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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1731970> since 2020-02-27T18:01:29Z

Published version:

DOI:10.1016/j.aca.2019.10.050

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Vacuum-assisted headspace sorptive extraction: Theoretical considerations and proof-of-concept extraction of polycyclic aromatic hydrocarbons from water samples.

Nicoleta Solomou[†], Carlo Bicchi[‡], Barbara Sgorbini[‡] and Elefteria Psillakis^{†,*}

[†] *Laboratory of Aquatic Chemistry, School of Environmental Engineering, Polytechniopolis, Technical University of Crete, GR-73100, Chania, Crete, Greece*

[‡] *Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, via Pietro Giuria 9, I-10125 Torino, Italy*

**Corresponding author e-mail: elia@enveng.tuc.gr (Elefteria Psillakis)*

ABSTRACT

The use of a thick sorbent coating in headspace sorptive extraction (HSSE) increases the amount of analytes extracted at equilibrium as well as the time needed to reach it. In this work we propose HSSE sampling under vacuum conditions to reduce equilibration times. A theoretical model is presented that describes the pressure dependence of the so-called vacuum-assisted HSSE (Vac-HSSE) method, and predicts the reduction in equilibration times when lowering the sampling pressure. We take advantage of the theoretical formulation to reach some general conclusions for HSSE on the relationship between the physical characteristics of the stir bar, uptake rates and equilibration times. The theoretical predictions were experimentally verified using water solutions spiked with naphthalene, acenaphthene and fluoranthene as model compounds. The effects of sampling temperature and extraction time under vacuum and regular pressure conditions were thoroughly investigated. The positive combined effect of heating the sample under low sampling pressure pointed that high humidity did not affect the performance of the extraction phase; an effect commonly recorded in headspace solid-phase microextraction. The extraction time profiles built at 25 and 55 °C visualized the substantial improvement in extraction kinetics with Vac-HSSE compared to the regular HSSE method. The results on naphthalene (assumed to evaporate relatively fast from the water sample) provided evidence that at 1 atm gas-sided resistance limited analyte uptake by the stir-bar and that this limitation could be effectively reduced by adopting the vacuum sampling approach. The accelerations of acenaphthene and fluoranthene suggested that gas-phase constraints limited both the evaporation and analyte uptake processes. Independent method optimization of HSSE under each pressure condition yielded a shorter sampling time for Vac-HSSE compared to the regular HSSE procedure (30 min vs. 60 min respectively). The analytical performances of the two optimized methods were evaluated and it was concluded that Vac-HSSE was performing similar (naphthalene and acenaphthene) or better (fluoranthene) than regular HSSE within half the sampling time needed.

Keywords: headspace sorptive extraction; vacuum-assisted; gas limitations; polycyclic aromatic hydrocarbons.

1. INTRODUCTION

Stir bar sorptive extraction (SBSE) was introduced in 1999 as a novel configuration for sorptive sampling [1]. The method used polydimethylsiloxane (PDMS) coated stir bars and consisted of a two-step procedure where target analytes were initially absorbed into the PDMS coating and then desorbed following a liquid or thermal desorption step. The stir bars were marketed under the name TwisterTM (Gerstel, Mülheim a/d Ruhr, Germany), and the method was successfully applied to the analysis of organic compounds and elemental speciation in a variety of samples including environmental, food and biological [2–6].

The headspace sampling mode was reported in 2001 by Tienpont et al. [7] and Bicchi et al. [8]. The procedure was termed headspace sorptive extraction (HSSE), and has mainly been used for studying the headspace composition of flavors and fragrances [2,9,10]. In HSSE, the stir bar was placed in the gas-phase with analytes transferring from the sample to the headspace and then into the sorbent phase. The use of a high PDMS volume as coating for the stir bar resulted in higher extraction capacities and recoveries than those obtained with other PDMS-based methods, *e.g.* headspace solid-phase microextraction (HS-SPME) using a 100- μ m PDMS fiber [10]. At the same time, HSSE needed longer times to reach equilibrium [5]; an effect also reported for the immersion sampling mode (SBSE) that was related to the thickness of the PDMS coating [11]. The theoretical principles of HSSE at equilibrium have been discussed at different occasions and were analogous to those applied in HS-SPME [2,3,12,13]. Next to these thermodynamic considerations, the kinetic aspects of the extraction process are equally important, especially because HSSE is mainly operated under non-equilibrium conditions. Nonetheless, to the best of our knowledge, a detailed discussion on the theoretical aspects of pre-equilibrium HSSE sampling has not yet been reported [3].

Different approaches are available to accelerate extraction kinetics in headspace sorbent-based methods, including *e.g.* stirring and/or heating the sample [9,12]. An alternative approach used to improve the extraction rates of analytes with a low affinity for the headspace, suggests headspace sampling under reduced pressure conditions. The approach was first applied to HS-SPME by Brunton *et al.* [14] and the effect was later confirmed by Darouzes *et al.* [15]. In 2012, Psillakis *et al.* presented the theoretical model describing the pressure dependence of the so-called vacuum-assisted HS-SPME (Vac-HS-SPME) for water [16] and (later) for solid matrices [17], and successfully applied the method to a variety of analytes and matrices [18–20]. Recently, Trujillo-Rodríguez *et al.* expanded the applicability of the vacuum-based

sampling approach to headspace single drop microextraction and reported shorter equilibration times for short chain free fatty acids [21]. In a subsequent report, Psillakis *et al.* [22] formulated the pressure dependence of the analyte evaporation and uptake processes taking place during this procedure.

