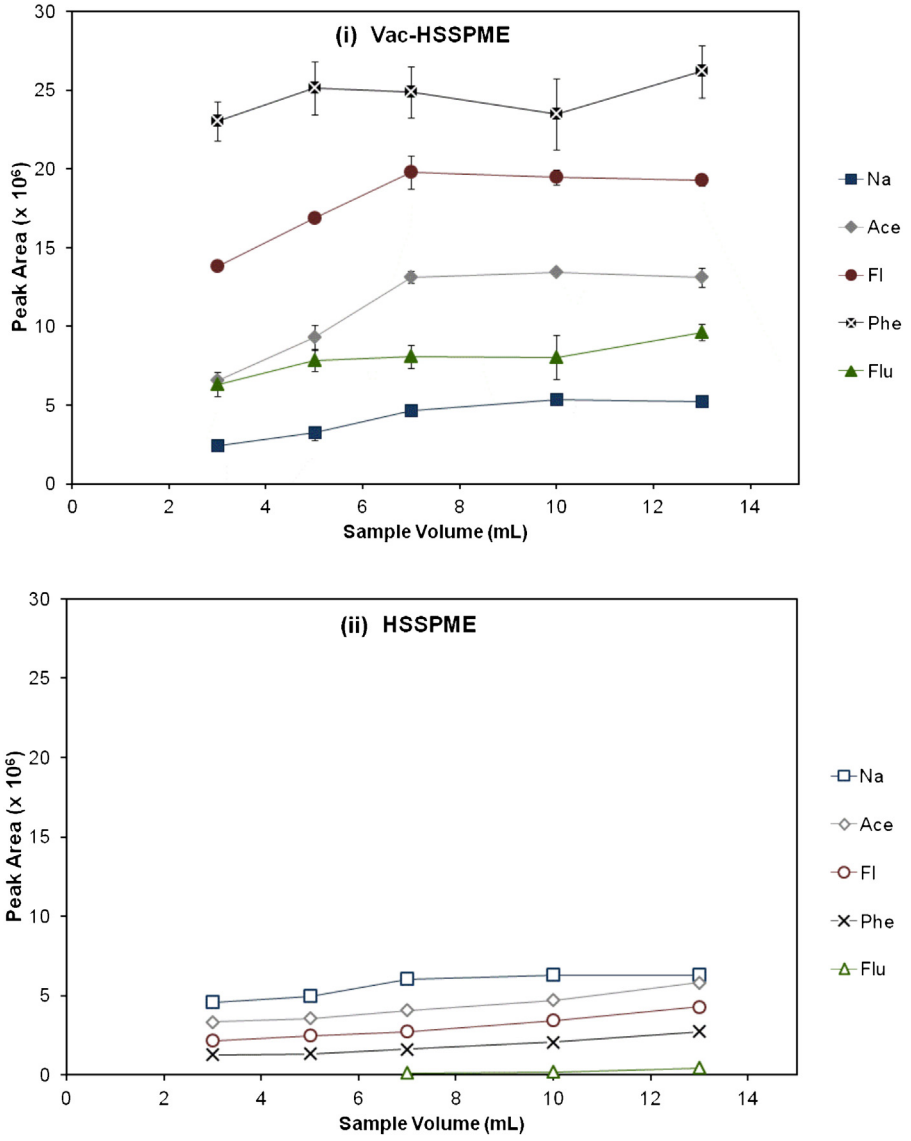

Fig. 3. Effect of sample volume on the extraction of PAHs obtained in the 22 mL modified sample vial (i) under reduced (Vac-HSSPME; filled symbols) and (ii) atmospheric (HSSPME; open symbols) pressure conditions. Other experimental conditions: aqueous samples spiked at 5 μg L^{-1}; 30 min sampling time; 1400 rpm agitation speed; 25 °C sampling temperature. Some error bars are too small to be visible as compared with the physical size of the symbol.

