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Ph.D. Thesis
Microextraction Under Vacuum Conditions

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Στην αγαπημένη μου μητέρα

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Περίληψη

Στην παρούσα διατριβή ερευνάται η δυνατότητα δειγματοληψίας ημι – πτητικών ενώσεων από τον υπερκείμενο χώρο σε υδατικά και στερεά δείγματα με χρήση της τεχνικής μικροεκχύλισης στερεής φάσης υπερκείμενου χώρου (HSSPME) κάτω από συνθήκες χαμηλής πίεσης. Αυτή η νέα μέθοδος ονομάστηκε μικροεκχύλιση στερεής φάσης υπερκείμενου χώρου υποβοηθούμενη από κενό (Vac – HSSPME).

Στο Κεφάλαιο 1 παρουσιάζονται οι τεχνικές προετοιμασίας δείγματος. Γίνεται αναλυτική παρουσίαση των τεχνικών στις οποίες η φάση δέκτης χρησιμοποιεί ελάχιστο ή καθόλου οργανικό διαλύτη. Ιδιαίτερη έμφαση δίνεται στις αρχές και τις παραμέτρους που επηρεάζουν τις δύο μεθόδους εφαρμογής της SPME (απευθείας και υπερκείμενου χώρου)

Στο Κεφάλαιο 2 παρουσιάζεται η δημοσιευμένη έρευνα με τίτλο: ***Μικροεκχύλιση στερεής φάσης υπερκείμενου χώρου υποβοηθούμενη από κενό: Βελτιωμένη εκχύλιση ημι – πτητικών ενώσεων με δειγματοληψία του υπερκείμενου χώρου σε συνθήκες μη ισορροπίας και χαμηλής πίεσης***. Σε αυτή τη δημοσίευση προτείνεται η εφαρμογή μιας νέας τεχνικής μικροεκχύλισης στερεάς φάσης υπερκείμενου χώρου (HSSPME) η οποία πραγματοποιήθηκε κάτω από συνθήκες ελαττωμένης πίεσης, σύμφωνα με την οποία όγκοι δείγματος που χρησιμοποιούνται στην κλασσική HSSPME (9 mL) εισάγονται για πρώτη φορά σε αεροστεγή και εμπορικά διαθέσιμη φιάλη μεγάλου όγκου (1000 mL) η οποία είχε εκκενωθεί από την παρουσία αέρα πριν την εφαρμογή της HSSPME. Η προτεινόμενη μέθοδος εξασφαλίζει επαναλήψιμες συνθήκες για την HSSPME και αποκλείει την πιθανότητα απώλειας των υπο – μελέτη ενώσεων. Παρουσιάζεται για πρώτη φορά ένα θεωρητικό μοντέλο της εξάρτησης της HSSPME από την πίεση σε συνθήκες μη – ισορροπίας. Αν και κατά τη διάρκεια της HSSPME η χαμηλή πίεση δεν αναμένεται να αυξήσει την ποσότητα της ουσίας που εκχυλίζεται σε κατάσταση ισορροπίας, αυξάνει όμως τους ρυθμούς εκχύλισης σε σύγκριση με την HSSPME υπό ατμοσφαιρική πίεση εξαιτίας της αύξησης των ρυθμών εξάτμισης υπό την παρουσία εκκενωμένου υπερκείμενου χώρου. Η επίδραση αυτή είναι πιο έντονη για τις ημι –

πτητικές ουσίες των οποίων οι ρυθμοί εξάτμισης ελέγχονται από την αντίσταση στη μεταφορά μάζας στο στενό αέριο φιλμ της υγρής/αέριας διεπιφάνειας. Εξετάζονται οι παράμετροι που επηρεάζουν την HSSPME κάτω από συνθήκες χαμηλής και ατμοσφαιρικής πίεσης και τα πειραματικά δεδομένα που συλλέγονται χρησιμοποιούνται για την επιβεβαίωση της θεωρίας/μοντέλου. Εξετάζεται επίσης και η χρήση του υπερβολικά μεγάλου υπερκείμενου όγκου. Η προτεινόμενη μέθοδος εφαρμόστηκε για την ανίχνευση χλωροφαινολών σε υδατικά δείγματα με γραμμικότητες καλύτερες από 0,9915 και όρια ανίχνευσης σε επίπεδα των ppt. Η επαναληψιμότητα κυμάνθηκε μεταξύ 3,1% και 8,6%.

Στο Κεφάλαιο 3 παρουσιάζεται η δημοσιευμένη έρευνα με τίτλο: ***Επίδραση της σταθεράς του νόμου Henry και των λειτουργικών παραμέτρων στην μικροεκχύλιση στερεής φάσης υπερκείμενου χώρου υποβοηθούμενης από κενό***. Σε αυτή τη δημοσίευση διερευνήθηκαν η επίδραση των ιδιοτήτων των οργανικών ενώσεων στόχων κ των παραμέτρων δειγματοληψίας (όγκος υπερκείμενης φάσης και ανάδευση δείγματος) στην αποτελεσματικότητα της προτεινόμενης μεθόδου Vac – HSSPME. Τα αποτελέσματα έδειξαν ότι σε θερμοκρασία δωματίου η HSSPME σε συνθήκες μη ισορροπίας βελτιώνεται δραματικά με την εφαρμογή κενού μέσα στην δειγματοληπτική φιάλη σε σύγκριση με συνθήκες κανονικής πίεσης. Προέκυψε ότι σε θερμοκρασία δωματίου η αύξηση των ρυθμών εκχύλισης που επάγεται από τη μείωση της ολικής πίεσης μέσα στη δειγματοληπτική φιάλη είναι σημαντική για τις ουσίες των οποίων η σταθερά του νόμου του Henry, K_H, είναι κοντά ή κάτω από το κατώφλι των τιμών για ουσίες χαμηλού K_H. Για αυτές τις ουσίες ο ρυθμός εξάτμισης εξαρτάται από την αντίσταση στη μεταφορά μάζας στο λεπτό αέριο στρώμα της διεπιφάνειας δείγματος/υπερκείμενου χώρου και μειώνοντας την ολική πίεση αυξάνουν οι ρυθμοί εξάτμισης και σαν αποτέλεσμα ταχύτερη συνολική διαδικασία εκχύλισης. Αντιστρόφως, για ουσίες με ενδιάμεση τιμή K_H, η Vac – HSSPME δεν αναμένεται να βελτιώσει τους ρυθμούς εξάτμισης σε σχέση με την κλασσική HSSPME δεδομένου ότι η αντίσταση στη μεταφορά μάζας στο λεπτό υγρό στρώμα παραμένει σημαντική. Σε συμφωνία με τη

θερμοδυναμική θεωρία, στην ισορροπία, η εκχυλιζόμενη ποσότητα της ουσίας από την SPME ίνα δεν επηρεάζεται από τις συνθήκες πίεσης μέσα στην δειγματοληπτική φιάλη. Επιπλέον, οι κινητικές εκχύλισης στην Vac – HSSPME για τις χαμηλού K_H ουσίες επηρεάστηκαν οριακά από την εφαρμοζόμενη αλλαγή του όγκου της υπερκείμενης φάσης καθώς οι ρυθμοί εξάτμισης αυξάνουν δραματικά κάτω από συνθήκες ελαττωμένης πίεσης και το δείγμα ανταποκρίνεται ταχύτερα στην πτώση της συγκέντρωσης στον υπερκείμενο χώρο σε σύγκριση με την κλασσική HSSPME. Στην ισορροπία όμως, αύξηση στον όγκο του υπερκείμενου χώρου μπορεί να οδηγήσει σε μείωση της ευαισθησίας για την Vac – HSSPME παρόμοια με την παρατηρούμενη κατά την κλασσική HSSPME. Όπως ήταν αναμενόμενο, η ανάδευση του υγρού δείγματος βελτίωσε την αποτελεσματικότητα της Vac – HSSPME. Η γραμμικότητα της μεθόδου ήταν καλύτερη από 0,998 και τα όρια ανίχνευσης σε επίπεδα των ppt. Η ακρίβεια της μεθόδου κυμάνθηκε μεταξύ 1,8% και 8,4%.