This study aims to investigate for the first time the applicability of the vacuum sampling approach to HSSE as a viable tool to accelerate extraction kinetics and reduce equilibration times. The resulting procedure was termed vacuum-assisted HSSE (Vac-HSSE). The theoretical model describing the HSSE pressure dependence under non-equilibrium conditions is here discussed. A comparative study between Vac-HSSE and regular HSSE (under atmospheric pressure) was carried out to demonstrate the benefits of adopting the vacuum approach. The use of three model polycyclic aromatic hydrocarbons (PAHs) as target analytes enabled exploring some new and important insights on HSSE. The effects of temperature and sampling time were thoroughly investigated and discussed under each pressure condition. Finally, the two methods were independently optimized and their analytical performance was assessed and compared.

2. Theoretical Considerations

During HSSE sampling from water samples, analytes transfer in three phases (water, headspace and PDMS coating) and across two interfaces (water/headspace and headspace/PDMS coating) until equilibrium between the three phases is reached [3]. Initially and as soon as the coated stir bar is exposed to the headspace above the aqueous sample, analyte uptake by the PDMS coating is linear with time. This is followed by a curvilinear region that finally moves to a constant phase, representing equilibrium conditions between all phases involved [23–25]. Current knowledge concludes that the amount of analyte extracted at equilibrium is independent on the total pressure in the sample vial, as partition coefficients and equilibrium concentrations are only affected at high operating pressures [16,18].

The pre-equilibrium aspects of the HSSE analytical system can be better understood by decoupling the system into two interfacial ones: (i) the evaporation step (water/headspace system), and (ii) the analyte uptake by the PDMS coating of the stir bar (headspace/PDMS system). The theory underlying the kinetics of the evaporation step is similar to that formulated for HS-SPME [5,7], and its pressure dependence has been demonstrated and experimentally

verified [16,18]. For this reason, the theoretical aspects concerning the headspace/PDMS interfacial system are only dealt with here.

The PDMS coating of the stir bar is considered as a single uniform phase. Accordingly, the overall mass transfer coefficient, k_o , controlling analyte uptake can be described using the two-film approach. The approach assumes uniformly mixed liquid and gas bulk phases, separated by two thin films of air and PDMS adjacent to the interface where analyte mass transfer is by molecular diffusion alone [26]. The two films are modeled as follows

$$\frac{1}{k_o} = \frac{1}{k_g} + \frac{1}{K_{eg}k_e} \quad (1)$$

where K_{eg} is the PDMS/headspace partition coefficient and k_g and k_e are the mass transfer coefficients for the gas- and PDMS-side boundary layers (no dimensions). In Eq. (1) the two films are modeled as two resistances in series where the overall resistance to analyte uptake ($1/k_o$) equals the sum of resistances of the gas-phase boundary layer ($1/k_g$) and the PDMS boundary layer ($1/(K_{eg}k_e)$) [24,27].

In general, the net rate of analyte accumulation in the PDMS coating can be represented by the following flux equation [28,29]

$$V_e \frac{dC_e}{dt} = k_o A_e \left(C_g - \frac{C_e}{K_{eg}} \right) \quad (2)$$

with t denoting time, V_e the volume of PDMS coating, C_g and C_e the analyte's concentrations in the gas-phase and PDMS respectively, and A_e the surface area of the PDMS coating. During the initial stage of linear uptake by the coated stir bar, C_e is very small and K_{eg} is large, resulting in a small analyte loss from the PDMS coating (C_e/K_{eg} term) [24,29]. The change in analyte concentration in the PDMS coating can then be simplified to

$$V_e \frac{dC_e}{dt} = k_o A_e C_g \quad (3)$$

Assuming constant values for C_g , then Eq. (3) can be integrated and expressed on a mass basis [23,24]

$$N_e = k_o A_e C_g t \quad (4)$$

which shows that in the linear region, the amount of extracted analyte, N_e , is directly proportional to the overall mass transfer coefficient and the area of the sampler. The latter relationship suggests that at an early stage of extraction, the large surface area of the PDMS coating will ensure high analyte uptake over time. It also suggests that during this stage, any other PDMS-based configuration having the same surface area with the PDMS coated stir bar will extract the same amount of analytes over time regardless of the coating volume [23].

As the analyte concentration in the PDMS coating increases, the loss from the PDMS coating becomes more important and uptake will become curvilinear [29]. At equilibrium, the net uptake will approach zero and rearrangement of Eq. (2) gives

$$N_{e(eq)} = K_{eg} V_e C_g \quad (5)$$

which shows that the final amount of analyte extracted at equilibrium, $N_{e(eq)}$, is directly proportional to the volume of the coating (and independent of A_e).

The complete uptake profile can be described by integrating Eq. (2) to yield

$$C_e = K_{eg} C_g \left(1 - e^{-\left(\frac{A_e}{V_e} \times \frac{k_o}{K_{eg}} \times t\right)} \right) \quad (6)$$

