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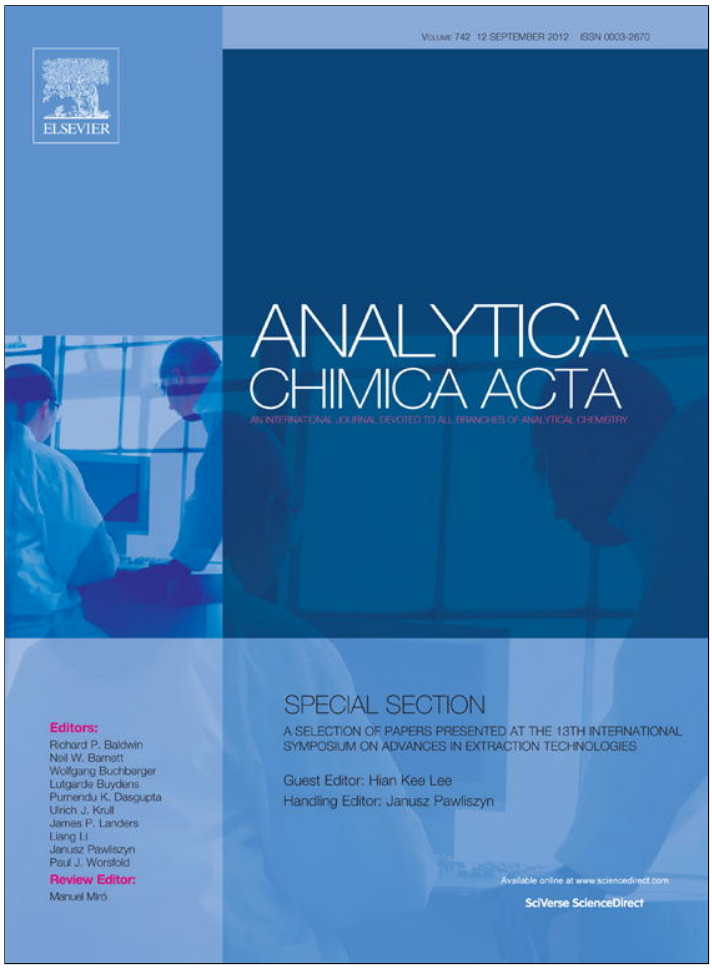

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Vacuum-assisted headspace solid phase microextraction: Improved extraction of semivolatiles by non-equilibrium headspace sampling under reduced pressure conditions

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ABSTRACT

A new headspace solid-phase microextraction (HSSPME) procedure carried out under vacuum conditions is proposed here where sample volumes commonly used in HSSPME (9 mL) were introduced into pre-evacuated commercially available large sampling chambers (1000 mL) prior to HSSPME sampling. The proposed procedure ensured reproducible conditions for HSSPME and excluded the possibility of analyte losses. A theoretical model was formulated demonstrating for the first time the pressure dependence of HSSPME sampling procedure under non equilibrium conditions. Although reduced pressure conditions during HSSPME sampling are not expected to increase the amount of analytes extracted at equilibrium, they greatly increase extraction rates compared to HSSPME under atmospheric pressure due to the enhancement of evaporation rates in the presence of an air-evacuated headspace. The effect is larger for semivolatiles whose evaporation rates are controlled by mass transfer resistance in the thin gas film adjacent to the sample/headspace interface. Parameters that affect HSSPME extraction were investigated under both vacuum and atmospheric conditions and the experimental data obtained were used to discuss and verify the theory. The use of an excessively large headspace volume was also considered. The applicability of Vac-HSSPME was assessed using chlorophenols as model compounds yielding linearities better than 0.9915 and detection limits in the low-ppt level. The repeatability was found to vary from 3.1 to 8.6%.

1. Introduction

The two most common solid-phase microextraction (SPME) sampling modes performed with the 'fiber-SPME' format are the direct and headspace approaches, depending on whether the SPME fiber is exposed directly to the sample matrix or to the headspace above it [1,2]. In particular, the headspace SPME (HSSPME) extraction mode protects the fiber coating from damage by hostile matrices or from excessive fouling, thus allowing for the analysis of complex matrices.

SPME sampling from the headspace above the sample in a closed three-phase system of a limited volume is a multi-stage process with analytes partitioning between the sample, headspace and fiber [3,4]. For most compounds, the rate limiting step is the transfer of analytes from the sample into its headspace, making extraction of volatile analytes faster than the one of semivolatiles [2,5]. Typically, equilibrium times for the less volatile compounds are shortened by applying agitation, but this approach is not always efficiently applied [6,7]. Increasing the sampling temperature was also found to have a significant effect on the extraction kinetics of the less volatile compounds. However, in some extreme cases raising the sample temperature resulted in decomposition of some compounds and/or creation of other components or artifacts [5]. More commonly though, elevated sampling temperatures decrease the analyte distribution constant between the sample matrix and the fiber coating and as a result the method sensitivity and analyte recovery at equilibrium were found to decrease [2].

