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ARTICLE *in* JOURNAL OF CHROMATOGRAPHY A · MAY 2012

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Effect of Henry's law constant and operating parameters on vacuum-assisted headspace solid phase microextraction

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ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 20 April 2012
Accepted 1 May 2012
Available online 10 May 2012

Keywords:
Reduced pressure sampling
HSSPME
Headspace volume
Agitation
Evaporation rates

ABSTRACT

Nonequilibrium headspace solid-phase microextraction (HSSPME) sampling under vacuum conditions may dramatically improve extraction kinetics compared to regular HSSPME at room temperature. This paper investigates the effects of organic analyte properties and sampling parameters (headspace volume and sample agitation) on vacuum-assisted HSSPME (Vac-HSSPME). It was found that at room temperature, acceleration effects on extraction rates induced by reducing the total pressure of the sample container are important for those compounds where the Henry's law constant, K_H, is close or below the reported threshold values for low K_H solutes. For these compounds evaporation rate is controlled by mass transfer resistance in the thin gas-film adjacent to the gas/sample interface and reducing the total pressure will increase evaporation rates and result in a faster overall extraction process. Conversely, for analytes with an intermediate K_H value, Vac-HSSPME is not expected to improve extraction rates compared to regular HSSPME given that mass transfer resistance in the liquid-film becomes important. In accordance with the theory, at equilibrium, the amount of analyte extracted by the SPME fiber is not affected by the pressure conditions inside the sample container. Furthermore, Vac-HSSPME extraction kinetics for low K_H analytes were marginally affected by the tested change in headspace volume as evaporation rates dramatically increase under reduced pressure conditions and the sample responds much faster to the concentration drops in the headspace when compared to regular HSSPME. At equilibrium however, increasing the headspace volume may result in a loss of sensitivity for Vac-HSSPME similar to that observed for regular HSSPME. As expected, stirring the liquid sample was found to improve Vac-HSSPME. Finally, the method yielded a linearity of 0.998 and detection limits in the ppt level. The precision varied between 1.8% and 8.4%.

1. Introduction

Since its introduction, solid-phase microextraction (SPME) has gained increasing acceptance in many areas, including applications in environmental, food, and drug analysis [1–4]. In particular, analytes in the headspace over a condensed phase are directly extracted and concentrated in the polymer film of the SPME fiber, which makes this technique advantageous over conventional techniques for headspace analysis [5,6].

In headspace SPME (HSSPME) three phases are involved (sample, headspace, and polymer film of the SPME fiber) that form two interfaces (sample/headspace and headspace/fiber) [5–7]. Analytes partition between the three phases and at equilibrium it is well established [1,6,8] that the amount of analyte extracted by the fiber (n_f^∞) can be calculated from

$$n_f^\infty = C_f^\infty V_f = \frac{K_f K_g V_s V_f}{K_f K_g V_f + K_g V_g + V_s} C_s^o \quad (1)$$

where K_f and K_g are equilibrium partition constants for the analyte between the headspace and the polymer film and between the condensed phase and its headspace respectively, V_f, V_g, and V_s are the volumes of the SPME polymer film, headspace and sample respectively, C_s^o is the initial concentration of the analyte in the sample matrix and C_f^∞ is the concentration of the analyte in the fiber coating at equilibrium.

The dynamic process of HSSPME sampling before partition equilibrium in a closed three-phase system of a limited volume is a multi-stage process [5,8]. Initially chemical equilibrium is allowed to establish between the aqueous solution and the headspace. Once the fiber is exposed to the headspace, it starts to sorb analyte molecules rapidly from the gas-phase. As soon as the headspace concentration of the analyte falls below the equilibrium level with

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http://dx.doi.org/10.1016/j.chroma.2012.05.006

respect to the aqueous phase, analyte molecules start to move from the liquid sample to the headspace. For semivolatiles, evaporation from the sample to its headspace is the rate-determining step for HSSPME causing the equilibration process to be slow [5]. Typically, equilibrium times are shortened by applying agitation or by increasing the sampling temperature; yet these parameters need to be carefully considered when optimizing HSSPME [9].