Eq. (6) is a form of a first order reaction where the time taken to attain equilibrium will be influenced by the coating, analyte properties and gas phase characteristics [28]. In particular, the time to effective equilibrium, $t_{95\%}$, defined as when C_e reaches 95% of its true equilibrium value can be estimated as

$$t_{95\%} = \frac{3 V_e}{A_e k_o} K_{eg} \quad (7)$$

From this equation, the relationship between the physical characteristics of the sorbent and the time to equilibrium is now clear: decreasing the surface area to volume ratio of the sorbent coating will increase the time needed to approach equilibrium. This explains the extended equilibration times recorded with SBSE compared to SPME (headspace and direct sampling modes). The 0.5-mm thick and 1-cm long PDMS coating of the stir bar has a 1 cm² surface area and a 24 μ L coating volume [11]. These values yield a A_e/V_e ratio that is four times lower than that of a 100- μ m thick and 1-cm long PDMS SPME fiber (surface area 0.1 cm² and 0.612 μ L coating volume [30]). Hence, when a coated stir bar is used for extraction, the larger PDMS volume will extract a larger amount of analyte at equilibrium, but the time needed to achieve the equilibrium will increase. It is also interesting to consider the case of thin film microextraction (TFME) where thin sheets of PDMS are used for extraction (*e.g.* the thin film considered in the past with a 5-cm² surface area on each side and a 63.5 μ L volume [11]). In this configuration, the PDMS volume is increased compared to the conventional SPME fiber and coated stir bar approaches. However, the maximization of the surface area when using a thin PDMS sheet yields a surface area to volume ratio that is comparable to that in SPME and close to four times larger than that in SBSE. For this reason, equilibration times recorded with TFME should (and were found to) be similar to those with SPME [31], and shorter than those recorded with SBSE (headspace and direct sampling modes) [11].

According to Eq. (1), K_{eg} is an important parameter to study for predicting the gas- or PDMS-sided limitations on uptake for a given analyte. For nonpolar organics, K_{eg} was assumed to be similar in magnitude to the octanol-air partition coefficient (K_{OA}) [24]. In particular, past reports discussing the theoretical aspects of passive air sampling suggested gas-sided limitations during

uptake of semivolatiles such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls [24,28], as for these analytes K_{OA} values were sufficiently large ($K_{OA} > 10^6$) to yield a negligible PDMS-sided resistance (*i.e.* the $1/(K_{eg} k_e)$ term in Eq. (1)) [24]. This implies that for gas-side limited analytes (where $k_o = k_g$) changes in the value of k_g will result in changes in the uptake rate throughout the pre-equilibrium stage (Eqs. (2)-(4) and (6)) as well as on the equilibration time. Conversely, for analytes with a small K_{eg} , extraction will be limited by diffusion in the PDMS phase (the $1/(K_{eg} k_e)$ term in Eq. (1) dominates) and any changes in k_g will not affect uptake rates by the PDMS. It is reminded, that analytes with small K_{eg} value will not have a high affinity for the extracting phase, and the use of PDMS coated stir bar might not be a sensitive tool for their detection and quantification.

In general, k_g is proportional to the diffusion coefficient in air (D_g) raised to some power, the value of which depends on the model assumed [16]. Among the different representations used for expressing D_g , the Fuller–Schettler–Giddings diffusivity correlation for binary mixtures of gases at low pressures shows that the value of D_g is inversely proportional to the total pressure in the gas phase [16]. This implies that reducing the total pressure will increase D_g and consequently k_g , leading to a reduced gas-sided resistance (expressed as $1/k_g$ in Eq. (1)). Accordingly, for analytes where gas-phase resistance controls analyte uptake, applying a low sampling pressure will accelerate the extraction kinetics during both the linear and curvilinear stages of the process. Moreover, Eq. (7) shows that any enhancements in k_g upon lowering the sampling pressure will also induce improvements in $t_{95\%}$, leading to shorter equilibration times between the headspace and the PDMS phases.

The following general conclusions can be reached by combining the theoretical aspects of the evaporation step (as discussed in [16,18]) and those applied during analyte uptake by the PDMS coated stir bar (as discussed here): (i) for analytes where gas-phase resistance controls analyte uptake (headspace/PDMS system) and/or evaporation (water/headspace system), reducing the sampling pressure will accelerate mass transfer in the thin gas film involved and reduce equilibration times, compared to 1 atm, (ii) for analytes where liquid-sided resistance controls the step(s) of analyte uptake (headspace/PDMS system) and/or evaporation (water/headspace system), reducing the sampling pressure will not affect the extraction kinetics (and as such the equilibration times) of the step(s) compared to standard pressure, and (ii) in cases where only one of the two steps is accelerated, then the magnitude of the effect of sampling under vacuum

will depend on the relative importance of the step involved *i.e.* accelerations will be recorded if the step involved is the rate-controlling step in the overall extraction kinetics.

3. MATERIALS AND METHODS

3.1. Chemicals, materials and samples

Naphthalene (Nap) and Acenaphthene (Ace) were purchased from Sigma-Aldrich (Steinheim, Germany) at a purity $\geq 99\%$ and Fluoranthene (Flu) from Riedel-de-Haën (Seelze, Germany) at a purity of 98.4%. A 100 mg L⁻¹ acetone stock solution of the three model analytes was prepared and used for preparing spiked water samples. The acetone stock solution containing the three target analytes was stored in an amber vial at -18 °C when not in use. Acetone (ACS Reagent grade) and acetonitrile (HPLC grade) was provided by Honeywell (New Jersey, USA). Deionized water was prepared on a Barnstead EASYpure II water purification system purchased by Thermo Scientific (Dubuque, USA). The 10-mm long stir bars (Twister™) were coated with a 0.5-mm thickness PDMS layer (24 µL) and were obtained from Gerstel (Mülheim an der Ruhr, Germany). New and used stir bars were conditioned by sonicating them in 1 ml of acetonitrile. The effect of matrix was studied using tap water collected from the University campus (Technical University of Crete, Greece) and secondary treated wastewater effluent (WWTP effluent) from the municipal wastewater treatment plant of Chania, Greece, serving approximately 70,000 inhabitants.