The possibility of using reduced pressure conditions during HSSPME sampling had been considered but overlooked. Brunton et al. [8] were the first to present their results on the positive effect of reduced pressure conditions on the HSSPME sampling of headspace volatiles from raw turkey meat homogenates and aqueous standards. According to their method, air evacuation of the headspace occurred after introducing the 25 mL sample in the 100 mL sampling apparatus. Subsequent HSSPME sampling for 30 min resulted in enhanced chromatograms compared to those obtained with regular HSSPME under atmospheric pressure. In 2005, Darouzès et al. [9] confirmed the positive effect of reduced pressure on the HSSPME sampling of ethylated derivatives of butyl- and phenyltin compounds. The authors evacuated the air from the

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50 mL sampling container in the presence of the 25 mL underivatized sample and reported that initiating the derivatization reaction after air evacuation minimized analyte losses and ensured more reproducible conditions for HSSPME. In an attempt to discuss the theory behind the positive effect of reduced pressure on HSSPME, the equilibrium partitioning process of HSSPME was considered despite the fact that none of the target analytes reached equilibrium within the sampling times tested [9]. Nevertheless, such theoretical considerations were not directly applicable since partial pressures and equilibrium concentrations appear to be independent of the sampling chamber's pressure conditions as the partition coefficients/Henry's constants are affected only at very high operating pressures. Hence, at equilibrium the amount of analyte extracted by the fiber under reduced and regular pressure sampling conditions is expected to be the same.

A new HSSPME sampling procedure carried out under reduced pressure conditions, termed vacuum-assisted HSSPME (Vac-HSSPME) is proposed here. According to the method samples are introduced for the first time into pre-evacuated large sample containers. Evacuating most of the air molecules before rather than after sample introduction (as seen in both previous contributions) ensures reproducible conditions for HSSPME and more importantly excludes the possibility of losing more volatile analytes already present in the sample due to air-evacuation of the headspace in the presence of the sample. The proposed procedure thus allows for the first time the HSSPME sampling under reduced pressure conditions of all compounds amenable to HSSPME regardless of their volatility. For the first time, a theoretical model is presented, demonstrating the pressure dependence HSSPME under non equilibrium conditions. Parameters influencing the HSSPME extraction process were controlled under both vacuum and atmospheric conditions and the results were used to discuss and verify the theory. The applicability of Vac-HSSPME was assessed using chlorophenols as model compounds since they are environmentally significant and cover a range of physicochemical properties (Table 1).

2. Theory

The principle behind HSSPME is the equilibrium partition process of the analyte between the three phases (sample or condensed phase, its headspace and the extraction phase of the SPME fiber) [10]. Assuming that sufficient sampling time has been allowed to reach equilibrium, it is well established [1,3,10] that the amount of analyte extracted by a liquid fiber is given by

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

where C_s^o is the concentration in the condensed phase prior to SPME fiber exposure, V_s, V_g and V_f are the volumes of the sample, gas and fiber coating, respectively, K_g is the gas-sample partition coefficient of the analyte defined as $K_g = C_g^{\infty}/C_s^{\infty}$ and K_f is the fiber coating-headspace partition coefficient of the analyte defined as $K_f = C_f^{\infty}/C_g^{\infty}$ with C_s^{∞}, C_f^{∞} and C_g^{∞} denoting the equilibrium concentrations of the analyte concentrations in the sample, fiber and gas phase, respectively. Based on the thermodynamic theory, partial pressures and equilibrium concentrations are independent of the total pressure as the partition coefficients/Henry's constants are affected only at high operating pressures ($P > 500$ kPa). Hence, at equilibrium the amount of analyte extracted by the fiber under reduced or regular pressure sampling conditions is expected to be the same. However, depending on the target analyte, the sampling pressure may affect the rate of extraction and consequently the dynamic response of the HSSPME sampling process.

In a closed three-phase system of a limited volume, HSSPME is considered as a multi-stage process that involves mass transfer in the three phases involved and across two interfaces (sample/headspace and headspace/fiber) [10–12]. Prior to SPME fiber insertion, it is reasonable to assume that the analyte(s) partition between the sample and the headspace and equilibrium has been reached. Once the fiber is exposed to the headspace, it starts to absorb analyte molecules rapidly from the gas phase. As a result, the concentration of analytes in the headspace falls rapidly and it is replenished by the analyte transferred from the sample to the headspace [3]. Typically, mass transfer in the headspace is considered a very fast process [12]. For semivolatile compounds, evaporation of the analyte from the sample to the headspace is the rate-determining step for HSSPME whereas the mass transfer at the headspace/SPME polymer interface is considered a relatively fast process [11,12].

In general, the evaporation of organic solutes from water is regarded as a first-order reaction and the variation of the concentration in the liquid phase (C_s) with time (t) is given by

$$C_s = C_s^o e^{-kt} \quad (2)$$

where k is the evaporation rate constant. Taking the chemical mass balance around the water body yields the following equation [13]

$$V_s \frac{dC_s}{dt} = -K_L A (C_s - C_i) \quad (3)$$

where C_i 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 is the overall mass transfer coefficient at the gas phase–sample interface.

Integration of Eq. (3) yields Eq. (2) with evaporation rate constant (k) defined as [14]

$$k = \frac{K_L}{L} \quad (4)$$

where L is the solution depth in a container with uniform cross section.