The possibility of using reduced pressure conditions during HSSPME sampling had been considered in the past but overlooked [10,11]. We recently proposed a new HSSPME sampling procedure, termed vacuum-assisted HSSPME (Vac-HSSPME), destined for the analysis of compounds whose mass transfer from the sample to the headspace is the rate-determining step [12]. The results showed that nonequilibrium HSSPME sampling under reduced pressure conditions may result in faster extraction kinetics due to the enhancement of evaporation rates in the presence of an air-evacuated headspace. We have also formulated a theoretical model demonstrating for the first time the pressure dependence of HSSPME sampling procedure under nonequilibrium conditions [12] by considering the evaporation of organic solutes from water as a first-order reaction and by taking the chemical mass balance around the water body expressed as [13,14]:

$$V_s \frac{dC_s}{dt} = -K_L A(C_s - C_i) \quad (2)$$

where C_i ($\mathrm{mol\,m^{-3}}$) is the concentration of the analyte at the water/air interface, A is the interfacial contact area between the sample and the gas phase and K_L ($\mathrm{m\,h^{-1}}$) is the overall mass transfer coefficient at the gas phase–sample interface related to the evaporation rate constant (k; $\mathrm{h^{-1}}$) by $K_L = kL$ with L denoting the solution depth (m) in a container with uniform cross section. Liss and Slater [15] and later Mackay and Leinonen [16] were the first to describe K_L using the two-film theory and the assumption that the overall resistance to mass transfer results from resistances through the two thin films (gas and liquid) adjacent to the gas–liquid interface, namely:

$$K_L = \left[\frac{1}{k_L} + \frac{RT}{K_H k_g}\right]^{-1} \quad (3)$$

where k_L and k_g ($\mathrm{m\,h^{-1}}$) are the liquid-film and gas-film mass-transfer coefficients respectively, K_H ($\mathrm{atm\,m^3\,mol^{-1}}$; $1\,\mathrm{atm} = 1.01 \times 10^5\,\mathrm{Pa}$) is the Henry's law constant defined as the ratio of partial pressure to aqueous concentration, T is the absolute temperature (K) and R is the gas constant ($8.2 \times 10^{-5}\,\mathrm{m^3\,atm\,mol^{-1}\,K^{-1}}$). For a high K_H organic solute, the major resistance to the mass transfer lies in the liquid-phase (*i.e.* $K_L \approx k_L$). Conversely, for a low K_H organic solute, the resistance to mass transport from the sample to its headspace is concentrated in the gas-phase (*i.e.* $K_L \approx K_H k_g/RT$). If the compound has an intermediate K_H value, both gas and liquid-phase mass transfer resistances are important. Evacuating most of the air from the sampling chamber prior to liquid sample introduction significantly reduces the total pressure of the system (P_{tot}) and increases the compound's molecular diffusion coefficient, D_g [17]. Given that k_g is proportionally related to D_g [18], reducing the pressure will also increase k_g and for low K_H compounds K_L will also increase given that for these compounds mass transfer resistance is concentrated in the gas-phase (*i.e.* $K_L \approx K_H k_g/RT$). This enhancement in evaporation rates results in a faster response of the sample to the concentration drops of analytes in the headspace as seen during the multi-stage process of nonequilibrium HSSPME sampling. Hence, for semivolatile compounds where evaporation from the condensed phase to its headspace is the rate-determining step and gas-phase mass transfer resistance controls the evaporation rate, HSSPME equilibrium is established faster when sampling under reduced pressure conditions.

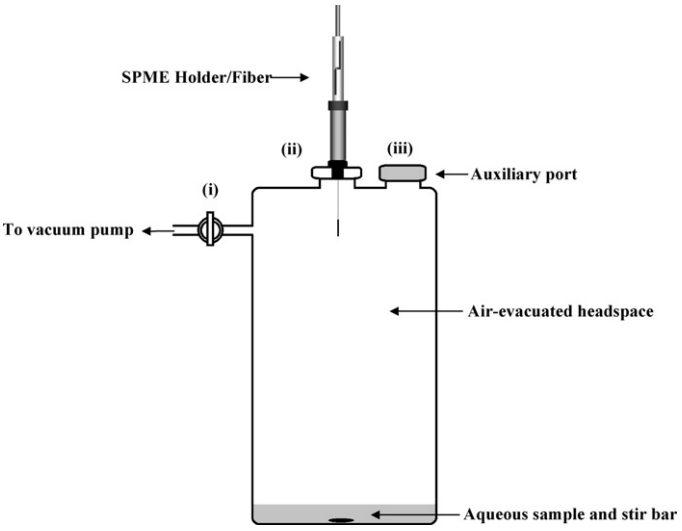

Fig. 1. Experimental setup used for Vac-HSSPME (the 1000 mL sampling vessel is shown here). The custom-made apparatus was equipped with three gastight ports: (i) one high vacuum glass stopcock used for connecting to a vacuum pump and prepare the sampling chamber for microextraction, (ii) one port equipped with a septum compatible with the needle of the SPME device and (iii) one auxiliary gastight port equipped with cap and septum offering additional and easy access to the sampling chamber.