3.2. Vac-HSSE and regular HSSE procedures

A 20 mL screw top vial (Restek, Bellefonte, USA) containing a magnetic polytetrafluoroethylene (PTFE) stir bar (15 mm x 5 mm; Sigma Aldrich) was placed on top of a magnetic and heating stir bath plate (Heidolph MR-Standard, Germany). The stir bar was then fixed on the inner top part of the vial wall with the help of two external magnets hooked on metallic binder clips. A tailor-made closure designed and constructed by the Laboratory of Aquatic Chemistry (Technical University of Crete) was used for all experiments. The closure was equipped with a cylindrical Thermogreen®LB-1 septum (Supelco) with half-hole (6 mm diameter × 9 mm length). Alternatively, the previously reported modified crimp-top Mininert®valve (Sigma-Aldrich) can be used to ensure gastight conditions inside the sampler

[29]. A VP 2 Autovac pumping unit (7 mbar = 0.007 atm ultimate vacuum without gas ballast) manufactured by Vacuubrand (Wertheim, Germany), was used to evacuate the air inside the sample container [32]. A 10 ml aqueous sample containing a known concentration of target analytes was introduced inside the vial through the septa using a gastight syringe (SGE, Australia). Agitation at 500 rpm was applied, initiating HSSE sampling for a preset period time and temperature. The effect of agitation was investigated during a preliminary set of experiments (tested values: 0, 500 and 1000 rpm). The results, shown in Fig. S1 in the supporting information showed that agitating the sample improved extraction efficiencies compared to the stagnant (standing) mode. Moreover, increasing the stirring speed from 500 rpm to 1000 rpm did not improve extraction efficiencies. However, the error associated to the measurements increased at 1000 rpm, most probably due to inconsistent agitation at high stirring speeds [33]. For this reason a stirring speed at 500 rpm was applied. When extraction was completed, the septum was pierced with an open-end disposable needle allowing pressure equilibration and easy opening of the closure. The stir bar was then removed with the help clean tweezers, dried with a lint-free tissue and transferred to a 350 μ L-glass insert vessel containing 150 μ L acetonitrile. The stir bar and solvent were sonicated for 15 min. Note that the glass inserts were placed in 2 mL vials containing a minimum amount of deionized water to afford successful propagation of ultrasound waves. Upon completion of the liquid desorption step, 40 μ L of the acetonitrile extract were used for analysis. It is noted that the liquid desorption step was optimized here during a preliminary study and the results are given in Fig. S2 in the supporting information [33,34]. The parameters controlled in this set of experiments were: desorption solvent volume (tested values: 150, 200 and 300 μ L), sonication time (tested values: 1 - 20min), and number of liquid desorption steps (1 to 3). The optimum conditions found were: one liquid desorption step, 150 μ L of acetonitrile and 15 min sonication time. These were similar to the optimum liquid desorption conditions reported in the past after immersion SBSE sampling of PAHs from water samples: (i) one desorption step sonication, 150 μ L of acetonitrile and 10 min sonication time [33] and (ii) one liquid desorption step, 200 μ L of acetonitrile and leaving the mixture for 15 min unattended [34].

For regular HSSE extraction the air-evacuation step was omitted and all extractions were performed under regular atmospheric conditions.

For both Vac- and regular HSSE, the closure's septum was replaced on a daily basis. Blanks were also run periodically to ensure the absence of carry over between runs. All extractions were run in triplicate.

3.3. High-performance liquid chromatography (HPLC) coupled to fluorescence detection

Separation and detection was performed on a HPLC (Shimadzu Corporation, Kyoto, Japan), equipped with two solvent delivery pumps (LC 10AD VP), a fluorescence detector (RF 10A XL), a Rheodyne manual sample injector valve with a 20- μ L loop (Chrom Tech Inc., MN, USA) and a Macherey-Nagel C18 (250 mm x 3.0 mm i.d., with 5 μ m particles size) (Macherey-Nagel, Duren; Germany). The mobile phase was acetonitrile : water 85:15 v/v and flow rate 1.0 mL min⁻¹. The selection of the excitation/emission pairs for detection were: 280/355 for Nap and Ace and 260/460 for Flu.

4. RESULTS AND DISCUSSION

4.1. Effects of temperature and extraction time on Vac-HSSE

Temperature is one of the most important experimental parameters to consider during HSSE method optimization [9]. Heating the sample will increase Henry's Law volatility constants, K_H , leading to higher headspace concentrations and shorter equilibration times. However, high sample temperatures may also reduce the PDMS-headspace partition coefficients and, as such, decrease the final amount of analyte accumulated at equilibrium [4]. Here, the effect of sample temperature was investigated from 25 to 65 °C under vacuum and atmospheric pressure (30 min extraction time) and the results are given in Fig. 1. The spiking concentration was set at 5 μ g L⁻¹, so as to ensure detection of all target analytes with regular HSSE at lower sampling temperatures. As can be seen, at each sampling temperature, Vac-HSSE greatly improved extraction efficiencies compared to regular HSSE and the effect was more important for Ace and Flu. A more accurate evaluation of the Vac-HSSE results, revealed an optimum temperature at 45 °C for Ace, whereas for Flu, heating the sample gradually increased the amount extracted and for Nap, no substantial changes in extraction efficiencies were recorded when heating the sample under low pressure conditions. With regular HSSE, the amount of Nap extracted seems to increase slightly when heating the sample at 55 °C. On the contrary, the beneficial effect of heating the sample at 1 atm was evident for Ace and Flu.