Liss and Slater [15] and later Mackay and Leinonen [16] were the first to describe K_L in the form of the following equation by using the two-film theory, a flux-matching boundary condition, and the assumption that overall resistance to mass transfer results from resistances through the two thin films (gas and liquid) adjacent to the gas–liquid interface, namely

$$K_L = \left[\frac{1}{k_L} + \frac{RT}{K_H k_g} \right]^{-1} \quad (5)$$

where k_L and k_g are the liquid- and gas-film mass-transfer coefficients, K_H is the Henry's law constant defined as the ratio of partial pressure to aqueous concentration, T is the absolute temperature and R is the gas constant. This approach has been widely applied to the problem of volatilization of chemicals from natural water bodies [14–21] and the results have shown that evaporation rates of chemicals can be controlled by mass transfer resistance in the liquid phase, gas phase, or a combination of both, depending on the value of K_H. The tendency for an organic solute to partition into the atmosphere is determined largely by its vapor pressure, yet it should always be recognized that high molecular weight hydrophobic substances, which have very low vapor pressures and hence low atmospheric concentrations, may still partition appreciably into the atmosphere as they also have low aqueous solubilities. The ratio of the concentration in the atmosphere to that in the water (*i.e.*, the air–water partition coefficient) may thus be large despite the low vapor pressure [22]. This partition coefficient can be expressed as the dimensionless Henry's law constant (K_H/RT) and used to predict the phase location of the resistance on mass transfer [16]. Hence, for a high K_H organic solute (K_H values greater than $\sim 5 \times 10^{-3}$ atm m^3 mol^{-1} [17]), the major resistance to the mass transfer lies in the liquid phase (*i.e.* $K_L \approx k_L$), whereas for a low K_H

Table 1
Main physicochemical properties of the chlorophenols studied (2,4-dichlorophenol (DCP); 2,4,6-trichlorophenol (TrCP); 2,3,4,6-tetrachlorophenol (TeCP); and pentachlorophenol (PCP)).

Compound	Molecular weight	Vapor pressure 25 °C (mm Hg)	K_H (atm m^3 mol^{-1})	pK_a	Log K_{ow}	Water solubility 25 °C (mg L^{-1})
DCP	163.00	0.09	4.29×10^{-6}	7.89	3.06	4500
TrCP	197.45	0.008	2.6×10^{-6}	6.23	3.69	800
TeCP	231.89	0.000666	8.84×10^{-6}	5.22	4.45	23
PCP	266.34	0.00011	2.45×10^{-8}	4.74	5.12	14

organic solute (typical threshold K_H values reported in the literature are 1.2×10^{-5} [17] or 1.6×10^{-4} atm m^3 mol^{-1} [16]), 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 resistance are important.

The mass transfer coefficient k_g is proportionally related to the compound's molecular diffusion coefficient (D_g) raised to some power m

$$k_g \propto D_g^m \tag{6}$$

The most likely values for m are 0.5, 2/3 and 1 [23,24]. In the past, gas phase controlled mass transfer experiments in stirred cells yielded m values of the order of 0.684 (which was subsequently corrected to 0.632) and 0.5 for low [24,25] and high [26] agitation speeds, respectively.

Furthermore, diffusivity correlations for binary mixtures of gases at low pressures can be estimated by a number of methods and regardless of the polarity of the analyte, they all show that D_g is inversely proportional to the total pressure (P) [27]. For example the method by Fuller–Schettler–Giddings [28], which was reported to be the most accurate correlation to use for nonpolar organic gases is given by

$$D_g = \frac{0.001 \times T^{1.75} \sqrt{(1/M_{air}) + (1/M_C)}}{P\left[\left(\sum V_{air}\right)^{1/3} + \left(\sum V_C\right)^{1/3}\right]^2} \tag{7}$$

where T is the absolute temperature, M_{air} and M_c are molecular weights for air and organic compound of interest, and V_{air} and V_c are the molar volumes of air and the compound.

Evacuating most of the air from the sampling chamber prior to liquid sample introduction will significantly reduce the total pressure of the system. For a given temperature and assuming a small effect of the air-related terms present in the diffusivity correlations (Eq. (7)) it is safe to conclude that reducing the total pressure of the system will increase D_g. Based on Eq. (6) this will increase k_g and for low K_H compounds this will result in a higher overall mass transfer coefficient values, K_L, compared to atmospheric pressure given that for these compounds $K_L \approx K_H k_g/RT$. It is thus suggested that for semi-volatile compounds where evaporation from the condensed phase to its headspace is controlled by the gas phase mass transfer coefficient, reducing the headspace pressure should enhance evaporation rates. This in turn implies that during the multi-stage process of non-equilibrium HSSPME sampling, reduced pressure conditions should result in a faster response of the sample to the concentration drop of analyte(s) in the headspace when compared to atmospheric pressure as analyte(s) will evaporate from the sample to the headspace faster and replenish their headspace concentration(s). Thus, for low K_H analytes where mass transfer from the sample to the headspace is the rate-determining step, HSSPME equilibrium is established faster when sampling under reduced pressure conditions.

3. Experimental

3.1. Chemicals

DCP was purchased from Fluka (Steinheim, Germany), TrCP and TeCP were obtained from Supelco (Bellefonte, PA) and PCP from Chem Service (West Chester, PA). All solvents were pesticide-grade (Merck, Darmstadt, Germany). Hydrochloric acid was used for pH adjustment and sodium chloride for increasing the ionic strength of the aqueous solutions. Deionized water was prepared on an EASYpure RF water purification system (Barnstead/Thermolyne, IA, USA). Individual stock standard solutions of each chlorophenol were prepared by weight in acetone and were used to prepare a standard stock solution (100 mg L^{-1}) in acetone containing all four chlorophenols. All stock solutions were stored in the dark at 4 °C. Working solutions were freshly prepared by dilution of the standard stock solution with deionized water.