Στο Κεφάλαιο 4 παρουσιάζεται η δημοσιευμένη έρευνα με τίτλο: ***Σμίκρυνση της μικροεκχύλισης στερεής φάσης υπερκείμενου χώρου υποβοηθούμενης από κενό***. Σε αυτή την έρευνα, έγινε δυνατή η σμίκρυνση της δειγματοληπτικής φιάλης σε ειδικά διαμορφωμένο φιαλίδιο των 22 mL και παρατηρήθηκε ότι οι αλλαγές στην τελική πίεση της εκκενωμένης από αέρα φιάλης πριν την εισαγωγή του δείγματος ήταν αρκετά χαμηλές και επέτρεψαν την ικανοποιητική απόδοση της Vac-HSSPME. Η διαμορφωμένη φιάλη των 22 mL χρησιμοποιήθηκε για την εκχύλιση πέντε αρωματικών υδρογονανθράκων. Μελετήθηκαν και βελτιστοποιήθηκαν μερικές πειραματικές παράμετροι. Για τις ουσίες των οποίων η αντίσταση στη μεταφορά στη μάζα στο λεπτό αέριο φιλμ της διεπιφάνειας αερίου/δείγματος ελέγχει τους ρυθμούς εξάτμισης, η μείωση της συνολικής πίεσης κατά τη διάρκεια της HSSPME μπορεί να βελτιώσει δραματικά τις κινητικές εκχύλισης μέσα στη διαμορφωμένη φιάλη των 22 mL. Η υγρασία αποδείχτηκε ότι επηρέασε την ποσότητα του ναφθαλενίου (ουσία ενδιάμεσης τιμής K_H) που εκχυλίστηκε από την πολυμερή ίνα στην θερμοδυναμική ισορροπία καθώς επηρέασε αρνητικά την εκχύλιση όλων των αναλυόμενων ουσιών σε υψηλές

εφαρμοζόμενες θερμοκρασίες δειγματοληψίας. Τα σημαντικά πλεονεκτήματα της διαμορφωμένης φιάλης είναι η αποτελεσματική ικανότητα εκχύλισης και καλή ευαισθησία που επιτεύχθηκαν σε συνθήκες θερμοκρασίας δωματίου και σε σύντομους χρόνους δειγματοληψίας. Για την φιάλη των 22 mL, η προτεινόμενη μέθοδος ήταν γραμμική, τα όρια ανίχνευσης σε επίπεδα των ng L^{-1} και σχετικές τυπικές αποκλίσεις που κυμάνθηκαν μεταξύ 1,3% και 5,8%. Οι υδατικές μήτρες δεν επηρέασαν την εκχύλιση με την Vac – HSSPME.

Το Κεφάλαιο 5 διερευνά την δυνατότητα χρήσης της Vac-HSSPME για την εκχύλιση πολυκυκλικών αρωματικών υδρογονανράκων από δείγματα χώματος. Διάφορες παράμετροι ελέγχθηκαν και βελτιστοποιήθηκαν. Οι βέλτιστες συνθήκες ήταν: δειγματοληψία του υπερκείμενου χώρου 2 g επιμολυσμένου χώματος και 2 mL απιονισμένου ύδατος για 30 min ενώ το μίγμα αναδευόταν στις 1400 rpm. Η Vac – HSSPME ήταν γραμμική σε εύρος συγκεντρώσεων 1 έως 400 ng g^{-1} (r^2>0,9478) και επαναλήψιμη (4.3 έως 10%, εκφρασμένη σε τιμές σχετικής τυπικής απόκλισης – RSD). Τα όρια ανίχνευσης κυμάνθηκαν σε επίπεδα των ng g^{-1} (0,003 – 0,233 ng g^{-1}). Για μία ακόμη φορά, η μέθοδος Vac-HSSPME αποδείχτηκε ιδιαίτερα ευαίσθητη και μεγάλης ακρίβειας κάτω από σύντομους χρόνους και υπό ήπιες θερμοκρασίες κατά τη διάρκεια της δειγματοληψίας.

Στο Κεφάλαιο 6 ανακεφαλαιώνονται τα αποτελέσματα της παρούσας έρευνας και παρουσιάζονται τα συμπεράσματα. Αξιολογούνται οι παράμετροι οι οποίες επηρεάζουν τη διαδικασία καθώς και η συνολική απόδοση της Vac-HSSPME. Στη συνέχεια, προτείνονται μελλοντικές κατευθύνσεις για την απλούστευση της προτεινόμενης μεθοδολογίας και την εφαρμογή της σε ευρύτερο πεδίο εφαρμογών.

Abstract

The present thesis investigates the possibility of sampling semi – volatile analytes from the headspace of aqueous or solid samples using headspace solid – phase microextraction (HSSPME) under reduced pressure conditions. The new procedure was termed vacuum assisted headspace solid phase microextraction (Vac – HSSPME).

In Chapter 1 sample preparation techniques are presented. A comprehensive review on solvent – free sample preparation techniques is given with emphasis to SPME principles and the parameters affecting the two sampling modes (direct and headspace).

Chapter 2 presents the published report entitled: **Vacuum-assisted headspace solid phase microextraction: Improved extraction of semivolatiles by non-equilibrium headspace sampling under reduced pressure conditions**. In this report, a new headspace solid-phase microextraction (HSSPME) procedure carried out under vacuum conditions was proposed 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%.

Chapter 3 presents the published report entitled: **Effect of Henry's law constant and operating parameters on vacuum-assisted headspace solid phase microextraction**. This paper investigated the effects of organic analyte properties and sampling parameters (headspace volume and sample agitation) on vacuum-assisted HSSPME (Vac-HSSPME). The results revealed 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, detection limits in the ppt level and precision varying between 1.8% and 8.4 %.

Chapter 4 presents the published report entitled: **Downsizing vacuum-assisted headspace solid phase microextraction**. In this study, we downsized the extraction

device to a 22 mL modified sample vial and concluded that changes in the final total pressure of the pre-evacuated vial following sample introduction were sufficiently low to allow efficient Vac-HSSPME sampling. The downsized extraction device was used to extract five low molecular weight polycyclic aromatic hydrocarbons and several experimental parameters were controlled and optimized. For those compounds whose mass transfer resistance in the thin gas-film adjacent to the gas/sample interface controls evaporation rates, reducing the total pressure during HSSPME sampling dramatically enhanced extraction kinetics in the 22 mL modified vial. Humidity was found to affect the amount of naphthalene (intermediate K_H compound) extracted by the fiber at equilibrium as well as impair extraction of all analytes at elevated sampling temperatures. All the same, the high extraction efficiency and very good sensitivity achieved at room temperature and within short sampling times comprised the most important features of Vac-HSSPME in this downsized extraction device. Analytically, the developed method was found to yield linear calibration curves with limits of detection in the low ng L^{-1} level and relative standard deviations ranging between 3.1 and 6.4 %. Matrix was found not to affect extraction.

Chapter 5 investigates the possibility of using Vac-HSSPME for extracting polycyclic aromatic hydrocarbons from soil samples. Several parameters were controlled and optimized. The optimum conditions found were: sampling the headspace of a 2 g spiked sandy sample and 2 mL of water for 30 min while stirring the sample at a 1400 rpm agitation rate. The application of Vac – HSSPME yielded good linearity in the range 1 to 400 ng g^{-1} (r^2>0.9478) and precision ranging between 4.3 to 10 % (expressed as RSD). The detection limits were in the low ng g^{-1} levels (0.003 – 0.233 ng g^{-1}). Overall, Vac-HSSPME method confirmed that very good sensitivity and precision could be attained within short sampling times and under mild sampling temperatures.

In Chapter 6 the results of the present study are summarized and conclusions are drawn. The parameters affecting Vac-HSSPME procedure as well as the overall performance of the method are evaluated. Future directions in the field are also given.

CHAPTER 1.

Introduction

1.1 Sample preparation

The analytical procedure consists of many steps, all contributing in the overall performance of the process. The major stages of an analytical process are depicted in Figure 1.1 and these are sampling, sample preparation, separation and quantitation, statistical evaluation of the results and decision making upon these results **[1]**. The sample preparation step is an extraction procedure that ensures the determination of very small amounts (very low concentrations) of chemicals in the environment. In sample preparation step, target analytes are isolated (extracted and separated) from the sample matrix, purified of co – extracted, non – target analytes (sample clean-up) and concentrated to finally be measured by highly selective and sensitive analytical equipment (such as gas chromatography/mass spectrometry, GC/MS). Traditional extraction techniques (such as liquid-liquid extraction and Soxhlet extraction), consume large amounts of toxic organic solvents, thus creating environmental hazards, which increases the risk of cancer and contributes to the depletion of the ozone layer. Some less – solvent – consuming procedures, such as solid phase extraction (SPE), new pressurized fluid extraction (PFE), hot – water extraction, microwave – assisted extraction and solid phase microextraction (SPME) are alternative methods **[1,2]**.