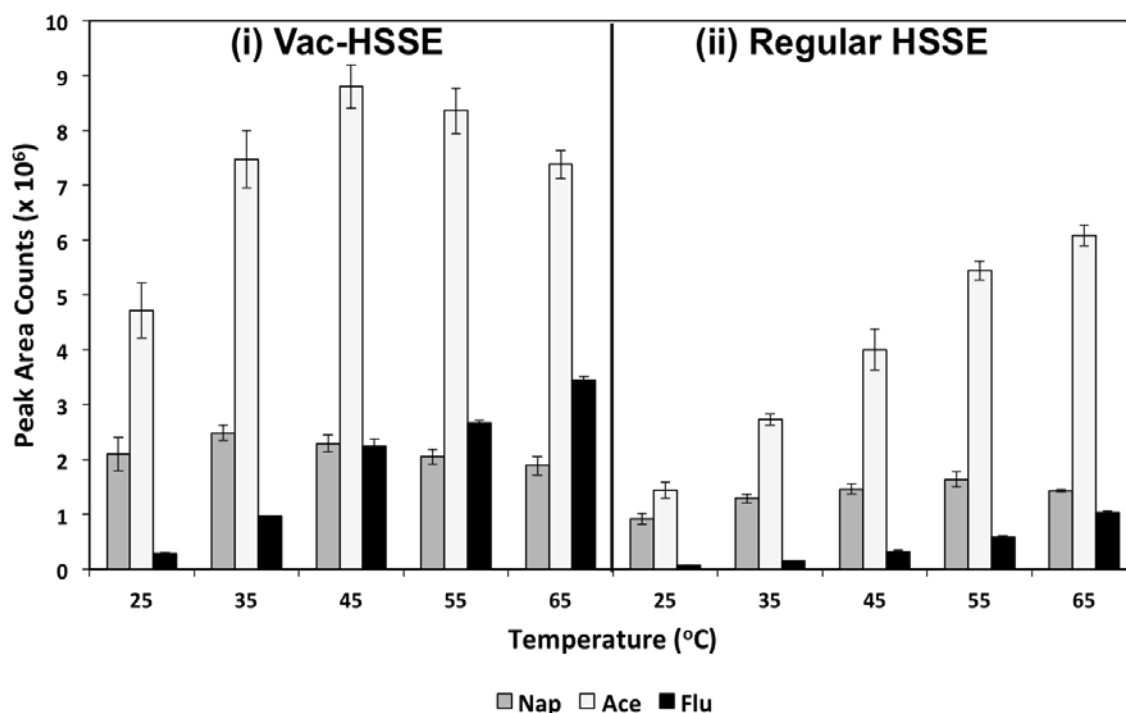
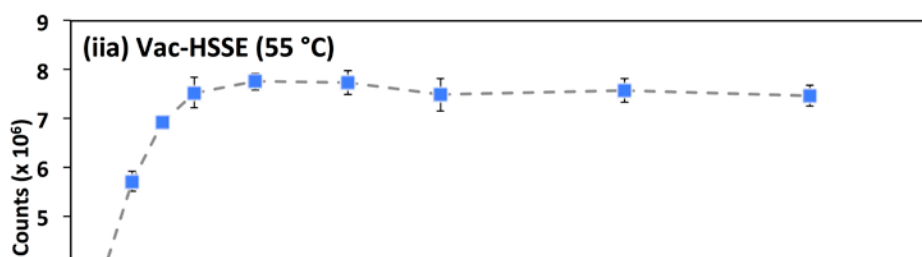
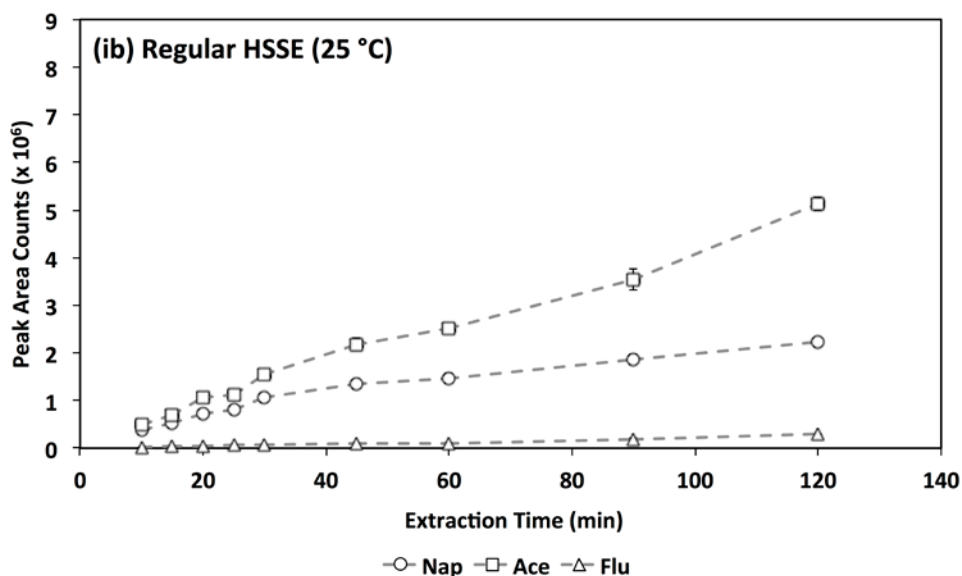
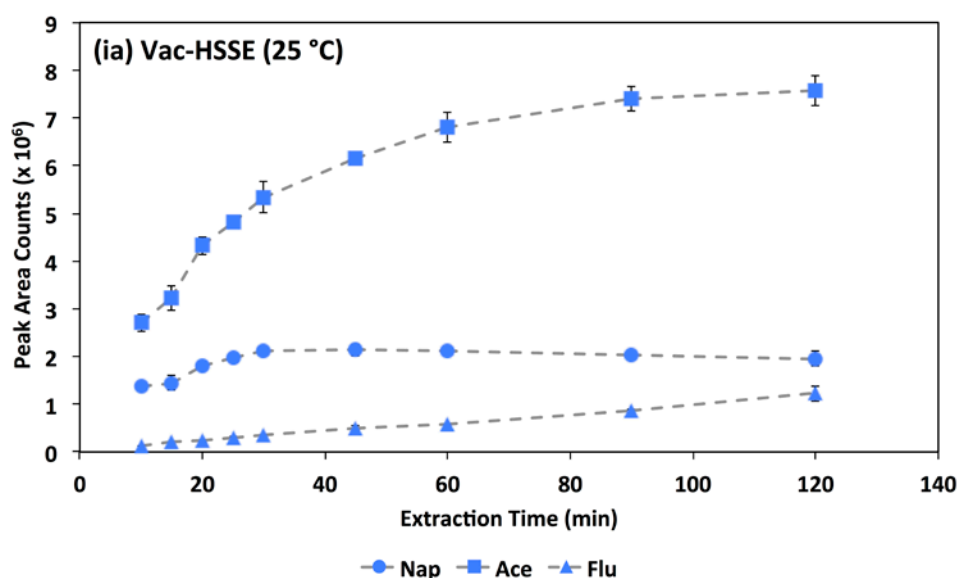