Evaporation rates from a condensed phase may be affected significantly by the organic solute's properties, such as the Henry's law constant [14]. On the other hand, headspace sampling parameters such as headspace volume and mixing of the condensed phase may have an effect on the amount of analyte extracted by the fiber [6,8,9]. Understanding the effects of both solutes' properties and sampling parameters on Vac-HSSPME is crucial for predicting, tuning and controlling the performance of the method so as to obtain enhanced sensitivity within short sampling times. This work gives new insights on the impact of these parameters on Vac-HSSPME and compares for the first time its performance with that of regular HSSPME during both the nonequilibrium and equilibrium stages of the sampling process.

2. Materials and methods

2.1. Chemicals

The three polycyclic aromatic hydrocarbons (PAHs) compounds used here together with some of their physicochemical properties are listed in Table 1. They were all purchased from Sigma–Aldrich (Steinheim, Germany) and were each >98%. A stock solution containing 500 mg $\mathrm{L^{-1}}$ of each target analyte in acetonitrile (pesticide-grade; Merck KGaA) 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 (EASYpure RF) supplied by Barnstead/Thermolyne (Dubuque, USA).

2.2. Vac-HSSPME procedure

Two custom-made glass sample containers having total volumes of 500 mL and 1000 mL were used as sampling chambers. Fig. 1 shows the cross-section of the 1000 mL apparatus. Each sampling vessel was equipped with three gastight ports: (i) a port equipped with high vacuum glass stopcock for connecting to the vacuum pump, (ii) a port equipped with a half-hole cylindrical

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

Compound	Molecular weight	Vapor pressure 25 °C (mmHg)[a]	K_H (atm m^3 mol^{-1})[b]	Log K_{ow}
Naphthalene	128.18	0.085	4.4×10^{-4}	3.30
Fluorene	166.22	0.0006	9.62×10^{-5}	4.18
Fluoranthene	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.

Thermogreen septum (Supelco, Bellefonte, PA) compatible with the needle of the SPME device and (iii) an auxiliary gastight port equipped with a black polypropylene open-hole cap and septum. The latter offered additional access to the sampling chamber and easy handling of the magnetic stir bar, overcoming thus problems associated with the small openings of the commercially available 1000 mL sampling chamber used in our previous studies where static HSSPME sampling mode was unavoidably applied in all cases [12]. For Vac-HSSPME, the sample container containing a Teflon-coated magnetic stir bar (10 mm × 5 mm) was air-evacuated after connecting the high vacuum glass stopcock with a vacuum pump (7 mbar ultimate vacuum without gas ballast; Vacuubrand GmbH & Co. KG, Model MZ 2C NT, Wertheim, Germany; 1 mbar = 100 Pa). Upon air evacuation, the glass stopcock was closed and the vacuum pump was disconnected. A 10 mL aqueous solution spiked at 10 μg L^{-1} was then introduced into the sampling chamber through the Thermogreen septum with the help of a 10 mL gastight syringe (SGE, Australia). The apparatus containing the sample and stir bar was then mounted on top of a stir plate (Heidolph, MR 3001K, Germany) placed inside a thermo-stated chamber/incubator (Elvem, Athens, Greece) maintained at 25 °C. Agitation at 1000 rpm (80% of the maximum speed) was applied and target analytes in the aqueous solution were left to equilibrate with the headspace for 15 min. Upon equilibration, the headspace would consist primarily of water and a very small amount of analytes and residual air. Based on the ultimate pressure limit of the vacuum pump used here (7 mbar; 1 mbar = 100 Pa) and the vapor pressure values of the target analytes (Table 1), it can be safely assumed that the final total pressure in the gas phase upon sample equilibration with the headspace would be slightly higher than that of pure water (less than 40 mbar in total at 25 °C; 1 mbar = 100 Pa). The needle of the SPME fiber/holder assembly (Supelco, Bellefonte, PA) was consequently introduced into the sampling chamber by piercing the Thermogreen septum and HSSPME sampling was performed for a preset period of time at 25 °C. Based on previous reports the 100-μm PDMS SPME fiber (Supelco, Bellefonte, PA) was used for extraction [19,20]. Unless otherwise stated in the text, sample agitation at 1000 rpm was applied during this step. When microextraction sampling was completed, the PDMS fiber was retracted and the SPME device was transferred to a gas chromatograph–mass spectrometer (GC–MS) for analysis. The pressure inside the sampling chamber was then equilibrated with atmospheric and the apparatus was emptied, washed and used for the next microextraction sampling. The Thermogreen septum was replaced daily to avoid pressure loss due to septum damage. All analyses were run at least in duplicates. It should be mentioned here that during the present studies the strong analytical response of the instrument did not point towards significant losses of target analytes due to sorption on the containers' walls.