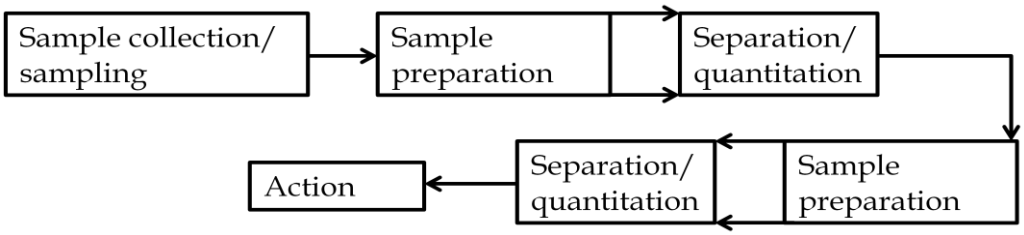

Figure 1.1. Major steps of an analytical process.

Extraction techniques can be classified according to their fundamental processes (Figure 1.2) **[3]**. Exhaustive extraction does not require calibration since most of the analytes are transferred to the sampler with the use of large volumes of the extraction phase. Reduction in time and solvent volumes in exhaustive techniques is accomplished by replacing batch equilibrium techniques with flow – through tecbniques **[3]**. In sorbent

trap and solid phase extraction (SPE), large volumes of sample are passed through a small cartridge, mass transfer is facilitated with the flow through the sorbent bed and finally analytes are desorbed into a small volume of solvent **[4,5]**. The drawbacks of sorbent in SPE are overloading or high carryover and batch – to – batch variation of the sorbents, resulting in poor reproducibility.

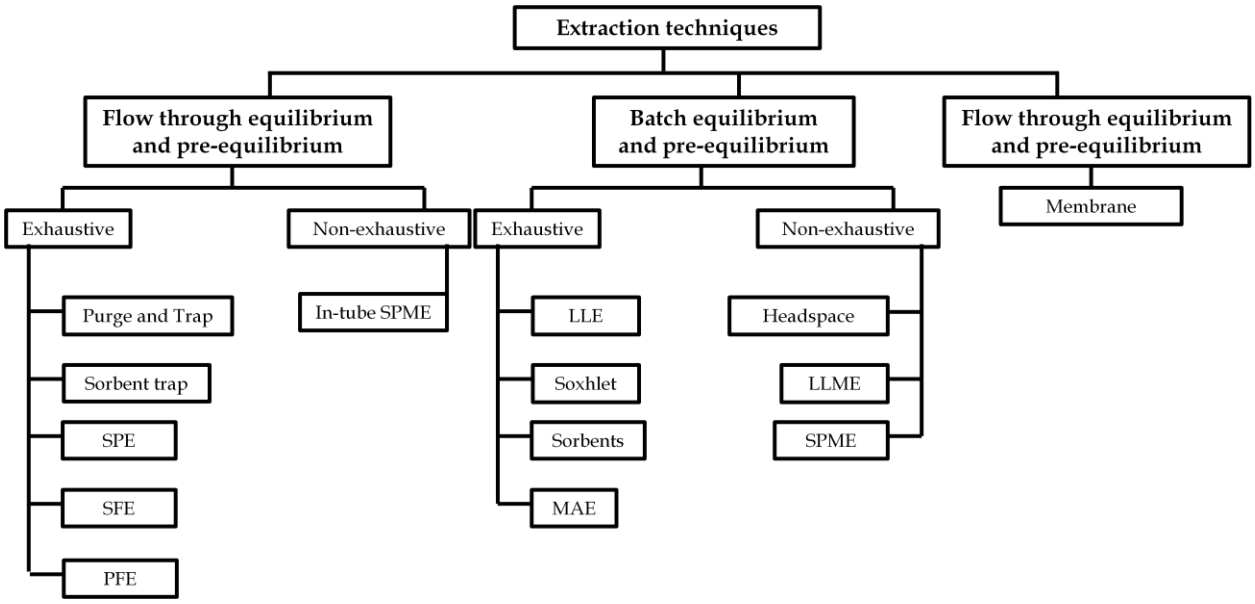

Figure 1.2. Classification of extraction techniques **[6]**.

In non – exhaustive techniques, the extraction phase has small capacity and is inadequate to remove most of the analytes from the sample matrix. Non – exhaustive techniques are classified into equilibrium, pre – equilibrium and permeation approaches **[7]**. Equilibrium techniques employ a small volume of the extraction phase relative to the large sample volume or a low partition coefficient between the extraction phase and the sample matrix. Pre – equilibrium approaches are performed when the extraction is terminated before the equilibrium between two phases. In membrane extraction sorption into and desorption out of the extraction phase occur simultaneously during the continuous transport of analytes through the membrane **[8]**.

1.2. Liquid based extractions

Extraction is a separation process based on the chemical differences in a mixture's components rather than in the physical. Liquid liquid extraction (LLE) involves the mixing of the solution with another solvent that is immiscible with the original. Yet, the solute that needs to be isolated is soluble in the solvent. After the mixing, two phases are formed because of the differences in the densities. Caution should be taken in the selection of the appropriate solvent to ensure better affinity of the solute toward the added solvent. The extraction process needs to be repeated two or three times and/or with the use of more than one solvent to achieve satisfactory mass transfer of the solute from the solution **[9]**.

LLE is usually performed using a separatory funnel where the compound will distribute between the two solvents. The success of this method depends upon the difference in solubility of a compound in various solvents. Most organic compounds are more soluble in organic solvents, while some organic compounds are more soluble in water (Figure 1.3).

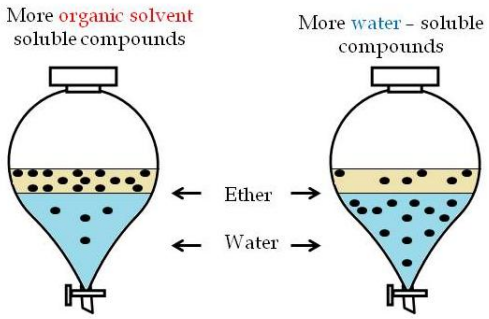

Figure 1.3. Schematic representation of the solubility of solutes between two immiscible solvents.

LLE has some drawbacks making it unprofitable to use. The successive extraction with expensive and toxic large volumes of organic solvents of high purity makes it tedious and time consuming while the formation of emulsion doesn't permit the automation of the procedure **[2]**.

Liquid based microextraction techniques

The need to overcome the drawbacks of the traditional LLE, lead to its miniaturization and the development of faster, simpler and inexpensive sample preparation techniques. These techniques employ smaller intitial sample sizes and offer the ability of the detection of very low analyte concentrations. In these non – exhaustive techniques, the analyte is extracted by a small volume of a liquid. The miniatuatized techniques of LLE are termed liquid – phase microextraction (LPME) techniques **[10,11-13]**.

The development of LPME techniques made it possible the great reduction in the volumetric ratio of the acceptor-to-donor phase. This can be achieved by using either immiscible liquid phases (solvent microextraction) or a membrane to separate the acceptor-donor phases (membrane extraction) **[10,14].**

Different ways of miniaturization of LLE causes a variety of modes of LPME. Some of these modes are SDME (single-drop microextraction) **[13,15-17]**, DLLME (dispersive liquid – liquid microextraction) **[18]**, hollow fiber based supported supported liquid membrane microextraction (liquid−liquid−liquid microextraction, LLLME) **[19]**, LPME-SFO (liquid phase microextraction based on solidification of floating organic drop) **[20]**, microextraction using immiscible liquid films including liquid – liquid microextraction (two-phase system) **[17]** and liquid – liquid – liquid microextraction using back-extraction (three-phase system) **[21,22]**, SLM (supported liquid membrane) **[23,24]**, MMLLE (microporous membrane liquid-liquid extraction) **[25,26]** and VALLME (vortex – assisted liquid – liquid microextraction) **[27]**. The term 'liquid-phase microextraction' was first introduced to describe two-phase systems in solvent microextraction **[17]**.

SDME, as a miniauturized version of the LLE, is a method in which the extraction solvent is a single drop. Jeannot and cantwell reported the SDME for first time by suspending an 8 µL organic solvent drop at the end of teflon rod immersed in the stirring sample. After extraction, solvent drop was removed from the end of the Teflon rod using a micro syringe and injected to analytical instrument. Figure 1.4 shows the

schematic SDME system. Some modifications were made by He and Lee **[17]** on primary reported method. In this version of SDME, teflon rod was replaced by a micro syringe (Figure 1.5). A 1 µL immiscible extracting solvent drop is exposed into the sample (liquid or gaseous) from a micro syringe. After establishment of distribution equilibrium the organic drop is retracted back into the micro syringe and is injected to the analytical instrument for determination of the analytes.