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. Before extraction, the pH and ionic strength of the samples were adjusted to 2 and 30% NaCl (w:v), respectively. HSSPME sampling of the unspiked environmental samples under both reduced and atmospheric conditions ensured that the samples were free of the target analytes.

3.2. Vac-HSSPME procedure

Fig. 1 shows the experimental setup for Vac-HSSPME. The 1000 mL glass sample container (Supelco, Bellefonte, PA) used here was equipped with two high vacuum glass stopcocks and a half-hole cylindrical Thermogreen septum (Supelco, Bellefonte, PA), which is compatible with the needle of the SPME device. For Vac-HSSPME, the sample container was initially air-evacuated after connecting one of the two glass stopcocks with a vacuum pump (7 mbar ultimate vacuum without gas ballast; Vacuubrand GmbH & Co. KG, Model MZ 2C NT, Wertheim, Germany) whilst keeping the other in the "off" position. Upon air evacuation, the glass stopcock was closed and the vacuum pump was disconnected. A 9 mL spiked aqueous solution with a pH = 2 and a 30% (w:v) NaCl content was then introduced into the sampling chamber through the

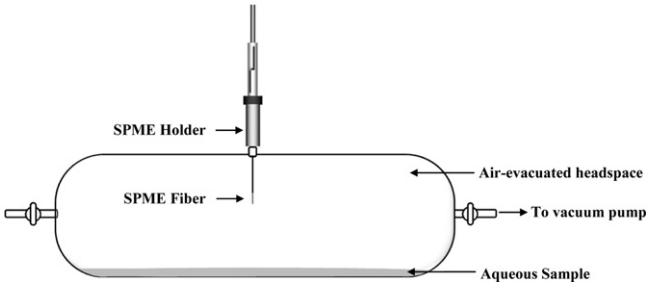

Fig. 1. Schematic representation of the experimental setup used for Vac-HSSPME.

Thermogreen septum with the help of a 10 mL gastight syringe (SGE, Australia). The sampling apparatus containing the sample was then secured on top of an orbital platform shaker (Heidolph, Model Unimax 1010 DT, Germany) placed inside a thermo-stated chamber/incubator (Elvem, Athens, Greece) maintained at a constant pre-set temperature value during the equilibration and sampling processes. Sampling temperatures could not exceed 45 °C due to limitations set by the incubator. Analytes in the aqueous solution were then left to equilibrate with the headspace for 10 min and orbital shaking (150 rpm; 30% of the maximum speed) was applied to accelerate mass transfer and facilitate equilibrium between the two phases. The time needed for this step was set by running duplicates for equilibration times ranging between 5 and 60 min under reduced and atmospheric pressure conditions. Upon sample equilibration, shaking was interrupted and the needle of the SPME fiber/holder assembly (Supelco, Bellefonte, PA) was introduced into the sampling chamber by piercing the Thermogreen septum of the sampling chamber. The SPME fiber was then exposed to the headspace above the sample for a preset period of time and HSSPME under reduced pressure conditions and at a constant temperature (35 °C unless otherwise stated in the text) was performed. Based on previous reports the 85 μm polyacrylate (PA) SPME fiber (Supelco, Bellefonte, PA) was chosen for extraction [29–33]. When microextraction sampling was completed, the PA fiber was retracted and the SPME device was transferred to a gas chromatographer–mass spectrometer (GC–MS) for analysis. The pressure inside the sampling chamber was then equilibrated with the atmospheric, and the sample container was emptied, washed and used for the next extraction. To avoid pressure losses due to septum damage, the Thermogreen septum of the sampling chamber was replaced daily. All analyses were run at least in duplicates.

For regular HSSPME, the same spiked aqueous sample was placed in the 1000 mL sampling chamber, the 22 or the 40 mL headspace glass vials (both vials were equipped with hollow caps and septum) and static HSSPME under atmospheric pressure was then performed with the rest of the experimental parameters set at the same values as those used for Vac-HSSPME.

3.3. GC–MS analysis

All analyses were carried-out on a Shimadzu GC-17A (Version 3) QP-5050A GC–MS system. The split/splitless injector operated at 280 °C, with the purge flow closed for 5 min. Helium (>99.999% pure) was used as a carrier gas at 1.0 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: 70 °C for 2 min, programmed to 190 °C at a rate of 8 °C min^{-1}, increased to 220 °C at a rate of 5 °C min^{-1} and then held for 6 min. A 6 min delay time was set for the detector. The ionization mode was electron impact (70 eV) and the interface temperature was set at 300 °C. The full scan mode (m/z 50–400) was used for all optimization experiments. The mass spectra obtained for each target analyte were used to determine their characteristic ions. The selected ion monitoring (SIM) mode was used as a sensitive tool for evaluating the analytical performance of the optimized Vac-HSSPME method.

4. Results and discussion

4.1. Preliminary investigations

For Vac-HSSPME, aqueous samples are introduced into sample containers that were previously air evacuated with the help of a vacuum pump. Evacuating the air from the sampling apparatus before rather than after sample introduction ensures repeatability of the process and eliminates the possibility of analyte losses due to air-evacuation of the headspace in the presence of the sample. Although, sample introduction in a pre-evacuated sample container generally results in pressure increments, changes in pressure were expected not to be significant as long as the sample to headspace volume ratio was kept low. The commercially available large volume (1000 mL) sample container used here could meet this criterion and by scaling-up the dimensions of the sampling chamber, sample loading could be increased to volumes commonly used in HSSPME (9 mL) whilst maintaining the vacuum conditions inside the vessel upon liquid sample introduction.