2.3. GC–MS analysis

A Shimadzu GC-17A (Version 3) QP-5050A GC-MS system was used for all analyses. The split/splitless injector operated at 260 °C, with the purge flow closed for 5 min. Helium (>99.999% pure) was used as the carrier gas at a 1.2 mL min^{-1} flow-rate. Separation was performed on a 30 m × 0.25 mm × 0.25 μm Equity™-5 capillary column (Supelco, Bellefonte, PA). The column oven was programmed as follows: 50 °C for 5 min, programmed to 160 °C at a rate of 10 °C min^{-1}, increased to 270 °C at a rate of 5 °C min^{-1} and then held for 2 min. The ionization mode was electron impact (70 eV) and the interface temperature was set at 320 °C. Results were recorded in the full scan mode in the range m/z = 50–350. Analytes were quantified using a five-point external calibration curve obtained by analyzing mixtures of PAHs standards.

3. Results and discussion

3.1. Predicting the performance of Vac-HSSPME: importance of K_H

Evaporation rates of chemicals can be controlled by mass transfer in the liquid-phase, the gas-phase or a combination of both, depending on the value of K_H. In this context, several previous reports suggest that mass transfer resistance in the liquid-phase controls more than 95% of the evaporation rate when the value of K_H (expressed as atm m^3 mol^{-1}; 1 atm = 1.01×10^5 Pa) is greater than about 5×10^{-3} atm m^3 mol^{-1} (1 atm = 1.01×10^5 Pa) [21,22]. If K_H is below the threshold K_H values reported in the literature for low K_H compounds (typical values: 1.2×10^{-5} [21,22] or 1.6×10^{-4} atm m^3 mol^{-1} (1 atm = 1.01×10^5 Pa) [16]) gas-phase resistance controls more than 95% of the evaporation rate. For a compound with an intermediate K_H (between the threshold values for low and high K_H), both gas and liquid-phase mass transfer resistances are important [14,21,22]. During the present investigations three low molecular weight PAHs compounds were used as model compounds since they are environmentally significant and range from intermediate to low volatile compounds. Accordingly, based on the K_H values given in Table 1, naphthalene represents the case of an intermediate K_H compound, fluorene lies on the border between intermediate and low K_H compounds and fluoranthene represents the low K_H class of compounds.

Figs. 2–4 show amongst others, the extraction time profiles obtained with HSSPME in the 500 mL sample container under vacuum and atmospheric pressure conditions. In particular, Fig. 2 shows that for naphthalene the extraction curves obtained with Vac- and regular HSSPME sampling, were essentially the same during the nonequilibrium (*i.e.* early sampling times) and equilibrium (*i.e.* later sampling times) stages of the process, with the analyte reaching equilibrium within roughly 20 min under both pressure conditions. For an intermediate K_H compound like naphthalene both gas and liquid-phase mass transfer resistances are expected to be significant. Indeed, for this compound liquid-phase resistance, which is independent of the pressure conditions in the headspace, appeared to be important since the presence of an air-evacuated headspace did not lead to obvious changes in the evaporation rate during the nonequilibrium stage of the sampling process. At equilibrium, the amount of naphthalene extracted by the fiber should be and was essentially measured to be the same under both pressure conditions. Regardless of the dominant resistance to mass transfer, the thermodynamic theory confirms that HSSPME equilibrium concentrations are independent of the total pressure as partition coefficients/Henry's constants are affected only at high operating pressures.

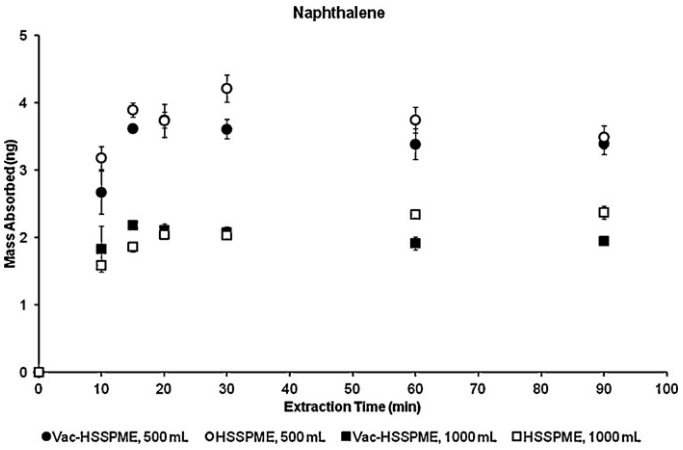

Fig. 2. Extraction time profiles for naphthalene obtained with the 500 (circles) and 1000 mL (squares) sample containers under reduced (Vac-HSSPME; filled symbols) and atmospheric (HSSPME; open symbols) pressure conditions. Other experimental parameters: 10 mL aqueous sample spiked at 10 $\mu g\,L^{-1}$; 1000 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.