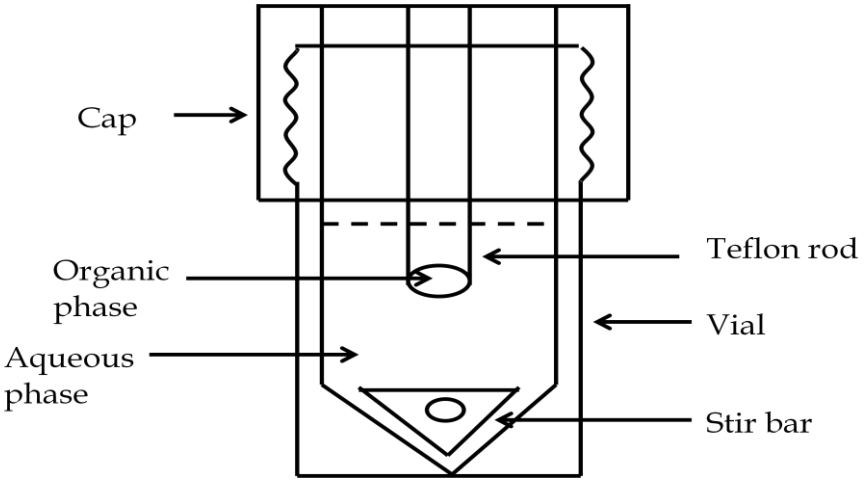

Figure 1.4. Illustration of SDME method reported by Jeannot and Cantwell **[15]**.

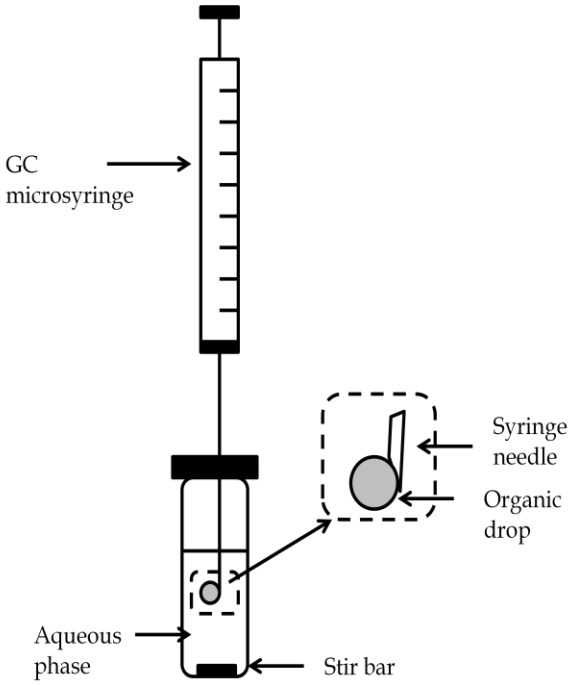

Figure 1.5. Schematic diagram of SDME using microsyringe **[17]**.

1.3. Solvent – free sample preparation techniques

The general principle of all sample preparation methods is the partitioning of analytes between the sample matrix and an extracting phase. Figure 1.6 classifies sample preparation techniques, that use little or no organic solvent, according to the extracting phases of gas, membrane and sorbent **[28]**.

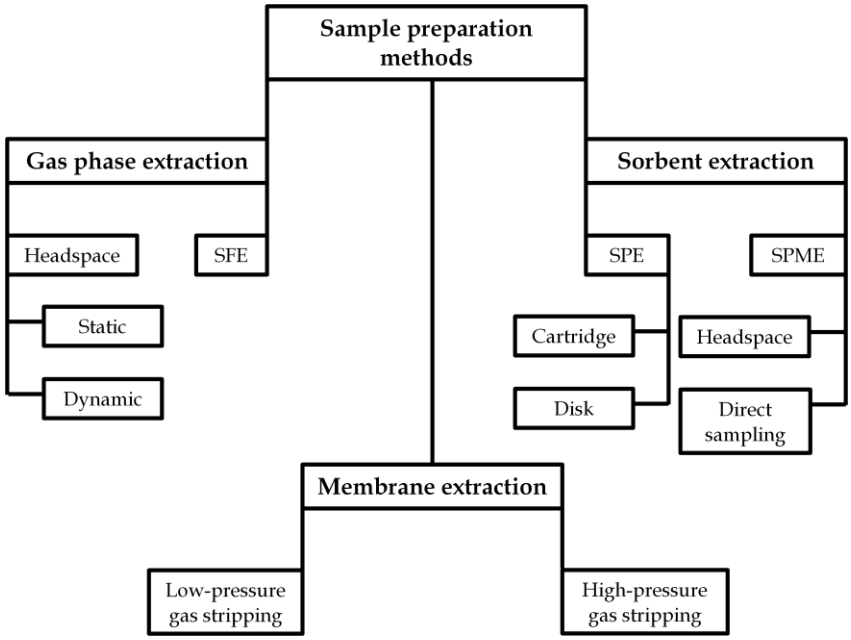

Figure 1.6. Classification of solvent – free sample preparation methods **[28]**.

Gas phase sample preparation methods. Gas phase sample preparation methods are described by the partitioning of analytes into a gas phase. In this partitioning, nonvolatile high molecular weight compounds are eliminated, preventing contamination of the separation column. Headspace mode has been widely used to analyze volatile compounds because the extracting phase (air, helium or nitrogen) is compatible with most instruments, such as gas chromatographs (GC). In the static headspace procedure, a sample is simply allowed to equilibrate with its headspace and then a small, well defined volume of the headspace is directly injected into a GC for analysis. This mode has been used for the analysis of volatile organic compounds (VOCs) in food, beverage, clinical and other samples **[29,30]**. The technique is low in sensitivity because it lacks a concentrating effect. Cannot achieve exhaustive extraction,

except in the case of very volatile gases, and therefore requires very careful calibration. In dynamic headspace mode take place multiple processes and allows quantitative removal of VOCs. The purge-and-trap approach of dynamic headspace mode has two steps **[31,32]**. The first step is to let a carrier gas purge through an aqueous sample to remove VOCs from the matrix. The second step is to quantitatively collect these compounds by using a cold or a sorbent trap. The drawbacks of this technique are tha formation of foam, carryover through analyses and the fact that the stripping flow rate is incompatible with the separation instrument.

The headspace mode can be extended to less volatile compounds with the combination of thermal desorption. By heating the sample to elevated temperatures, analytes are thermally desorbed from the matrix and partition efficiently into the gas phase. Heat can be applied in the analyses of samples containing solids, such as clay soil, however, thermally unstable substances and a high moisture content in the desorbed gas mixture frequently prevent the use of the thermal desorption approach. Supercritical fluid extraction (SFE) is a gas – based sample preparation method that uses liquid such as compressed carbon dioxide as an extracting phase and is capable of removing less volatile compounds at ambient temperatures. Supercritical fluids possess both gas like mass transfer and liquid like solvating characteristics **[33-35]**. The drawback of SFE is the use of heavy equipment, such as an expensive high – pressure fluid delivery system and a high purity gas source, both making the field analysis difficult. Since this technique can extract nonvolatile compounds at ambient temperatures, it is useful for the analysis of thermally unstable analytes and matrices.

Membrane extractions. Membrane extraction consists of two simultaneous processes: extraction of analytes from the sample matrix by the membrane material and extraction of analytes from the membrane by a stripping phase. There is little knowledge concerning this method on its use for sample separation for chromatography, while it has been developed for mass spectrometry (MS) over the last three decades. The permeated analytes are transferred with nitrogen stripping gas from the surface of a flat

polymeric membrane to a bed of activated charcoal **[36]**. The compounds are desorbed into a GC for analysis after switching of a valve. Although, many early methods used supported membrane sheets, most recent developments of membrane extraction techniques have focused on the use of hollow fibers **[37-39]**. Hollow fiber membrane modules are simpler to make because a hollow fiber is self – supporting. Compared to membrane sheets and headspace methods, hollow fibers provide a higher ratio of surface area to volume for the stipping gas, which allows a more sufficient extraction. Membrane extraction can be directly combined with MS or GC to perform continuous monitoring **[38,40]**.

The membrane can be fitted conveniently into a flowing stream. The transport of analytes through the membrane adds selectivity to the sample preparation process and the membrane protects the separation column from high molecular weight compounds, with an additional sorbent concentration advantage. Membrane extraction can be applied to volatile compound analysis, as well as to higher molecular weight compounds by using higher temperatures or microporous membranes with various pore diameters **[1]**.

Sorbent extractions. The concept of using an adsorbent material to extract trace organic compounds from an aqueous sample was developed in the 1980s, and its applications have been extensively reviewed **[41,42]**. Sorbents can be used to extract organic compounds from various matrices including water, air and soil. A sorbent that has high affinity towards organic compounds will retain and concentrate those compounds from a much diluted aqueous or gaseous sample. Many sorbents are specifically suited for the extraction of different groups of organic compounds with various degrees of selectivity. One widely used sorbent technique is SPE and its miniaturization lead to SPME.