Fig. 1. Effect of temperature on (i) Vac-HSSE and (ii) regular HSSE. Experimental conditions: 10 mL aqueous samples spiked at $5 \mu\text{g L}^{-1}$; 30 min sampling time; 500 rpm agitation speed. Some error bars are too small to be visible as compared with the physical size of the symbol.

An important point to consider in Vac-HSSE is the successful combination of the effects of temperature and low pressure. This was not the case when low pressure conditions were applied to HS-SPME sampling of PAHs from aqueous solutions [35], where higher sampling temperatures unexpectedly affected mass loading of PAHs into the SPME fiber. It was assumed that the high amount of water molecules interacted with the PDMS coating of the SPME fiber, changing its properties. In the past, the use of PDMS as a material in permeation-type passive air samplers resulted in a low permeability towards water vapor and a low energy of activation of permeation towards gas phase organic compounds [9,12]. Accordingly, water uptake and the possibility of sorbent saturation with water were assumed to be very small. Moreover, no significant variations in the analyte uptake rate from the gas phase were observed when high humidity [36] or temperatures were applied [36,37] on PDMS acting as a coating or membrane material. It therefore appears that the non-ideal performance of the PDMS fiber during Vac-HS-SPME at elevated temperatures is related to the small thickness of the fiber coating compared to that of a PDMS coated stir bar. At high humidity conditions, any surface irregularities/uncoated sites of the SPME fiber (due, for instance, to its repeated use) might act

as a spot for water adsorption and suppress analyte uptake [36]. Here, the relatively high PDMS volume and layer thickness of the stir bar, prevented water molecules interfering with analyte uptake during Vac-HSSE sampling, and afforded effective coupling of the effects of temperature and low pressure.



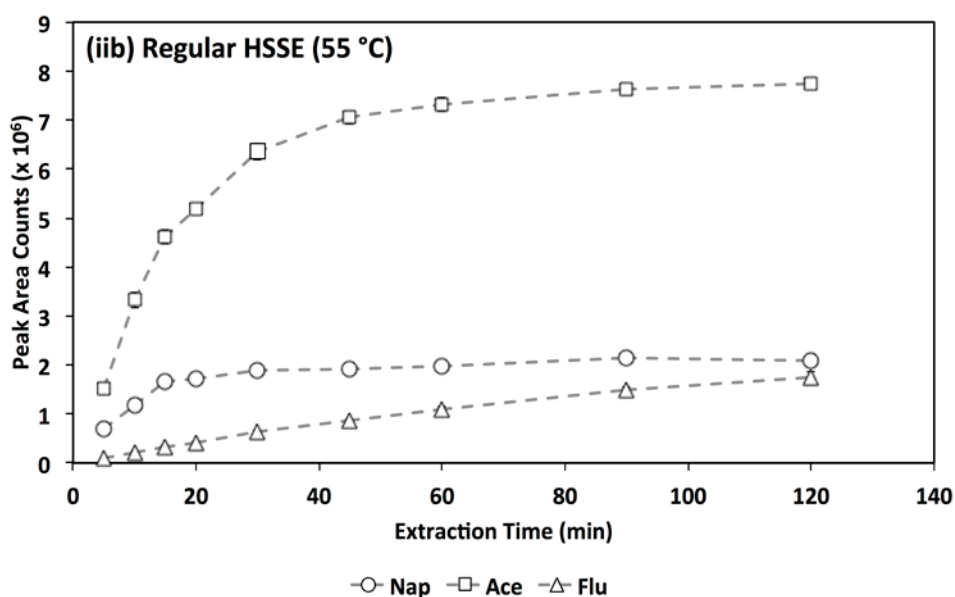


Fig. 2. Extraction time profiles for the three model PAHs obtained at 25 °C (ia) under vacuum (filled symbols) and (ib) regular (open symbols) pressure conditions and at 55 °C (iia) under vacuum (filled symbols) and (iib) regular (open symbols) pressure conditions. Experimental conditions: 10 mL aqueous samples spiked at 5 $\mu\text{g L}^{-1}$; 500 rpm agitation speed. Some error bars are too small to be visible as compared with the physical size of the symbol.