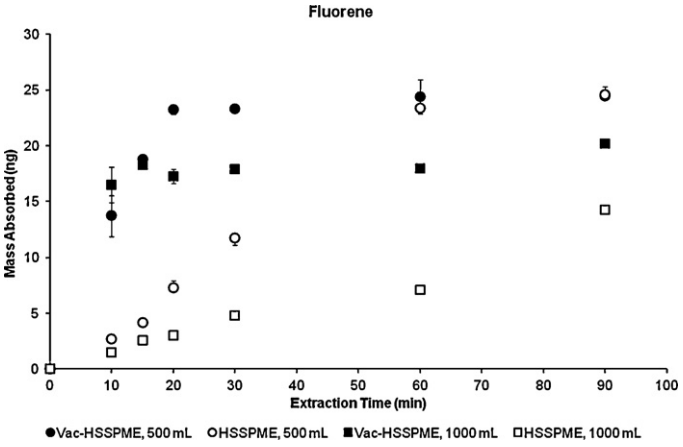

Fig. 3. Extraction time profiles for fluorene obtained with the 500 (circles) and 1000 mL (squares) sample containers under reduced (Vac-HSSPME; filled symbols) and atmospheric (HSSPME; open symbols) pressure conditions. Other experimental parameters: 10 mL aqueous sample spiked at 10 $\mu g\,L^{-1}$; 1000 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.

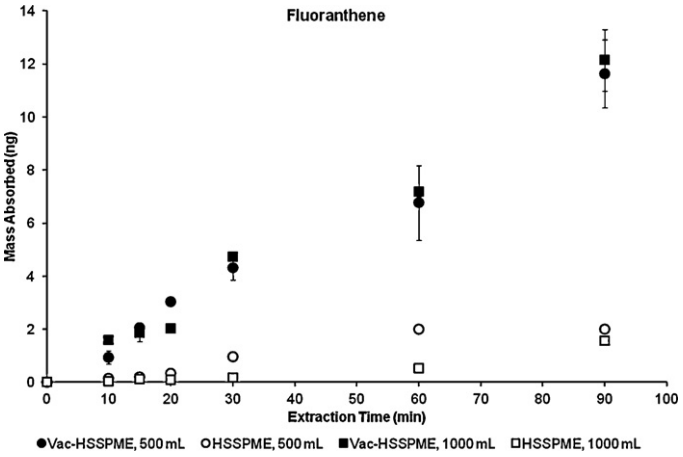

Fig. 4. Extraction time profiles for fluoranthene obtained with the 500 (circles) and 1000 mL (squares) sample containers under reduced (Vac-HSSPME; filled symbols) and atmospheric (HSSPME; open symbols) pressure conditions. Other experimental parameters: 10 mL aqueous sample spiked at 10 $\mu g\,L^{-1}$; 1000 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.

On the other hand, for fluorene, a compound with a K_H lying on the border between intermediate and low K_H compounds, enhanced extraction kinetics were recorded with Vac-HSSPME over regular HSSPME during the nonequilibrium stage of the HSSPME process (Fig. 3). Gas-phase resistance controlled the evaporation rate for this compound and a clear transition from slow to fast equilibration was observed upon reducing the total pressure of the sample container. In particular, the Vac-HSSPME extraction time profile clearly showed the two-stage nature of the HSSPME process as the analyte reached equilibrium within approximately 20 min of sampling. Regular HSSPME revealed that this compound was at equilibrium only after sampling the headspace for 90 min. The latter was evidenced by comparing the amount of analyte extracted by the fiber under reduced and regular pressure conditions throughout the sampling times tested. Based on the theory the Vac-HSSPME/HSSPME mass ratio should be equal to unity when HSSPME sampling attains equilibrium under both pressure conditions since at equilibrium the amount of analyte extracted by the fiber will be the same regardless of the pressure conditions inside the sample container. Indeed, at 20 min (*i.e.* as soon as equilibrium was attained with Vac-HSSPME) the amount of fluorene extracted with Vac-HSSPME was more than 3 times larger compared to regular HSSPME and this relative enhancement leveled off to 1 after sampling the headspace for 90 min (*i.e.* as soon as equilibrium was also attained with regular HSSPME).