Solid phase extraction. SPE was introduced in early 1970 and developed during 1980-90 **[43]**. Analytical laboratories use SPE to concentrate and purify samples for analysis.

Solid phase extraction can be used to isolate analytes of interest from a wide variety of matrices, including urine, blood, water, beverages, soil, and animal tissue **[44-46]**. Due to the complexity of the various matrices and the demands of the chromatographic analysis extraction facilitates the dissolution of the analytes in a suitable solvent while removing from the solution as many interfering compounds as possible **[2,47]**.

SPE uses the affinity of solutes dissolved or suspended in a liquid (known as the mobile phase) for a solid through which the sample is passed (known as the stationary phase) to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase. The portion that passes through the stationary phase is collected or discarded, depending on whether it contains the desired analytes or undesired impurities. If the portion retained on the stationary phase includes the desired analytes, they can then be removed from the stationary phase for collection in an additional step, in which the stationary phase is rinsed with an appropriate eluent **[43]**.

The stationary phase comes in the form of a packed syringe – shaped cartridge, mounted on top of the SPE apparatus (**Figure 1.7**). The manifold allows multiple samples to be processed by holding several SPE media in place and allowing for an equal number of samples to pass through them simultaneously. A typical cartridge SPE manifold can accommodate up to 24 cartridges, while a typical disk SPE manifold can accommodate 6 disks, thus making SPE a faster extraction method than LLE. Most SPE manifolds are equipped with a vacuum port. Application of vacuum speeds up the extraction process by pulling the liquid sample through the stationary phase. The analytes are collected in sample tubes inside or below the manifold after they pass through the stationary phase **[10]**.

SPE cartridges and disks are available with a variety of stationary phases, each of which can separate analytes according to different chemical properties. Most stationary phases are based on silica that has been bonded to a specific functional group. Some of these

functional groups include hydrocarbon chains of variable length (for reversed phase SPE), quaternary ammonium or amino groups (for anion exchange), and sulfonic acid or carboxyl groups (for cation exchange) **[43]**.

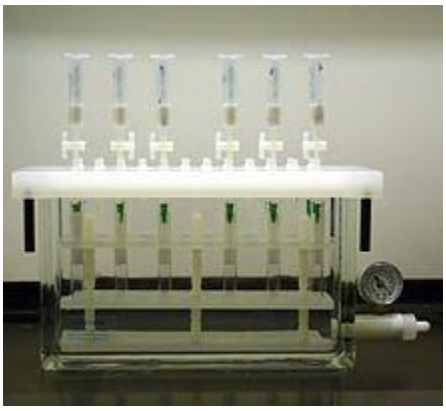

Figure 1.7. A typical solid phase extraction manifold. The cartridges drip into the chamber below, where tubes collect the effluent. A vacuum port with gauge is used to control the vacuum applied to the chamber.

SPE employs many stages to accomplish the desired purification of the extract thus adding complexity and difficulty in mastering the method. It requires lengthy efforts to result in method development and is a quite costly method since it involves considerable consumption of organic solvents that have to be further discarded.

1.4. Solid phase microextraction

SPME was developed by Pawliszyn et al. **[1,28]** in an attempt to address the need to facilitate rapid sample preparation both in the laboratory and on-site where the investigated system is located. SPME was originally named after the first experiment that used an SPME device, which involved extraction on solid fused – silica fibers. Then, it was later renamed to be a reference to the appearance of the extracting phase in relation to a liquid or gaseous donor phase, even though it is recognized that the extraction phase is not always technically a solid. SPME combines sampling, isolation, concentration and sample introduction into one step **[47,48]**. In the technique, a small amount of extracting phase that is dispersed on a solid support is exposed to the sample for a well defined period of time. In one approach, a partitioning equilibrium between

the sample matrix and the extraction phase is reached. In this case, convection conditions do not affect the amount extracted. In a second approach that uses short – time pre – equilibrium extraction, if convection or agitation or both are constant, then the amount of analyte extracted is related to time. Quantitation can then be performed based on timed accumulation of analytes in the coating. Figure 1.8 illustrates several implementations of SPME that have been considered. These mainly include open – bed extraction concepts such as coated fibers, vessels, and agitation mechanism disks, but in – tube approaches are also considered. Some implementations better address issues associated with agitation, and others better address the ease of implementing sample introduction to the analytical instrument **[46,49]**.

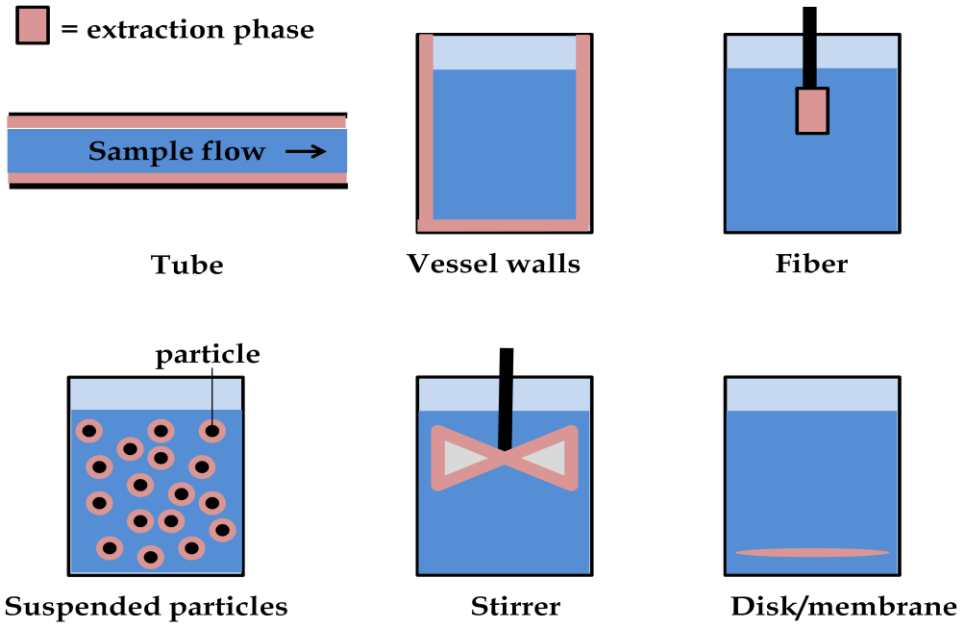

Figure 1.8. SPME configurations.

The "fiber-SPME" format is the most common form of the technique for sampling directly the sample matrix or the headspace above it **[50,51]**. The extraction procedure implemented in SPME starts with the exposure of the fiber coating to a liquid or a gaseous sample long enough for the amalytes to partition between the sample and the extraction phase. Then, the fiber is withdrawn into the needle of the SPME device (Figure 1.9) and introduced into an injector of a gas chromatographer (GC). The

analytes are thermally desorbed and analyzed. The simplicity of this procedure minimizes the loss of analyte due to multi – step process. After the desorption of the analytes into the injector and the protection of the fiber into the needle it can be reused for subsequent extractions. The ease of the SPME apparatus makes the technique amenable for field extraction.

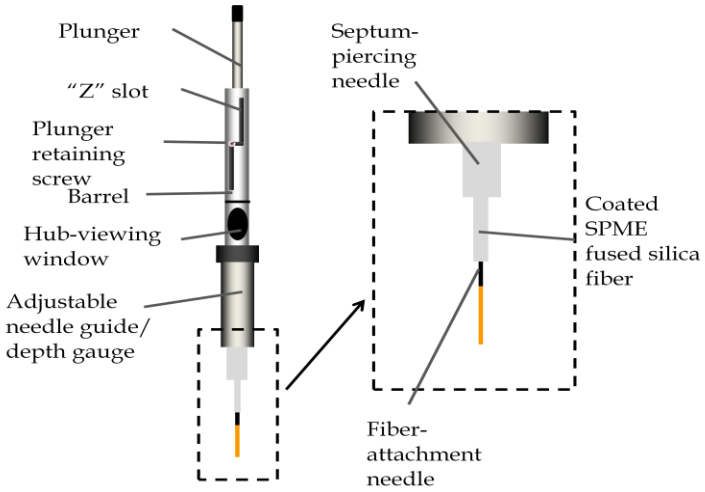

Figure 1.9. Design and enlarged view of the commercial SPME device.