The effect of sampling time was initially investigated at 25 °C under vacuum and atmospheric pressure conditions. The spiking concentration was again set at 5 $\mu\text{g L}^{-1}$, so as to allow detection of Flu with regular HSSE at lower sampling times. The results, depicted in Fig. 2(ia) and 2(ib), verified our theoretical predictions and a remarkable improvement when sampling under

vacuum vs. atmospheric pressure was recorded during both the linear and curvilinear stages of extraction. With Vac-HSSE, Nap reached equilibrium after only 30 min of sampling, whereas with regular HSSE equilibrium was assumed to be between 90 and 120 min, since the Vac-HSSE/HSSE peak area ratios were close to unity (*i.e.* Vac- and regular HSSE have the same extraction efficiencies at equilibrium). The long HSSE equilibration time required for Nap at 1 atm was in agreement with a past report aiming at the determination of PDMS-air partition coefficients using regular HSSE [37]. This study monitored concentrations in air and on the PDMS coating of a stir bar and reported a 90 min equilibration time for naphthalene at temperatures between 20 and 30 °C.

Fig. 2 (ia) and 2(ib), also show that the extracted amount of Ace under vacuum gradually increased with time, and the extraction rate appears to slow down close to 120 min, *i.e.* as Ace slowly approached equilibrium. On the contrary, with regular HSSE, Ace was still away from equilibrium even after 120 min (Vac-HSSE/HSSE peak area ratio equals 1.5 at this time point). The extraction time profiles obtained for Flu, the most hydrophobic and least volatile analyte tested here, were away from equilibrium at both investigated pressures. However, the improvement in extraction efficiency with Vac- over regular HSSE was important at each sampling time tested (*e.g.* at 120 min the Vac-HSSE/HSSE peak area ratio for Flu was 4).

Comparison of the present results to those obtained with HS-SPME can give some important insights into the HSSE process. Past studies reported a rather fast equilibration for HS-SPME sampling of Nap from water solutions under regular and low pressure conditions [35,38]. These short equilibration times were associated to the relatively fast evaporation rate of Nap from the water sample [38], taken that equilibration between the headspace and the SPME fiber polymer interface during HS-SPME is assumed to be fast for semi-volatiles [25]. This conclusion was in agreement with a past report stating that for Nap, evaporation from water was largely controlled by liquid-phase resistance (82.2 %) [39], and was therefore, expected to be relatively independent of the total pressure in the sampling vial [38]. Here, the extraction time profiles under each pressure were substantially different: Nap reached equilibrium at about 30 min with Vac-HSSE, whereas equilibrium with regular HSSE was assumed to be between 90 and 120 min. Assuming that Nap evaporates relatively fast from the water sample, this finding suggested that the rate-limiting step in HSSE was located in the headspace/PDMS system. Moreover, the acceleration in extraction rates recorded when lowering the sampling pressure highlighted that mass transfer from the headspace to the PDMS coating of the stir bar was pressure-dependent (*i.e.* the major resistance was gas-sided) and that these gas-phase constraints could effectively

be reduced by lowering the total pressure. The latter effect was predicted by the theory presented here, as for this gas-side limited analyte (where $k_o = k_g$) reducing the value of k_g was expected to increase analyte uptake by the PDMS phase rate throughout the pre-equilibrium stage (Eqs. (2)-(4) and (6)) and reduce its equilibration time (Eq. (6)). It is noted that air-sided resistance to diffusion into passive samplers has been demonstrated for PAHs in general [23,40]. This implies that gas-phase limitations were also present during Ace and Flu uptake by the PDMS coated stir bar and that they could be effectively reduced when sampling under vacuum. However (and as will be discussed in the following section), for Ace and Flu, evaporation is also expected to be a slow step in extraction and reducing the sampling pressure will induce additional accelerations in the overall extraction kinetics.

HS-SPME enables to predict the effect of low sampling pressure on extraction rates using the so-called K_H criterion, where the K_H values of target analytes are compared to some threshold values [18,38]. This type of evaluation should be used with caution with the HSSE sampling approach. According to the K_H criterion, the extraction kinetics of Nap was not expected to be affected when lowering the sampling pressure, since its K_H value suggested a liquid-sided resistance to evaporation, which is independent of the total pressure [18]. Nonetheless, a significant improvement in the extraction rate of Nap was recorded with Vac-HSSE, as gas-sided resistance controlled analyte uptake in the headspace/PDMS system. For Ace and Flu (low K_H compounds), the K_H criterion predicted gas-sided control of the evaporation step [18] and low evaporation rates from water samples. Taken that Ace and Flu have K_{OA} values sufficiently large ($K_{OA} > 10^6$) to yield a negligible PDMS-sided resistance (*i.e.* the $1/(K_{eg} k_e)$ term in Eq. (1)), air-sided resistance to diffusion into the PDMS coated stir bar should be present [22,23,40] that can also be removed by lowering the sampling pressure. Accordingly, for Ace and Flu, lowering the sampling pressure was expected to accelerate both the evaporation (water/headspace system) and analyte uptake (headspace/PDMS system) HSSE steps.