with V_s and V_g denoting the volumes of the sample and headspace, respectively. The lowest value P_{evac} can attain is the ultimate pressure limit of the vacuum pump used (in our case 7 mbar; 1 mbar = 100 Pa). Based on this value and the vapor pressure values of the target analytes (Table 1), it can be safely assumed that for a low sample to headspace volume ratio the final total pressure in the gas phase upon sample equilibration will be ultimately slightly higher than that of pure water and less than 40 mbar in total at 25 °C. The markedly small sample to headspace volume ratio achieved with the 500 and 1000 mL vessels used in our previous studies could meet this criterion and changes in pressure upon sample introduction and equilibration were not expected to be significant [3,4]. During the present investigations, the use of a 22 mL vial resulted in a sample to headspace volume ratio that could no longer be neglected. Nevertheless, as long as P_{evac} is sufficiently low, changes in the final total pressure, P, upon different sample volumes introduction will not be significant. For example, introducing a 7 mL aqueous sample in the pre-evacuated 22 mL vial should result in an approximate 1.5-fold increment in P_{vac} leading to minor changes in the final total pressure despite the substantial increase in sample to headspace volume ratio.

To demonstrate the above assumption the effect of aqueous sample volume on Vac- and regular HSSPME was investigated within the range from 3 to 13 mL after a 30 min sampling time at 25 °C and the results are given in Fig. 3. As seen, with the exception of Na, HSSPME sampling under reduced pressure conditions enhanced extraction kinetics for each sample volume when compared to regular HSSPME. Hence, pressure changes induced for sample to headspace volume ratios commonly used in HSSPME are sufficiently low to allow efficient Vac-HSSPME sampling. In fact, even after introducing 13 mL of the aqueous sample the Vac-HSSPME/HSSPME peak area ratio still remained important (2.2, 4.5, 9.6 and 22 for Ace, Fl, Phe and Flu, respectively).

A closer look in Fig. 3 shows that for Vac-HSSPME the amount of extracted analyte gradually increased for sample volumes up to 7 mL and then remained, to some extent, constant. In general, the flat part of the curves does not necessarily mean saturated absorption, especially if the analyte's concentration is low. When