Fluoranthene had the lowest K_H value investigated here. By using the K_{ow} value as an indicator of hydrophobicity, it is clear that the hyperhydrophobic gas/water interface is the preferred location for this compound [23,24]. Based on the low K_H and large K_{ow} values of fluoranthene a long HSSPME equilibration time was expected [6]. Indeed, Fig. 4 illustrates that equilibrium was not attained under both pressure conditions even after sampling the headspace for 90 min. Nevertheless, with a K_H value well below the reported threshold values for low K_H compounds, gas-phase resistance controlled the evaporation rate and HSSPME sampling under reduced pressure conditions dramatically enhanced extraction kinetics when compared to regular HSSPME. Furthermore, the positive effect of reduced pressure sampling conditions remained important even after sampling the headspace for 90 min as evidenced by the Vac-HSSPME/HSSPME ratio throughout the sampling times tested (*e.g.* approximately 9 and 6 at 15 and 90 min respectively).

Overall, for compounds whose mass transfer at the liquid/gas interface is the rate determining step for HSSPME, Vac-HSSPME will perform better than or similarly to HSSPME depending on the location of the dominant resistance to evaporation. The K_H values in particular may be used to predict the performance of Vac-HSSPME. When working at room temperature, 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. For these compounds gas-phase resistance dominates and evaporation rates will be significantly improved in the presence of an air-evacuated headspace. Hence, HSSPME extraction rates will dramatically increase leading to enhanced sensitivity within short sampling times. On the other hand, for analytes with an intermediate K_H value, Vac-HSSPME is not expected to improve extraction rates compared to regular HSSPME since liquid-phase resistance (which is independent of the pressure conditions in the headspace) has become important for evaporation rates.

3.2. Effect of headspace volume during Vac-HSSPME

In regular HSSPME, headspace volume can have a significant effect on equilibration times (extraction kinetics) [8]. If the headspace capacity, K_gV_g, is sufficiently large, the analyte is

extracted almost exclusively from the gaseous phase and equilibration can be very fast provided that the amount of the analyte extracted by the fiber at equilibrium is negligible compared with the amount present in the headspace equilibrated with the sample and that only a very small amount of the analyte has actually to be transported from the liquid sample through the headspace to the fiber coating. Conversely, for a small headspace capacity the up taken analyte must be replenished by a significant amount of analyte molecules evaporating from the liquid phase. At any given moment there can only be so many molecules in the headspace, depending on the K_g value and the headspace acts in this case as a bottleneck for analyte transport to the fiber causing the equilibration process to be very slow [8]. For semivolatiles, the general suggestion for HSSPME is that the size of the headspace volume should not be very large given that equilibrium is established more quickly with the coating when the headspace volume is smaller [6,8]. Furthermore, at equilibrium and according to Eq. (1), increasing the headspace volume may result in a loss of sensitivity given that the K_gV_g term is present in the denominator. This is the case for volatile analytes whose K_g values are usually close to unity and the K_gV_g term can be neglected only when the headspace volume is close to zero [8]. Semivolatile analytes on the other hand, have much lower K_g values that may lead to a negligibly small K_gV_g term; yet this assumption must be always verified [8]. For Vac-HSSPME, the use of large sample containers results into excessive headspace volumes that may affect K_gV_g depending on the K_g value of the analyte. However, for those compounds that exhibit improvement in extraction rates with Vac-HSSPME, the effect of reduced pressure conditions is expected to dominate over any effect of headspace volume on extraction kinetics. Under vacuum conditions evaporation rates of these analytes are greatly increased and the sample responds much faster to the concentration drops in the headspace leading to enhanced extraction rates and significant reduction in equilibration times. Nevertheless, the thermodynamic theory predicts that at equilibrium, Vac-HSSPME will behave similarly to regular HSSPME and depending on the compound, increasing the headspace volume will cause a loss of sensitivity.

Figs. 2–4 compare the extraction time profiles obtained with Vac-HSSPME and regular HSSPME after sampling the headspace of the same sample (10 mL aqueous solutions spiked at 10 $\mu g\,L^{-1}$) contained in a 500 or 1000 mL sampling vessel (yielding 490 and 990 mL headspace volumes respectively). As seen (Fig. 2), for naphthalene the extraction time profiles for Vac- and regular HSSPME were essentially the same in each sample container. Moreover, the curves obtained with the two vessels looked similar in that the analyte reached equilibrium relatively fast regardless of the pressure conditions and headspace volume. As discussed earlier, naphthalene is the most volatile compound investigated here and equilibration is expected to be fast and independent of the total pressure. The high K_H value (compared to the rest of analytes) combined with the presence of an excessively large headspace volume (compared to typical HSSPME applications) resulted in a sufficiently large headspace capacity and consequently relatively short equilibration times. The difference between the extraction curves obtained with the two containers lied in the amount of analyte extracted at a certain time before reaching the equilibrium as decreased extraction rates were recorded with the 1000 mL sample container most probably reflecting the decrease in stirring efficiency when using a larger sampling vessel [20]. Interestingly, the reduced agitation conditions attained in the 1000 mL vessel did not lead to longer equilibration times. Although the headspace to fiber partition coefficients for semivolatile compounds are usually high (K_f > 1000 for the compounds studied in this work [20,25]), it was assumed that for naphthalene increasing the headspace from 490 to 990 mL resulted in a headspace capacity that was much larger than that of the fiber so that the fiber could efficiently extract the analyte from the gas-phase alone [8]. At equilibrium, a loss of sensitivity was recorded as the total amount of analyte extracted by the fiber decreased with increasing headspace volume. The K_gV_g term for this compound could not be neglected in Eq. (1) explaining thus the loss of sensitivity for the larger vessel.