In general, stirring of the solution is expected to increase evaporation rates and consequently enhance the amount of analyte extracted by the fiber during HSSPME regardless of the pressure conditions inside the sampling vessel. The strong mixing of the water body produces turbulence, which results in frequent exchanges between the surface layer and the bulk aqueous phase enabling compounds to reach the interface faster [14]. Nonetheless, acceleration effects on the 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 [34]. Initial investigations showed that it was difficult to apply sample agitation during Vac-HSSPME sampling. The small openings of the commercially available glass sample container allowed only the use of very small magnetic stir bars that did not lead to efficient sample agitation. The possibility of applying orbital shaking during Vac-HSSPME was also investigated and shaking speeds up to 120 rpm were found to enhance extraction. However, the rotating tray agitation mechanism could lead to SPME needle damage [35] or vacuum loss due to the mechanical stress applied on the SPME needle. It was therefore decided to use the simple case of static HSSPME sampling mode for all experiments, which may be more instructive when considering the effect of different parameters on extraction kinetics.

4.2. Comparison of Vac-HSSPME with regular HSSPME

Based on the K_H values of the model compounds used here (Table 1) evaporation rates were expected to be controlled by gas phase mass transfer resistance (*i.e.* $K_L \approx K_H k_g/RT$) [16,17]. As discussed in the theory, for such compounds reducing the pressure in the headspace will increase D_g and consequently k_g and result in a higher overall mass transfer coefficient at the gas phase–sample interface (K_L) for each target analyte. Non-equilibrium HSSPME sampling of chlorophenols under vacuum conditions is thus expected to enhance the amount of analyte extracted by the fiber when compared to regular HSSPME, since the aqueous sample will respond faster to the temporary concentration drops in the gas phase during the multi-stage process of HSSPME sampling. To provide experimental evidence on the theoretically predicted enhancement on evaporation rates and consequently improvement of the non-equilibrium HSSPME sampling under vacuum conditions, 9 mL spiked aqueous samples were placed in the 1000 mL container and static HSSPME under both vacuum and atmospheric pressure conditions was performed. It was assumed that changes in K_H values due to the low pH and high ionic strength conditions of the spiked aqueous solution were relatively not important [36]. As seen (Fig. 2), for a short 10 min sampling at 25 °C, the amount of analyte extracted by the fiber when using Vac-HSSPME was 3.0–8.3 times larger than that obtained with HSSPME under atmospheric pressure with the lowest relative enhancement value recorded for the least volatile and more hydrophobic analyte examined here, PCP, which is generally considered to be "trapped" in the hyperhydrophobic water/air interface [37].

The general suggestion for HSSPME is that the size of the headspace volume should not be very large because extraction

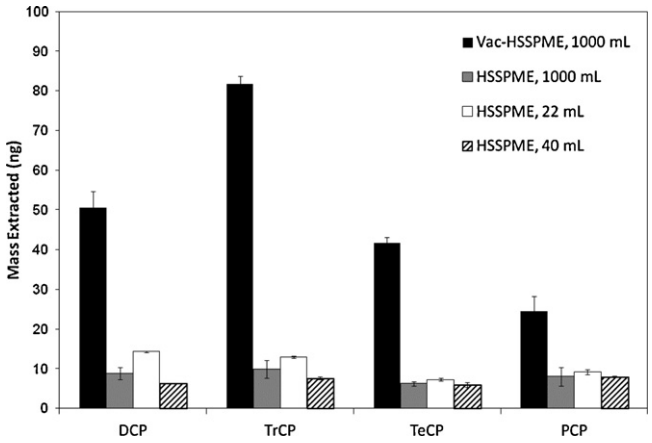

Fig. 2. Comparison of extraction efficiencies obtained for the same sample with Vac-HSSPME using the 1000 mL sample container (Vac-HSSPME, 1000 mL) and HSSPME under atmospheric pressure using the 22 mL (HSSPME, 22 mL) and 40 mL (HSSPME, 40 mL) headspace vials and the 1000 mL sample container (HSSPME, 1000 mL). Other experimental parameters: 9 mL aqueous sample spiked at 100 $\mu g\,L^{-1}$ with each chlorophenol; pH = 2; 30% NaCl (w:v); 25 °C sampling temperature; static HSSPME sampling for 10 min.

efficiency increases with decreasing headspace volume [1] given that equilibrium is established more quickly with the coating when the headspace volume is smaller [3]. Based on this, typical HSSPME applications under atmospheric pressure commonly make use of 22 mL or 40 mL headspace vials. During the present investigations, the 1000 mL sample container used for extracting the 9 mL spiked aqueous samples resulted in an exceptionally large headspace volume. To exclude the possibility that the relative enhancements of Vac- over regular HSSPME found earlier were the outcome of a sensitivity loss due to the presence of a large headspace volume during regular HSSPME, 9 mL samples were then placed in 22 and 40 mL headspace vials and each time a 10 min static HSSPME sampling at 25 °C and under atmospheric pressure was performed. The results (Fig. 2) showed once again that non-equilibrium HSSPME sampling was significantly improved under vacuum conditions compared to atmospheric pressure regardless of the sampling vessel volume used for regular HSSPME.