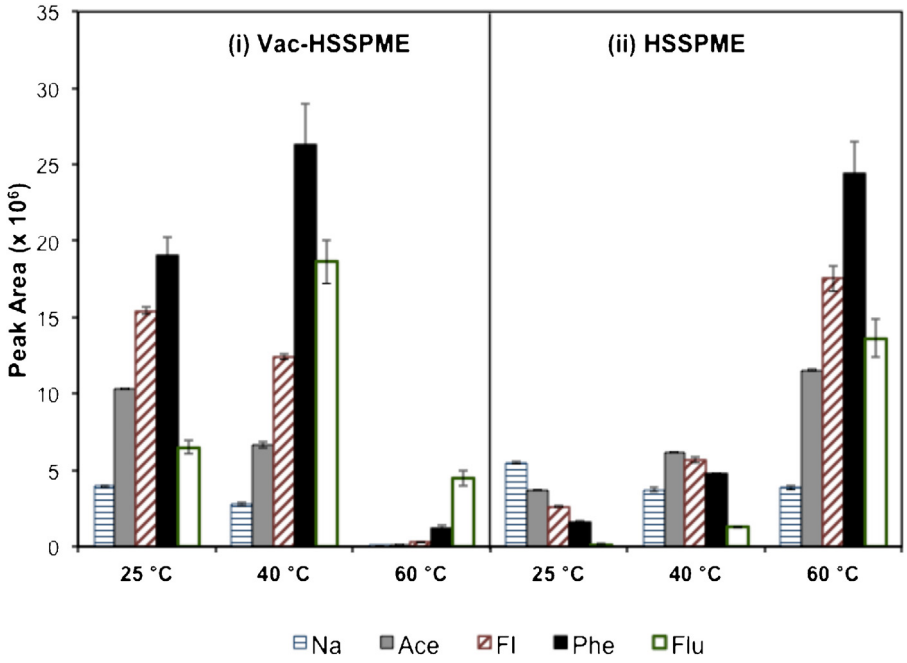

Fig. 4. Effect of temperature on the extraction of PAHs obtained in the 22 mL modified sample vial (i) under reduced (Vac-HSSPME) and (ii) atmospheric (HSSPME) pressure conditions. Other experimental conditions: 7 mL aqueous samples spiked at 5 μg L^{-1}; 30 min sampling time; 1400 rpm agitation speed; 25 °C sampling temperature. Some error bars are too small to be visible as compared with the physical size of the symbol.

the concentration change after absorption is no longer significant, SPME absorption is practically independent of sample volume [21]. It therefore appears that for Vac-HSSPME, the presence of an air evacuated headspace accelerated extraction kinetics and curves leveled off for almost all analytes. On the other hand for regular HSSPME, with the exception of Na, the amount of analyte extracted by the fiber increased with increased sample size throughout the volumes tested. This is consistent with the fact that for the less volatile analytes, higher sensitivities (i.e. shorter equilibration times) can be obtained during regular HSSPME by increasing the aqueous phase volume, because for these analytes reducing the headspace volume increases the concentration gradient in the headspace and it takes less time to diffuse through the headspace [9,22]. As expected for the more volatile Na, the amount absorbed by the fiber increased with regular HSSPME for water volumes up to 7 mL and then SPME adsorption became practically independent of the sample volume [22]. Based on the present findings it was decided to use a 7 mL sample volume for all subsequent experiments.

3.3. Effect of agitation

Strong mixing of the condensed phase is expected to increase evaporation rates and consequently enhance the amount of analyte extracted by the fiber. Mixing the water body produces turbulence which results in frequent exchanges between the surface layer and the bulk aqueous phase [23]. Compounds may thus quickly reach the interface and, depending on their gas resistances, leave the solution surface faster when compared to the stagnant mode. Acceleration effects on evaporation rates induced by stirring the solution may be larger for the high K_H compounds than for the low K_H compounds due to evaporation resistances being concentrated in the liquid and gas phase, respectively [24] as long as they are distant from equilibrium [11].

During the present studies, the effect of sample agitation on Vac-HSSPME was investigated after exposing the fiber for 30 min to the headspace of 7 mL water samples spiked at 5 μg L^{-1} with each target analyte and agitated at different stirring speeds (namely: 0, 500, 1000 and 1400 rpm). The results (not shown here) confirmed our previous observations, in that agitation improved the amount extracted under reduced pressure conditions with stirring enhancements between the turbulent (1400 rpm) and static mode reaching values of 1.6, 3.7, 6.7, 6.7 and 10 for Na, Ace, Fl, Phe and Flu, respectively. As expected, improvement in Na extraction was not so pronounced given that this compound reached equilibrium fast. It was therefore decided to use the maximum stirring speed (i.e. 1400 rpm) for all subsequent experiments.

3.4. Effect of temperature

Heating the sample typically results to a faster overall HSSPME procedure. As temperature increases, diffusion coefficients and Henry's Law constants increase, leading to higher headspace concentrations and shorter equilibration times. However, elevated sample temperatures can impair recovery by shifting both the sample-headspace and the fiber-headspace equilibrium to favor the headspace phase [16].

Combining the effects of temperature and reduced pressure in Vac-HSSPME, was expected to enhance even further the kinetics of the extraction up to a certain temperature above which the effect of temperature would dominate and basically control the extraction. The reason for this is that the vapor pressure of water, P_w, increases exponentially with temperature (when heating the sample) leading to a considerable increment in the final total pressure, P, according to Eq. (1).