For fluorene (Fig. 3), the extraction time profiles under reduced and atmospheric pressure conditions in the two sample containers were somewhat different. In particular, with Vac-HSSPME fluorene reached equilibrium in the 500 or 1000 mL sample containers at roughly the same time scale and extraction rates were essentially the same in both vessels. As discussed earlier for fluorene gas-phase resistance dominates and extraction kinetics under reduced pressure conditions is expected to be independent of the tested change in headspace volume. As the evaporation rate of the analyte is greatly increased under vacuum conditions, fast replenishment of the analyte's headspace concentration occurs leading to an enhanced extraction rate and a significant reduction in equilibration time. At equilibrium a loss of sensitivity for the large sample vessel was recorded with Vac-HSSPME. Although the K_H (and consequently K_g) value of fluorene was smaller than that of naphthalene the use of excessively large headspace volumes during the present studies resulted in a K_gV_g term that could not be neglected in Eq. (1). Thus, at equilibrium increasing the headspace volume reduced the amount of analyte extracted by the fiber. On the other hand, regular HSSPME sampling of fluorene was an overall slower process with the analyte being at equilibrium only when using the 500 mL container and after sampling the headspace for 90 min. It was assumed that the use of a smaller container increased the concentration gradient in the headspace and reduced the equilibration time [6]. Differences in stirring efficiency may also account for this observation [20].

As illustrated in Fig. 4, extraction kinetics with Vac-HSSPME for fluoranthene was once again not affected by the tested change in headspace volume and the effect of reduced pressure conditions dominated throughout the experiment. With regular HSSPME analyte transport from the sample to the fiber was a much slower process. The small K_H and large K_{ow} values of this analyte implied that at the sampling times tested only a small number of molecules could be present in the headspace making the extraction process to be very slow [6,8]. Nonetheless, the use of a smaller sample container somewhat improved extraction rates when sampling under atmospheric pressure in a nonequilibrium situation.

In summary, Vac-HSSPME extraction kinetics of the lower K_H analytes investigated here (fluorene and fluoranthene), appeared to be independent of the tested change in headspace volume. During nonequilibrium Vac-HSSPME sampling, evaporation rates were greatly increased and the analytes replenished their headspace concentration much faster when compared to atmospheric pressure sampling conditions. This resulted in faster overall HSSPME extraction process which was not affected by the tested change in headspace volume. At equilibrium however, Vac-HSSPME behaved similarly to regular HSSPME and for those analytes where the K_gV_g term that could not be neglected in the calculation of the amount of analyte extracted by the fiber, a loss of sensitivity was recorded at equilibrium. For the intermediate volatility compound (naphthalene), Vac- and regular HSSPME were affected by the tested change in headspace volume in a similar manner since extraction was independent of the total pressure conditions during both the nonequilibrium and equilibrium stages of the process.

3.3. Effect of agitation during Vac-HSSPME

HSSPME equilibration times for hydrophobic compounds can be significantly shortened when agitation is used [1,9], as high stirring speeds decrease the thickness of the boundary layers (by creating convection primarily in the aqueous and secondarily in