Regarding the results obtained with the two headspace vials, the expected decrease on the amount of analyte extracted by the fiber with increasing the headspace volume was recorded (Fig. 2). However, despite the substantial change in headspace volume, a significant loss in sensitivity was not recorded for the HSSPME experiment performed in the 1000 mL sample container under atmospheric pressure. Placing the same sample size, in a 1000 mL horizontal cylindrical sampling chamber rather than a vertical cylindrical vial of a much smaller volume (such as the 22 and 40 mL headspace vials) greatly increased the sample/headspace interfacial area (A) and reduced the depth of the sample solution (L) at the same time. Based on the theory the latter resulted in increased evaporation rates ultimately enhancing the amount of analyte extracted by the SPME fiber under non-equilibrium HSSPME conditions.

4.3. Investigations on the effect of temperature on Vac-HSSPME

Increasing the sample temperature can increase the headspace capacity and/or analyte diffusion coefficients, which leads to an increase in the rate of extraction or the rate of mass transfer onto the fiber coating [2,5]. Hence, for HSSPME (under both reduced and atmospheric conditions), heating the sample is expected to enhance even further extraction up to the point where elevated sample temperatures will result in significantly decreased headspace/fiber distribution constants. Fig. 3 shows the extraction curves obtained for all tested compounds after Vac-HSSPME sampling at temperatures ranging from 25 to 45 °C and for sampling times varying between 10 and 40 min. As can be seen, for the more volatile DCP and TrCP the effect of temperature on Vac-HSSPME is marginal. For these compounds, the overall high evaporation rates were not significantly affected within the relatively small temperature and sampling time ranges tested here. A positive effect of temperature on Vac-HSSPME was gradually recorded for TeCP, which became more pronounced for the least volatile compound examined here (PCP) and for increased sampling times. The effect of temperature on Vac-HSSPME for these two low vapor pressure compounds became clear as evaporation rates were notably affected even at early sampling times and for the small temperature range tested here. It appears therefore that during Vac-HSSPME, the effect of temperature is more pronounced for the less volatile compounds whereas for organic solutes with overall high evaporation rates the effect is diminished. A 35 °C sampling temperature was used for all subsequent studies as it provided enhanced sensitivity for the less volatile compounds without working close to the maximum limits set by the incubator.

4.4. Investigations on the enhancement of extraction rates

Based on theory, for the same sample size and headspace volume, the amount of analyte extracted at equilibrium will be the same regardless of the pressure inside the container. The difference when sampling under reduced pressures, lies in the speed of extraction, *i.e.* the time needed to attain partition equilibrium. In general, equilibration times are controlled by octanol/water partition coefficients and Henry's constants [10]. For compounds with small K_H, a reasonable equilibration time can still be reached if their K_{ow} values are small. However, most semivolatile compounds, like the studied chlorophenols, have small K_H values and large K_{ow} values, which lead to long equilibration times during HSSPME sampling [10].

The extraction time profiles for each chlorophenol were then determined at 35 °C under both vacuum and atmospheric pressure conditions and the results are given in Fig. 4. As seen, even after sampling the headspace for 150 min none of the target analytes reached equilibrium under both pressure conditions. The results however, clearly showed that Vac-HSSPME greatly improved extraction rates compared to regular HSSPME. Moreover, the magnitude of the positive effect on extraction rates varied between target analytes and was once again related to their ability to partition into the headspace. In particular, the amount of DCP extracted after a 10 min sampling was 2.0 times larger when using Vac-HSSPME compared to regular HSSPME (Fig. 4). This relative enhancement decreased with increased sampling times, reaching a value of 1.3 times after 150 min of sampling, *i.e.* as DCP approached equilibrium where according to the theory the amount of analyte extracted by the fiber is the same regardless of the pressure conditions inside the sample container. Based on the K_{ow} values of chlorophenols (Table 1), it is reasonable to assume that DCP will reach equilibrium faster than the rest of the analytes tested here. It can be thus concluded that for DCP reduced pressure conditions may improve HSSPME only at early sampling times far ahead of equilibrium. Analogous decreases in relative enhancement over time were also recorded for TrCP and TeCP (Fig. 4). However, the relative enhancement of Vac-HSSPME over regular HSSPME was 2.2 and 2.9 for TrCP and TeCP, respectively, for a 150 min headspace sampling, implying that they were more distant from equilibrium conditions than DCP as evidenced by their K_{ow} values. On the other hand, the amount of PCP extracted by the fiber under vacuum was constantly larger than that extracted under atmospheric pressure throughout the sampling times tested (Fig. 4) and the Vac-HSSPME/HSSPME ratio was 4.0 and 3.8 after 10 and