1.4.1. Principles of SPME

In SPME, a small amount of the extracting phase associated with a solid support is placed in contact with the sample matrix for a predetermined amount of time. If the time is long enough, concentration equilibrium is established between the sample matrix and the extraction phase. When equilibrium conditions are reached, exposing the fiber for a longer amount of time does not accumulate more analytes. SPME is not an exhaustive extraction. In fact, the objective of the experiment is to produce full breakthrough as soon as possible, because this indicates that equilibrium extraction has been reached.

SPME is considered a multiphase equilibration process. There can be implemented either two phases consisting of the sample, which is usually an aqueous phase, and the extraction phase of the SPME fiber coating, or three phases consisiting of the sample, the fiber and gaseous headspace of the sample. The sample is considered as a

homogenous matrix. SPME is completed when the analyte reaches distribution equilibrium between the sample matrix and the fiber coating and extended extraction time will not result in further increase in the amount extracted **[49]**.

In the case of the **two phase system** equilibrium conditions can be described as:

$$n = \frac{C_0 V_f V_s K_{fs}}{V_f K_{fs} + V_s} \tag{1.1}$$

where n is the mass of the analyte extracted by the coating, C_0 is the initial concentration of the analyte in the aqueous solution; V_f and V_s are the volumes of the fiber coating and the aqueous solution, respectively; K_{fs} is the coating/sample matrix partition coefficient. This equation describes the partitioning equilibrium when liquid polymeric phases are involved, such as PDMS **[52]**.

The extraction can be interrupted before the equilibrium is reached but in order to acquire reproducible data, constant convection conditions and extraction time are necessary. According to Equation 1.1, after the equilibrium is reached, the amount of the analyte extracted is directly proportional to the sample concentration

When the sample volume is very large $V_f K_f K_g \langle\langle V_s$, Equation 1.1 can be simplified to:

$$n = C_0 V_f K_{fs} \tag{1.2}$$

According to this equation, the amount of the analyte extracted is independent of the volume of the sample, which makes it the advantage of the SPME technique for field applications. The fiber is exposed directly to ambient air, water, or the production stream and the amount of the extracted analyte corresponds directly to its concentration in the matrix **[49]**.

1.4.2. Extraction modes with coated fiber SPME

SPME sampling can be performed in three basic modes: (a) direct extraction, (b) headspace extraction, and (c) extraction with membrane protection. **Figure 1.10** illustrates the differences between these modes **[49]**.

In **direct extraction mode** (**Figure 1.10**a), the coated fiber is inserted into the sample and the analytes are transported directly from the sample matrix to the extracting phase. To facilitate rapid extraction, some level of agitation is required to transport the analytes from the bulk of the sample to the vicinity of the fiber. For gaseous samples, the natural flow of air (e.g., convection) is frequently sufficient to facilitate rapid equilibration for volatile analytes, but for aqueous matrices, more efficient agitation techniques such as fast sample flow, rapid fiber or vial movement, stirring, or sonication are required to reduce the effect of the depletion zone produced close to the fiber as a result of slow diffusional analyte transport through the stationary layer of liquid surrounding the fiber.

In the **headspace mode** (**Figure 1.10**b), the analytes are extracted from the gas phase equilibrated with the sample. The primary reason for this modification is to protect the fiber from adverse effects caused by nonvolatile, high – molecular – weight substances present in the sample matrix (e.g., humic acids or proteins). The headspace mode also allows matrix modifications (including pH adjustment) without affecting the fiber.

In the **third mode** (SPME with **membrane protection**, **Figure 1.10**c), the fiber is separated from the sample with a selective membrane, which lets the analytes through while blocking the interferences. The main purpose for the use of the membrane barrier is to protect the fiber against adverse effects caused by high – molecular – weight compounds when very dirty samples are analyzed. Although, extraction from headspace serves the same purpose, membrane protection enables the analysis of less volatile compounds. The extraction process is substantially slower than direct extraction because the analytes need to diffuse through the membrane before they can reach the

coating. The use of thin membranes and an increase in extraction temperature result in shorter extraction times.

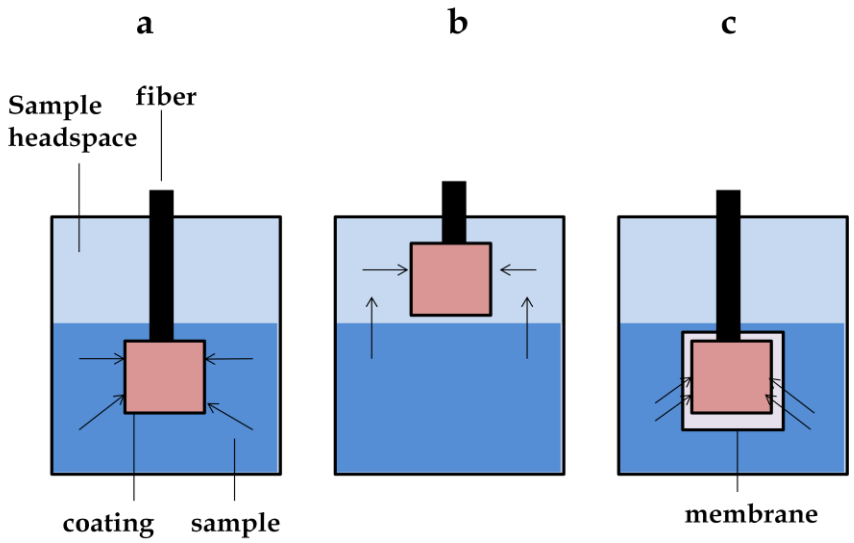

Figure 1.10. Modes of SPME operation: a) direct extraction, b) headspace SPME, and c) membrane – protected SPME **[49]**.

1.4.3. SPME fibers

SPME fiber materials that are more commonly used are polydimethylosiloxane (PDMS), polyacrylate (PA), Carboxen (CAR), polyethylene glycol (PEG, or polyethylene oxide, PEO, or Carbowax, CW) and divinylbenzene (DVB). For better selectivity, coatings of blended materials are also commercially available, such as PDMS/DVB, PDMS/CAR and CW/DVB **[53]**. A list of the commercially available fiber/coatings and their recommended use is given in Table 1.1. These fibers have a poor affinity for polar analytes which makes it difficult to extract polar analytes from polar media such as water **[54]**.There can also be found coatings utilizing sol – gel technology and crown – ethers and calix[4]aranes that have been developed to enhance the extraction of polar analytes **[55-57]**.

The selection of fiber depends on the analyte, in accordance with the general rule "like dissolves like". Typical thickness of the coatings is from 7 – 150 μm. The thicker the phase, the larger is the amount extracted resulting, though, in longer extraction times.

Thick coating is also ideal for highly volatile analytes while thin layers are a better choice for less volatile compounds.

Table 1.1. Commercially available SPME coatings (Supelco, Bellefonte, PA)

Stationary phase	*Recommended use*
Polydimethylsiloxane (PDMS)	
100 µm/non-bonded	*Volatiles*
30 µm/non-bonded	*Non-polar semivolatiles*
7 µm/bonded	*Moderately polar to non-polar semivolatiles*
Polydimethylsiloxane/divinylbenzene (PDMS/DVB)	
65 µm/partially cross-linked	*Polar volatiles*
60 µm/partially cross-linked	*General purpose (for HPLC only)*
Polydimethylsiloxane/carboxen (PDMS/carboxen)	
75 - 85 µm/partially cross-linked	*Trace-level volatiles*
Divinylbenzene/carboxen (DVB/carboxen)	
50/30 µm	*Flavor compounds (volatiles and semivolatiles), odor compounds*
Carbowax/divinylbenzene (CW/DVB)	
70 µm/partially cross-linked	*Alcohols polar analytes*
Carbowax/template Resin (CW/TPR)	
50 µm/partially cross-linked	*Surfactants, polar analutes (for HPLC)*
Polyacrylate (PA)	
85 µm/partially cross-linked	*Polar semivolatiles*

The fibers are also characterized as polar and non – polar. The PDMS fiber has a non – polar coating while the PA and PEG have a polar coating **[46]**.

The fiber coatings are characterized as liquid and solid depending on the sorption mechanism towards the bulk of the fiber (Figure 1.11). In liquid coatings, the analytes partition onto the extraction phase, where the molecules are solvated by the coating

molecules. Their diffusion coefficients allow the molecules to penetrate the whole volume of the coating within a reasonable extraction time, depending on the thickness. Partitioning between the sample matrix and the extraction phase occurs. As liquid fibers are considered the PDMS, the PA and the PEG and extraction made through absorption. In the case of solid coatings, the well defined crystalline structure, which if dense, reduces substantially the diffusion coefficients within the structure. Compounds with weaker affinity are observed at short extraction times. At prolonged extraction times displacement of analytes with lower affinity occurs. This effect is associated with the fact that there is only limited surface area available for adsorption **[58,59]**. As adsorption fibers are considered the blended coatings.