An alternative approach to understand the combined effect of reduced pressure and temperature is to consider the fact that HSSPME sampling under reduced pressure conditions will also affect the mole fraction of the analyte in the headspace, y_i, which is strongly dependent on the total pressure in the headspace (P). In particular

$$y_i = \frac{P_i}{P} = \frac{P_i}{P_i + P_w + P_{air}} \quad (3)$$

Table 2
Linearity, detection limits, repeatability, and average relative recoveries from tap water and secondary treated wastewater (WW) effluent for chlorophenols – with Vac-HSSPME.

Compound	Conc. range ($\mu g\,L^{-1}$)	r^2	LODs ($\mu g\,L^{-1}$)	Repeatability (% RSD)[a]	Relative recoveries	
					Tap[b]	WW effluent [b]
Na	0.2–10	0.9960	0.027	5.2	105(1.1)	99(1.8)
Ace	0.1–10	0.9993	0.013	3.6	102(4.3)	99(3.9)
Fl	0.1–10	0.9997	0.015	1.3	106(2.5)	101(1.3)
Phe	0.1–10	0.9979	0.014	5.4	105(3.1)	104(3.1)
Flu	0.2–10	0.9929	0.021	5.8	104(3.7)	97(4.0)

[a] Spiking level 0.25 $\mu g\,L^{-1}$; $n = 5$.
[b] Spiking level 1 $\mu g\,L^{-1}$; % RSD values given in parentheses; $n = 5$.

where P_{air} denotes the atmospheric pressure following the aqueous sample introduction in regular HSSPME. The relative increase in the mole fraction of the analyte, E_y, in the gas phase when sampling under vacuum relative to the atmospheric pressure is then given by

$$E_i = \frac{y_{i,vac}}{y_i} = \frac{P_i + P_w + P_{air}}{P_i + P_w + P_{vac}} \quad (4)$$

and represents the enhancement in analyte collisions with the fiber as it is proportional to the ratio of the mole fractions. A numerical example of the strong temperature dependence of HSSPME extraction kinetics can be given by calculating the E_y values at different temperatures. At 25 °C the value of E_y will take values up to ~38 meaning that the fiber coating is expected to "uptake" analyte gas molecules much faster when working under vacuum conditions relative to atmospheric pressure since the portion of analyte molecules in the air-evacuated headspace colliding with the fiber at 25 °C will be ultimately 38 times larger than the portion of analyte molecules colliding with the fiber in the presence of air. As the saturation pressure of water depends strongly on temperature, the values of E_y are reduced to ~19 at 40 °C and 7.8 at 60 °C, demonstrating that the positive effect of working under vacuum conditions on extraction kinetics will be reduced when increasing the temperature.

In our previous report we were able to investigate the effect of temperature on Vac-HSSPME over a small temperature range (from 25 to 45 °C) due to limitations of the experimental setup and a positive effect of temperature was reported for the less volatile chlorophenol compounds [3]. During the present investigations, the use of a 22 mL modified vial allowed us to examine sampling temperatures from 25 to 60 °C. For comparison, HSSPME extractions were performed under both vacuum and atmospheric pressure conditions and the results are given in Fig. 4. As seen, for a 30 min Vac-HSSPE sampling, heating the sample gradually decreased mass loading of the more volatile Na, Ace and Fl (all expected to be at equilibrium) until the point (60 °C) where extraction was found to be practically impaired. On the other hand, for Phe and Flu, increasing the temperature from 25 to 40 °C improved extraction; yet a further increase to 60 °C drastically restricted extraction (Fig. 4). As mentioned earlier, during Vac-HSSPME the water vapor in the headspace is close to saturation. Increasing the temperature greatly increases humidity and challenges even more the fiber. Since more water molecules are available to partition with the PDMS fiber [12] the fiber's characteristics are changed, thus impairing mass loading of the analytes [13,20].