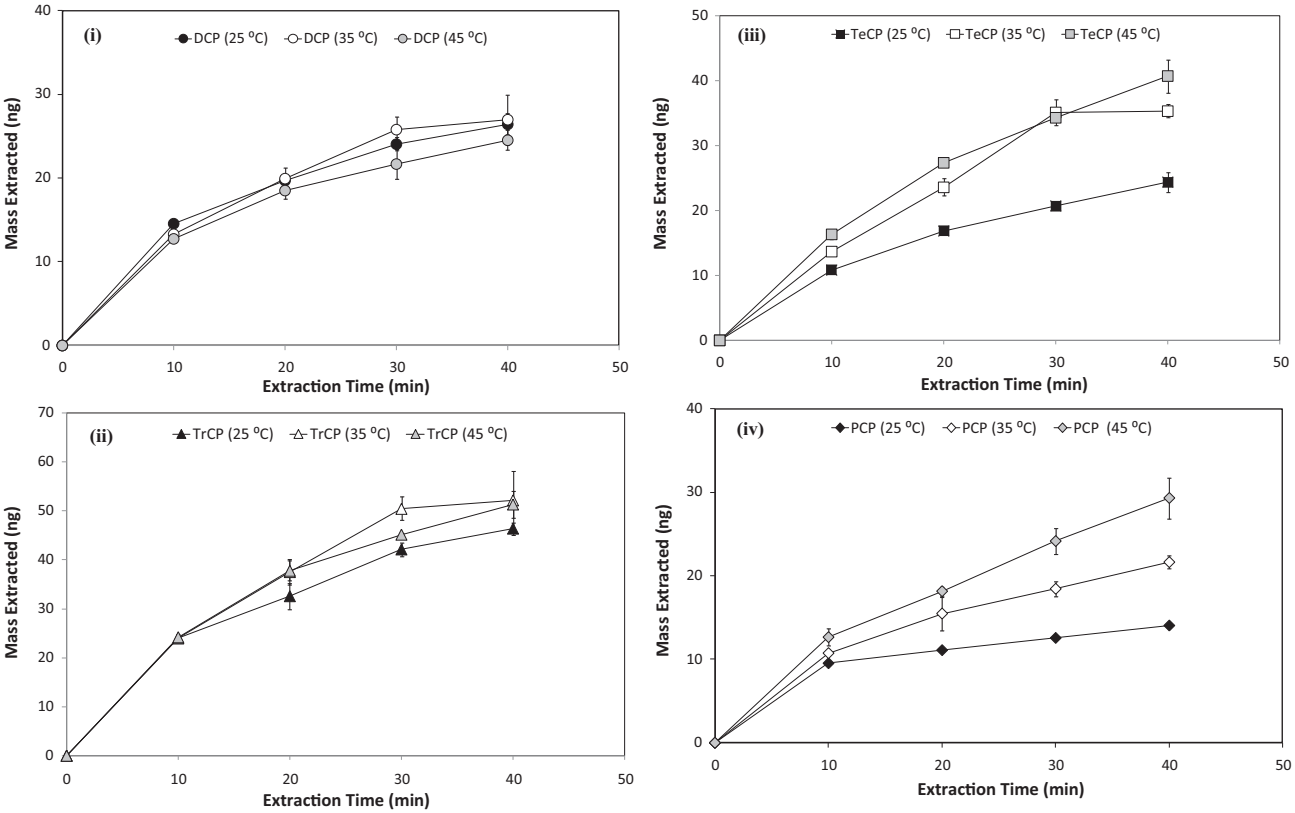

Fig. 3. Extraction time profiles obtained with Vac-HSSPME at 25, 35 and 45 °C for (i) DCP, (ii) TrCP, (iii) TeCP and (iv) PCP. Other experimental parameters: 9 mL aqueous sample spiked at 20 μg L^{-1} with each chlorophenol; 1000 mL sample container; pH = 2; 30% NaCl (w:v); static HSSPME.