There is a substantial difference in performance between the liquid and solid coatings (Figure 1.11). A comparison with adsorptive versus absorptive equilibrium extraction is useful. In both cases, the extraction process begins by the adsorption of analytes at the extraction phase–matrix interface, and then diffusion of analytes into the bulk of the extraction – phase follows. If the diffusion coefficients of the analytes in the extraction phase are high, then the analytes partition fully between the two phases, and absorptive extraction is accomplished. This process is aided by thin extraction phase coatings or the convection of the sample matrix (if flowing liquid). However, if the diffusion coefficient is low, the analyte remains at the interface and adsorption results. The principle advantage of absorption extraction (partitioning) is a linear isotherm over a wide range of analyte and interference concentrations, because the property of the extraction phase does not change substantially until the extracted amount is approximately 1% of the extraction phase weight. However, in adsorption extraction, the isotherm is highly nonlinear for higher concentrations when the surface coverage is substantial. This causes a particular problem in the equilibrium methods because the response of the fiber for the analyte at high sample concentrations depends on the concentrations of both analytes and interferences. The advantages of the solid sorbents include higher selectivity and capacity for polar and volatile analytes **[49]**.

The SPME fibers are coated with a liquid polymer and/or a porous solid sorbent by immobilization of fused silica fibers as non – bonded, bonded, partially cross – linked or highly cross – linked films. Non – bonded films are stable when used with some water – miscible organic solvents, but they might swell when used with nonpolar solvents. Bonded phases are stable with all organic solvents except for some nonpolar solvents. Partially cross – linked phases are stable in most water – miscible organic solvents and some nonpolar solvents. Highly cross – linked phases are equivalent to partially cross – linked phases, except that some bolding to the core may occur **[58]**.

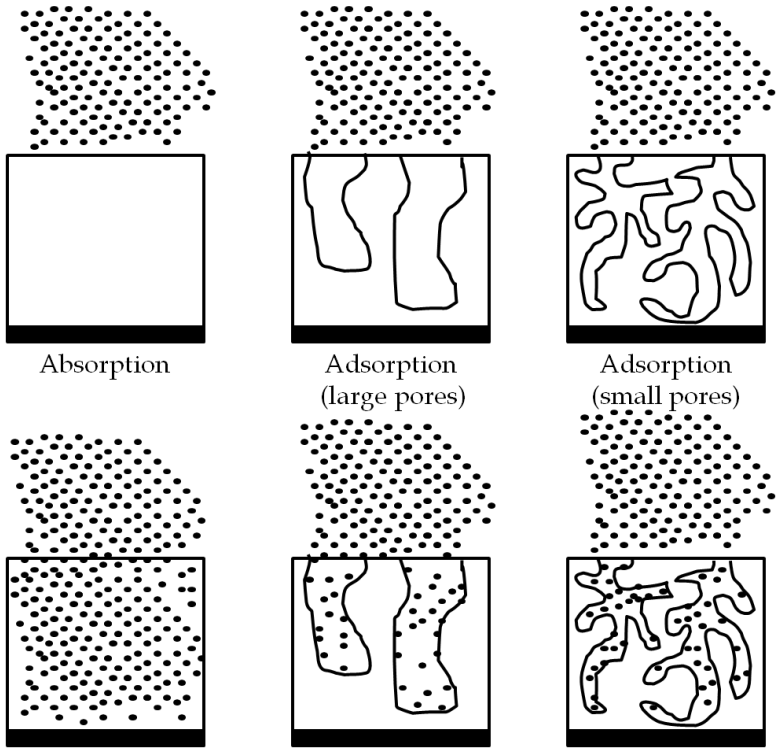

Figure 1.11. Schematic representation of absorptive versus adsorptive extraction and adsorption in small versus large porous **[49]**.

1.5. Headspace solid – phase microextraction (HSSPME)

The headspace mode of the SPME technique makes it possible to expand to more complex samples which contain solid or high molecular weight materials such as soil and sludge **[59]**.

In HSSPME mode a fused silica fiber coated with polymeric organic liquid is introduced into the headspace above the sample. The volatilized organic analytes are extracted and concentrated in the coating and then transferred to the analytical instrument for desorption and analysis. This modification of the solid – phase microextraction method shortens the time of extraction and facilitates the application of this method to analysis of solid samples. At ambient temperature, the headspace SPME technique can be used very effectively to isolate compounds with Henry's constants above 90 $atm \cdot cm^3\ mol^{-1}$ (i.e., three – ring PAHs or more volatile analytes) and can also be used to sample less volatile compounds if high sensitivity can be achieved without reaching equilibrium. The equilibration time for less volatile compounds can be shortened significantly by agitation of both aqueous phase and headspace, reduction of headspace volume, and increase in sampling temperature.

The geometry of the SPME headspace extraction is illustrated in **Figure 1.12**a. An aqueous sample contaminated with organic compounds is transferred to a closed container with headspace. Chemical equilibrium is allowed to establish between the aqueous solution and the headspace, and then a fused silica fiber coated with a thin layer of a selected liquid organic polymer is inserted into the headspace portion of the container (the fiber does not have any direct contact with the aqueous phase). The fiber's liquid coating starts to absorb organic analytes from the headspace. Analytes undergo a series of transport processes: from water to gas phase and eventually to the coating, until the system finally reaches equilibrium. The diffusion process occurs not only in the axial direction but also in the radial direction as well. A simple one – dimensional diffusion model, as illustrated in **Figure 1.12**b, is capable of providing sufficient insight into this diffusion problem. In the model illustrated in **Figure 1.12**b,

diffusion only occurs in one direction (x – axis); a is the thickness of the polymeric coating, b-a is the length of the headspace; and c-b is the length of the analytes aqueous solution.

Though the headspace SPME technique can be used for analyzing organic compounds in various matrices and the fiber coating can be solid or liquid, its equilibrium theory and kinetic theory can be better understood by examining a three phase system in which a liquid polymeric coating, a headspace, and an aqueous solution are involved. The amount of analytes absorbed by the liquid polymeric coating is related to the overall equilibrium of analytes in the three – phase system. Since the total amount of an analyte should be the same during the extraction, we have

$$C_0 V_s = C_f^{\infty} V_f + C_s^{\infty} V_s + C_g^{\infty} V_g \tag{1.3}$$

where C_0 is the initial concentration of the analyte in the aqueous solution; C_f^{∞}, C_s^{∞} *and* C_g^{∞} are the equilibrium concentrations of the analyte in the coating, the aqueous solution, and the headspace, respectively; V_f, V_s and V_g are the volumes of the coating, the aqueous solution, and the headspace, respectively. If we define coating/gas partition coefficients as $K_f = C_f^{\infty}/C_g^{\infty}$ and gas/water partition coefficient as $K_g = C_g^{\infty}/C_s^{\infty}$, the amount of the analyte absorbed by the coating (i.e., the capacity of the coating), $n = C_f^{\infty} V_f$, can be expressed as

$$n = \frac{C_0 V_f V_s K_f K_g}{V_f K_f K_g + V_g K_g + V_s} \tag{1.4}$$

where n is the mass of the analyte extracted by the coating, C_0 is the initial concentration of the analyte in the aqueous solution; V_f, V_s and V_g are the volumes of the coating, the aqueous solution, and the headspace, respectively; K_f is the coating/gas partition coefficient and K_g the gas/water partition coefficient.

Equation 1.4 describes the mass absorbed by the polymeric coating after equilibrium has been reached. The driving force in a multiphase equilibrium is the difference among an analyte's chemical potentials in the three phases. In a three phase system, at equilibrium conditions, the amount of analyte extracted is independent of the location of the fiber in the system. As long as the volume of the fiber coating, headspace and sample are kept constant the fiber can be placed either in the headspace or directly in the sample. The three terms in the denominator of Equation 1.4 give a measure of the analyte capacity of each phase: fiber ($V_f K_f K_g$), headspace ($V_g K_g$) and sample (V_s). Assumingly that the vial containing the sample is fully filled with the aqueous matrix (no headspace), the term $V_g K_g$ in the denominator can be eliminated resulting in Equation 1.1 (two phase system).