As expected for regular HSSPME, heating the sample improved extraction (Fig. 4). However, when sampling under atmospheric pressure conditions, a 60 °C sample temperature is necessary in order to reach the maximum peak area values attained with Vac-HSSPME (at 25 or 40 °C and depending on the analyte). It should be emphasized however that regardless of the adverse effect of higher temperatures on Vac-HSSPME, one of the most important features of Vac-HSSPME is that high extraction efficiency and very good sensitivity can be achieved under mild extraction conditions and that includes extraction at room temperature. In cases where higher sensitivity is needed then fine tuning of the Vac-HSSPME method can be achieved by simply increasing the sampling time. Based on the above discussion it was decided to use a 25 °C as sampling temperature.

3.5. Validation of the method

The main analytical parameters of merit were determined for the newly proposed extraction approach. The analytical curve was constructed by extracting for 30 min at 25 °C the headspace of 7 mL aqueous solutions stirred at 1400 rpm and spiked with all target analytes using five concentration levels ranging from 0.1 or 0.2 depending on the analyte to 10 $\mu g\,L^{-1}$ (Table 2). The calculated calibration curves gave a high level of linearity for all target analytes with correlation coefficients (r^2) ranging between 0.9929 and 0.9997. The repeatability of the proposed method, expressed as relative standard deviation (RSD), was evaluated by extracting five consecutive aqueous samples spiked at 0.25 $\mu g\,L^{-1}$ with each target analyte and was found to range between 1.3 and 5.8% (Table 2). The limits of detection (LODs) were also determined and were found to be in the low ng L^{-1} level (Table 2) and, as expected, they were better than those reported in the 500 mL sample container [4]. Analyte recoveries from tap and secondary treated wastewater effluent samples spiked at 1 $\mu g\,L^{-1}$ ranged between 102–106% and 97–104%, respectively (Table 2), relative to the amount extracted from pure water samples, demonstrating that matrix did not affect Vac-HSSPME extraction.

Regular HSSPME sampling of PAHs from water samples has been investigated on several occasions [25]. To the best of our knowledge, the majority of these reports discuss and compare the effect of different extraction parameters on headspace and direct

Table 3
Summary of Vac-HSSPME and other published HSSPME procedures used for the determination of PAHs in water samples.

Fiber	Sample volume (mL)	Extraction time (min)	Extraction temperature (°C)	Salt addition	Analytical instrument	LODs ($\mu g\,L^{-1}$)	Reference
PDMS	7	30	25	No	GC–MS-IT	0.013–0.027	This work
PA[a]	20	60	50	No	GC-FID	0.09–0.20	[26]
PLAC[b]	50	30	80	12 g	GC-FID	0.03–0.15	[27]

[a] Polyacrylate 85 μm.
[b] Porous layer of activated charcoal; laboratory made.

immersion HSSPME sampling modes [9,10,25] and only few of them present the analytical performance of developed HSSPME procedures [26,27]. Table 3 summarizes LODs and the main experimental conditions under which they were obtained for Vac-HSSPME and previously reported regular HSSPME methods. Since each method uses different fibers, sample volumes, analytical instrumentations and ionic strength, care should be taken when comparing their analytical performances. Nevertheless, with Vac-HSSPME very good sensitivity is achieved whilst extracting small sample volumes for short sampling times, at room temperature and without adding salt to the water samples.

4. Conclusions

Downsizing Vac-HSSPME has been made possible. This is the first work indicative of the automation potential of such an efficient methodology destined for environmental laboratories that constantly seek high sample throughput and short sample turnaround time to overcome the large number of samples both from the point of view of energy use and analyst time. The proposed approach offers ease in handling and significant analytical performance. Very good sensitivity and precision can be achieved within shorter sampling times and under milder conditions (i.e., lower temperatures) relative to regular HSSPME. The behavior of Vac-HSSPME for naphthalene (intermediate K_H compound) observed in the large sample containers was not recorded in the small 22 mL sample vial.

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