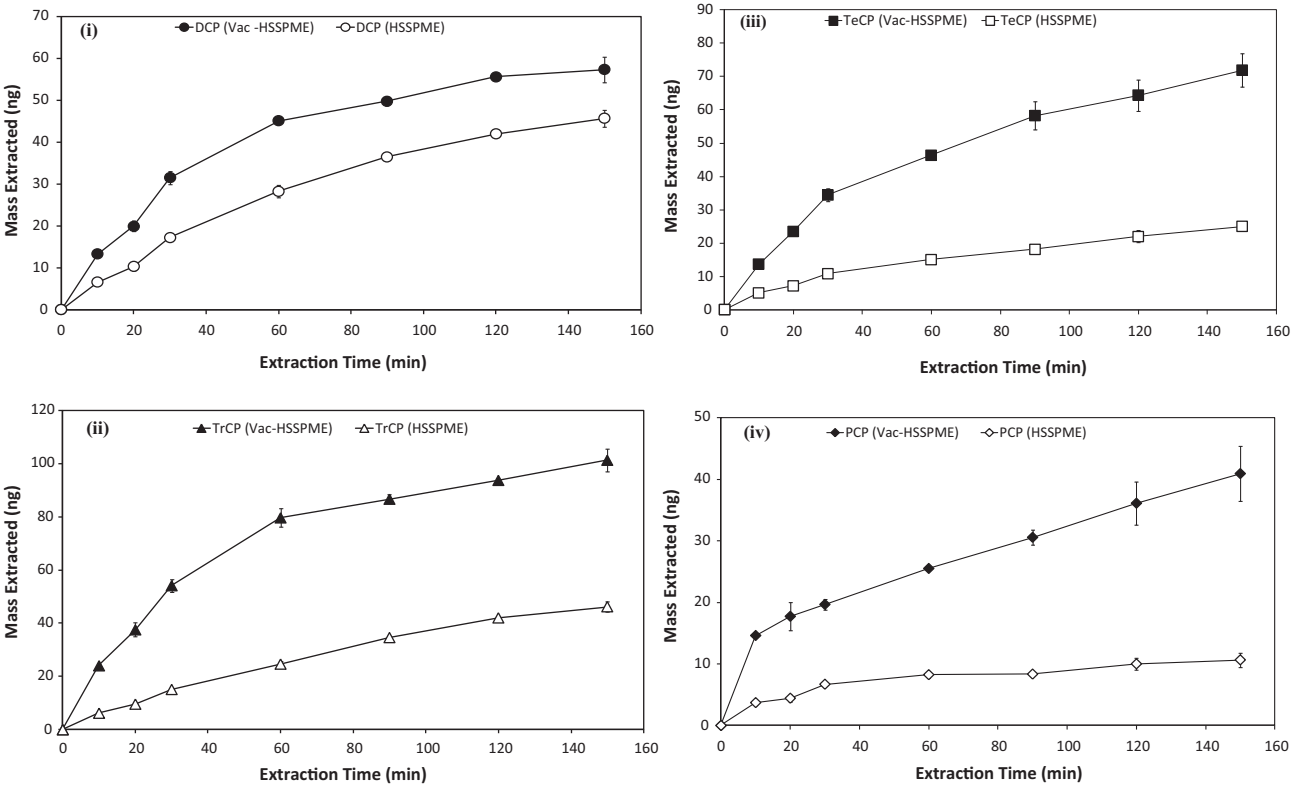

Fig. 4. Extraction time profiles obtained with the 1000 mL sample container under reduced (Vac-HSSPME) and atmospheric (HSSPME) pressure conditions for (i) DCP, (ii) TrCP, (iii) TeCP and (iv) PCP. Other experimental parameters: 9 mL aqueous sample spiked at 20 μg L^{-1} with each chlorophenol; pH = 2; 30% NaCl (w:v); static HSSPME at 35 °C.

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)	Relative recoveries[a]	
					Tap	WW effluent
DCP	0.050–10	0.9981	0.019	5.0	92 (5.1)	92 (6.0)
TrCP	0.050–10	0.9999	0.019	3.1	94 (8.1)	91 (9.1)
TeCP	0.050–10	0.9988	0.018	5.7	91 (9.6)	91 (4.2)
PCP	0.250–10	0.9915	0.111	8.6	104 (9.5)	89 (4.4)

[a] Spiking level 0.250 $\mu g\,L^{-1}$; % RSD values given in parentheses; $n = 5$.

150 min sampling, respectively. For compounds such as PCP, long equilibration times are expected and the positive effect of reduced pressure remained important even after prolonged sampling times distant however from equilibrium. On the whole, Vac-HSSPME greatly improved HSSPME sampling under non-equilibrium conditions. Short sampling times are sufficient for Vac-HSSPME given that the reduced pressure conditions inside the sampling vessel enhance evaporation rates and consequently increases the amount of analyte adsorbed per unit time, resulting in faster extraction kinetics and enhanced sensitivity without sacrificing analysis time.

4.5. Application of Vac-HSSPME

The purpose of this final section was to evaluate for the first time the analytical performance of HSSPME under reduced pressure conditions. Taking into consideration the 10 min incubation time, extraction time was set at 30 min as a compromise between high-throughput analysis and good sensitivity. The linearity of Vac-HSSPME was then determined by extracting under set experimental conditions (1000 mL sample container; pH = 2; 30% NaCl (w:v); static HSSPME for 30 min at 35 °C) 9 mL aqueous solutions spiked at concentrations ranging from 0.050 to 10 $\mu g\,L^{-1}$ for DCP, TrCP and TeCP and 0.250 to 10 $\mu g\,L^{-1}$ for PCP. The SIM mode was used as a sensitive tool for these measurements. All compounds showed good correlation with coefficients of determination (r^2) higher than 0.9915 (Table 2). The repeatability of Vac-HSSPME method, expressed as relative standard deviation (RSD), was evaluated after extracting five consecutive aqueous samples spiked at 0.250 $\mu g\,L^{-1}$ and the RSD values found ranged between 3.1 and 8.6% (Table 2). The limits of detection (LODs) defined for a signal-to-noise of three (S/N = 3) ranged between 0.018 and 0.111 $\mu g\,L^{-1}$ for the static HSSPME approach used here (Table 2).

The effect of matrix on Vac-HSSPME was evaluated in tap and secondary treated wastewater effluent samples. The relative recoveries found (defined as the ratio of the concentrations found in real and deionized water samples all spiked with the same amount of analytes) for a spiking level of 0.250 $\mu g\,L^{-1}$ are given in Table 2 and the results showed that the matrix did not affect extraction.

5. Conclusions

The pressure dependence of the HSSPME approach under non equilibrium conditions has been formulated. It was demonstrated that for low K_H analytes, where mass transfer from the sample to the headspace is the rate-determining step, HSSPME extraction rates increase when sampling under vacuum conditions due to the enhancement of evaporation rates. Therefore, higher extraction efficiency and sensitivity can be achieved with Vac-HSSPME within short sampling times and under mild conditions (*e.g.* lower temperatures). Introducing aqueous samples into air-evacuated sample containers ensured reproducible conditions for HSSPME sampling and excluded the possibility of analyte losses. The proposed procedure enables for the first time sampling under reduced pressure conditions of all compounds amenable to HSSPME, regardless of their volatility. The capabilities and potential applications of this simple and easy-to-use HSSPME approach need to be further explored.

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