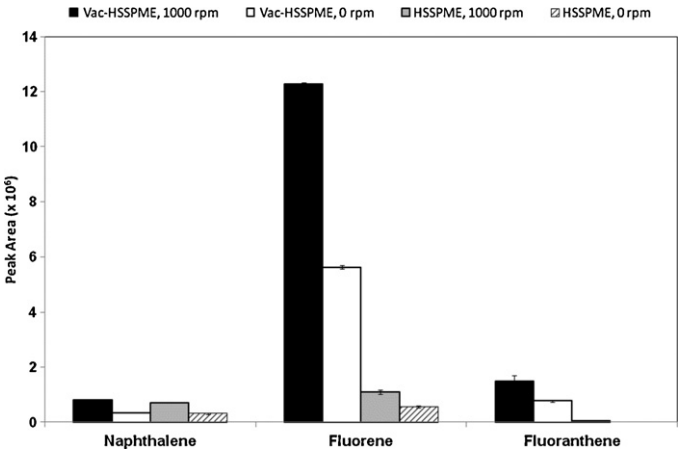

Fig. 5. Effect of agitation: extraction efficiencies obtained with Vac-HSSPME under turbulent (Vac-HSSPME, 1000 rpm) and static (Vac-HSSPME, 0 rpm) conditions and regular HSSPME under turbulent (HSSPME, 1000 rpm) and static (HSSPME, 0 rpm) conditions. Other experimental parameters: 500 mL sample container; 10 mL aqueous sample spiked at 10 $\mu g\,L^{-1}$ with each PAH; 25 °C sampling temperature; 10 min sampling time. Some error bars are too small to be visible as compared with the physical size of the symbol.

the headspace), thus reducing the diffusion time of solutes [6,20]. Fig. 5 shows the signals after HSSPME under normal and reduced pressure conditions and at two mixing regimes for the aqueous phase (0 and 1000 rpm). The three PAHs were extracted in a nonequilibrium situation (10 min) from the 500 mL sampling vessel containing 10 mL aqueous solution spiked at 10 $\mu g\,L^{-1}$. For naphthalene and fluorene, stirring the condensed phase was found to improve extraction rates compared to the stagnant mode under each pressure condition. In fact stirring enhancements were found to be similar whether sampling under vacuum or atmospheric pressure conditions. In the case of fluoranthene the turbulent/stagnant ratio could be calculated only for Vac-HSSPME as this compound was not detected after regular HSSPME sampling in the static mode. Clearly, for this low K_H compound resistance to mass transport at the sample/headspace barrier was dominant.

It should be mentioned here that with the exception of naphthalene, enhanced performance was obtained with Vac-HSSPME (whether in the static or turbulent mode) compared to regular HSSPME thus confirming the beneficial effect of sampling low K_H compounds in an air-evacuated headspace. As discussed earlier naphthalene is not affected by the pressure conditions in the headspace and for this analyte differences between Vac- and regular HSSPME at each agitation condition were found to be marginal.

3.4. Application of Vac-HSSPME

The linearity of the method was investigated by extracting aqueous standards with increasing concentrations over a range from 0.5 and 10 $\mu g\,L^{-1}$. A 10 mL spiked aqueous sample was placed in the 500 mL sampling vessel and stirred at 1000 rpm. Each time Vac-HSSPME extraction was performed for 30 min at a constant temperature (25 °C). All compounds showed good correlation with 0.998, 0.998 and 0.999 coefficients of determination (r^2) for naphthalene, fluorene and fluoranthene respectively. The precision of the method, expressed as relative standard deviation (RSD), was determined by performing five consecutive extractions from aqueous samples with a concentration of 10 $\mu g\,L^{-1}$ and the RSD values found were 2.7, 1.8 and 8.4% for naphthalene, fluorene and fluoranthene respectively. The limits of detection (LODs) defined for a signal-to-noise of three (S/N = 3) were 0.09, 0.02 and 0.08 $\mu g\,L^{-1}$ for naphthalene, fluorene and fluoranthene respectively.

4. Conclusions

The present work demonstrated that for those compounds whose mass transfer at the gas/liquid interface is the rate determining step, nonequilibrium Vac-HSSPME may perform better than regular HSSPME at room temperature depending on the location of dominant resistance to evaporation rate. In particular, the K_H value may be used to predict the performance of Vac-HSSPME. For analytes close or below the reported threshold values for low K_H solutes, extraction kinetics are considerably improved with Vac-HSSPME compared to regular HSSPME, as evaporation rates for these analytes dramatically increase under reduced pressure conditions and consequently the sample responds much faster to the concentration drops in the headspace. For these compounds the faster replenishment of the analytes' headspace concentrations also explained the fact that extraction kinetics was largely not affected by the tested change in headspace volume. Conversely, for intermediate K_H solutes where liquid-phase resistance to mass transfer becomes important, Vac-HSSPME will not lead to obvious improvements in extraction rates compared to regular HSSPME. At equilibrium, the amount extracted by the SPME fiber is independent of the pressure conditions inside the sample container and depending on the K_H value of the target analyte increasing the headspace volume may result in a sensitivity loss. However, the present findings suggest that within short sampling times Vac-HSSPME will result in enhanced sensitivity compared to regular HSSPME. Finally, stirring the liquid sample was found to improve even further Vac-HSSPME.

Acknowledgement

The authors thank the Technical University of Crete for the financial support.

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