The extraction time profiles were then constructed at 55 °C and the results are reported in Fig. 2(iia) and 2(iib). As expected, heating the aqueous sample accelerated the extraction process under each pressure condition and the effect was more pronounced with sampling under vacuum. In particular, an important enhancement in extraction rates was recorded with Vac-HSSE, where all target analytes reached equilibrium within the sampling times tested (15 min for Nap, 30 min for Ace and 90 min for Flu). Under regular pressure conditions, Nap reached equilibrium at about 20 min and Ace was approaching equilibrium close to 120 min of sampling (Vac-HSSE/HSSE peak area ratio was close to 1). Flu was away from equilibrium under each

pressure condition. However, the positive effect of sampling under vacuum was again important at each sampling time tested (e.g. at 120 min the Vac-HSSE/HSSE peak area ratio was 1.8).

In order to evaluate the analytical performances Vac-HSSE and regular HSSE, the optimum conditions for each method were set as: the sampling temperature was set as a compromise at 55 °C for both sampling pressures and sampling times were set as 30 min for Vac-HSSE and 60 min for regular HSSE.

4.2. Analytical performance of the optimized Vac-HSSE and regular HSSE procedures

The performances of Vac-HSSE and regular HSSE methods were evaluated after extracting samples for 30 and 60 min, respectively, at 55 °C. Selected quality analytical parameters are given in Table 1. The results show that the linearity ranges of Vac- and regular HSSE for Nap and Ace were between 100 and 5000 ng L⁻¹. For Flu, a wider linearity range was found with Vac-HSSE compared to regular HSSE, reflecting the improvement in extraction rates discussed above. The determination coefficients (r^2) ranged from 0.9995 to 0.9999 with Vac-HSSE and from 0.9992 to 0.9997 with regular HSSE. The limits of detection (LODs) were estimated as three times the signal-to-noise ratio, and were verified by performing extractions at those levels. Table 1 shows that LOD values with the proposed Vac-HSSE procedure were similar for Nap and Ace or lower for Flu to those obtained with regular HSSE in half of the sampling time needed for regular HSSE. Similar conclusions could be reached for the estimated limits of quantification (LOQ) defined as ten times the signal-to-noise ratio (estimated values not given in Table 1). Intra-day precision for both methods, estimated as the relative regular deviation (RSD) at 1000 ng L⁻¹ (Table 1) ranged from 1.2 % to 3.1 % with Vac-HSSE sampling, and between 1.2 % to 6.5 % for regular HSSE. Initial Vac-HSSE analyses of tap and wastewater effluent samples did not show detectable amounts of the target analytes and were therefore used for recovery studies. The relative recoveries, defined as the ratio of the concentrations found in real and deionized water samples spiked with the same amount of analytes, were then determined and the results are given in Table 1. For both types of real world water samples, matrix did not affect extraction, also reflecting the relatively simple nature of matrices examined here.

5. CONCLUSIONS

The effect of low pressure is a new experimental parameter to consider during method optimization in headspace (micro)extraction. For the first time, this work extended the applicability of the vacuum-based sampling approach to HSSE. The theoretical model describing the pressure dependence of Vac-HSSE was here discussed and the reduction in equilibration times was predicted. Inducing accelerations in HSSE is important, since the low surface area to volume ratio typically results in long equilibration times at 1 atm compared to other PDMS-based methods (*e.g.* SPME). The relatively high PDMS volume and layer thickness used for coating the stir bars, prevented water molecules interfering with analyte uptake during Vac-HSSE sampling, and allowed effective coupling of the effects of temperature and low pressure. Moreover, the extraction time profiles demonstrated the substantial improvement in kinetics when adopting the Vac-HSSE sampling approach. Target analytes could more rapidly reach equilibrium, and the optimum sampling time with Vac-HSSE was half of that found with regular HSSE. The analytical performance of Vac-HSSE was similar or better than that of regular HSSE in a shorter sampling time. The results on naphthalene revealed for the first time that gas-phase limitations can control extraction kinetics and that this type of constraints can effectively be reduced with sampling at a low pressure. For Ace and Flu, gas-sided limitations were assumed to control both the evaporation and analyte uptake processes and sampling under vacuum was found to accelerate the overall extraction kinetics.

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Table 1. Analytical performances of the optimized Vac-HSSE and regular HSSE procedures.

Analyte	Vac-HSSE						Regular HSSE					
	Linear range (ng L ⁻¹)	<i>r</i> ² (n=5) ^a	LOD (ng L ⁻¹)	Precision (%) ^b	Relative Recovery		Linear range (ng L ⁻¹)	<i>r</i> ² (n=5) ^a	LOD (ng L ⁻¹)	Precision (%)	Relative Recovery	
					(RSD, %) ^c						(RSD, %) ^c	
					Tap water	WWTP effluent					Tap water	WWTP effluent
Nap	100-5000	0.9999	43	1.2	102 (3)	99.9 (3)	100-5000	0.9997	44	1.2	102 (3)	98 (6)
Ace	100-5000	0.9998	6	2.5	101 (3)	101 (3)	100-5000	0.9997	5	2.4	102 (1)	105 (4)
Flu	100-5000	0.9995	35	3.1	99.8 (5)	99.4 (7)	250-5000	0.9992	101	6.5	95.8 (7)	97.0 (8)

^a Correlation coefficient, number of calibration points (n) in parenthesis.

^b Intra-day precision expressed as relative standard deviation (n=5).

^c Spiking level 1000 ng L⁻¹; % RSD values given in parentheses; n = 3.