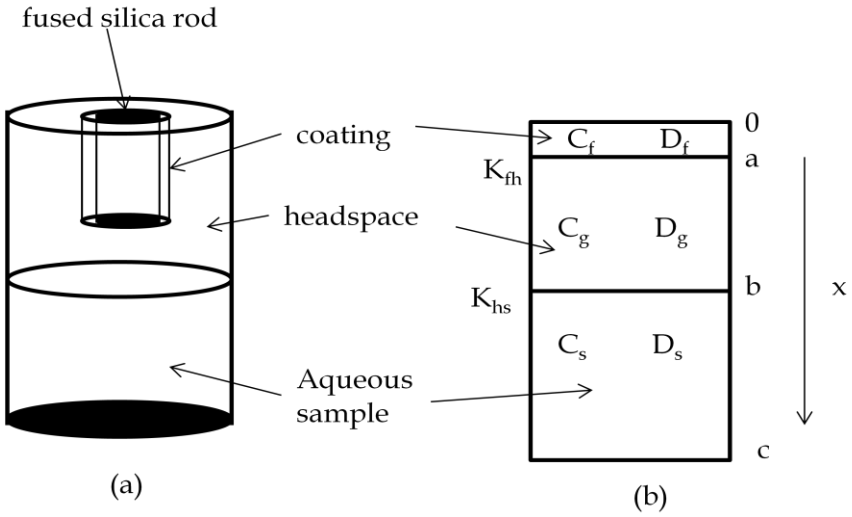

Figure 1.12. (a) Geometry of the headspace SPME method. (b) One – dimensional model of the three – phase diffusion process; K_{fh} and K_{hs} are the coating/gas and gas/water partition coefficients, respectively: D_f, D_h, & D_s are the diffusion coefficients of the analyte in the coating, the headspace, and water, respectively; C_f, C_h & and C_s, are the concentrations of the analyte in the coating, the headspace, and water, respectively; a, b-a, and c-b are the thicknesses of the coating, the headspace, and aqueous phase, respectively **[59]**.

The headspace SPME technique is based on the equilibrium of analytes among the involved phases. Equation 1.4 gives the mass of analytes absorbed by the liquid

polymeric coating when the equilibrium has been achieved. The kinetics of the mass transport, in which analytes move from the aqueous phase to the headspace and finally to the coating, must also be addressed because it is this process that determines the sampling time of the headspace SPME technique.

1.5.1. Effect of extraction parameters

Thermodynamic theory predicts the effects of modifying certain extraction conditions on partitioning and indicates the parameters to control for reproducibility. This theory can be used to optimize the extraction conditions with a minimum number of experiments and correct for variations in the extraction conditions without the need to repeat calibration tests under the new conditions. For example, SPME analysis of outdoor air may be done at ambient temperatures that can vary significantly. The relationship that predicts the effect of temperature on the amount of analyte extracted allows for calibration without the need for extensive experimentation.

Effect of sample volume

The effect of sample volume on quantification and precision of results can be neglected only in rare cases. Extraction kinetics in headspace analysis is dependent on the headspace capacity. If it is sufficiently large, the analyte is extracted almost exclusively from the gaseous phase, and equilibration can be very fast. On the other hand, this causes a loss of sensitivity. In order to avoid errors or poor precision, care should be taken to ensure that the volumes of samples and standard solutions for calibration are the same **[60,61]**.

The amount of the analyte extracted by the fiber at equilibrium in a three – phase system is the same independently of where the fiber is located, be it in the headspace or the liquid. The amount of analyte extracted by the fiber regardless of where the fiber is located can be calculated from the Equation

$$n = C_f^{\infty} V_f = \frac{V_f V_s K_f K_g}{V_f K_f K_g + V_g K_g + V_s} C_s^0 \tag{1.5}$$

For volatile compounds K_g is usually close to 1, which means that headspace volume can be neglected only when it is close to zero (a two – phase system). Semivolatile compounds have much lower values of K_g. Therefore, the $K_g V_g$ term may be negligibly small. However, such an assumption should always be verified. It is the combination of K_g and K_f K_g for a given compound that determines the magnitude of the effect of the sample volume on the amount extracted in three – phase systems with headspace.

Assuming that less than 1% of the initial amount present in the sample is extracted by the fiber, i.e., $n = C_f^{\infty} V_f \leq 0.01\ C_0 V_s$, Eq. (1.5) can yield

$$V_s \geq \frac{99 V_f K_f K_g}{1 + \alpha K_g} \tag{1.6}$$

where $\alpha = V_g/V_s$. From Eq. (1.6) can be calculated the minimum sample volume that does not affect the amount of the analyte extracted by the fiber.

If the analyte has a very high affinity for the SPME polymer phase, that means that $K_g K_f$ is very large and $K_g K_f V_f >> V_g K_g + V_s$ and Eq. (1.6) becomes

$$n \approx V_s C_s^0 \tag{1.7}$$

This is the situation for exhaustive extraction, which is highly unlikely to occur.

Effect of temperature

The concentration of semivolatiles in the gaseous phase at room temperature is small, and headspace extraction rates for those compounds are substantially lower. An increase in temperature results in an increase of the analytes Henry's constant, an

increase in the diffusion coefficient, and a decrease of the amount extracted at equilibrium **[49,62]**.

Elevated extraction temperatures can have a dramatic improvement in extraction speed. By increasing the sampling temperature, the headspace – sample partition coefficient K_g also increases resulting in faster equilibrium times and replenishment of the headspace during extraction. There is a clear transition from slow to fast equilibrium of a sample containing analytes for which the headspace capacity at room temperature is small.

The drawback in excessive increase of the sampling and extraction temperature is the restriction of the fibre – headspace partition coefficient, K_f, and therefore the coating capacity ($K_gK_fV_f$). The system can result in faster equilibrium times sacrificing sensitivity. The amount of the analyte extracted from the sample matrix will be smaller in the short equilibration time of elevated temperature compared to the one under excessive equilibration time of lower temperature conditions **[46, 49,61,62]**.

For this reason care should be taken for the extraction temperature to be optimized to achieve rapid headspace extraction of semivolatile analytes with regard to sensitivity. A way to minimize this drawback of elevated temperatures is the optimization of all parameters influencing extraction efficiency.

Effect of agitation

For compounds that have a large coating/sample partition coefficient (K_fK_g) the sampling time can be relatively long. Extraction times are substantially reduced when sampling analytes indirectly from the headspace above the sample. The diffusion of analytes in the vapor phase is four orders of magnitude higher than in the aqueous phase. A rapid equilibrium between aqueous and vapor phase can be achieved by constantly stirring the aqueous sample to generate a continuously fresh surface. Moreover, by sampling from the headspace, SPME technique can be extended to more complex samples which contain solid or high molecular weight materials such as soil and sludge **[46,59]**.

High stirring rate of the sample not only agitates the aqueous phase well but may also create the convection in the headspace. It is important in such experiments to ensure constant agitation conditions and acceptable extraction times in order to obtain good precision **[46,49,59]**.

Effect of sample matrix

Water solubilities of organic pollutants and pesticides are among the most important physical properties controlling the transport and fate of the chemicals in aquatic systems. Their magnitudes determine not only the individual limiting loads in water but also such partition constants as the soil sorption coefficients and bioconcentration factors. Low concentrations of dissolved and/or suspended particulate – bound natural organic matter in water can significantly enhance the solubility and stability of many "hydrophobic" organic compounds. Dissolved cosolutes in relatively dilute solutions enhance the solubility of solutes that are more water insoluble than themselves. The cosolute produces an enhancing effect on solute solubility either by changing the solvency of the medium or by direct solute interaction either by adsosption or by partitioning (solubilization) **[46,63-65]**.

Significant solubility enhancements of relatively water – insoluble soluted by dissolved organic matter (DOM) of soil and aquatic origins may be described in terms of a partition – like interaction of the solutes with the microscopic organic environment of the high – molecular – weight DOM species. The apparent solute solubilities increase linearly with DOM concentration and show no competitive effect between solutes. The effectiveness of DOM in enhancing solute solubility appears to be largely controlled by the DOM molecular size and polarity. For the solute, the important properties for solubility enhancement are very low solubility in water and significant compatibility with the organic phase.

In the headspace mode, used in the present study, the analytes need to be transported through the barrier of air before they can reach the coating. In this modification, the

fiber coating is protected from damage by high – molecular mass and other non – volatile interferences present in the sample matrix, such as humic materials or proteins. Also, with the headspace mode modification of the sample matrix is allowed, such as change of the pH and salinity, without damaging the fiber. And since equilibrium concentration is independent of the fiber location in the sample/headspace system, the amounts of analytes extracted into the fiber at equilibrium will be the same using direct and headspace modes. The headspace mode serves better in sensitivity for volatile compounds and eliminates the introduction of moisture and oxygen in the GC injection port.

Effect of pH adjustment

Adjustment of the pH of the sample can improve the sensitivity of the method for basic and acidic analytes. This is related to the fact that unless ion – exchange coatings are used, SPME can only extract neutral nonionic species from water. By properly adjusting the pH, weak acids and basis can be extracted by the SPME fiber. To make sure that at least 99% of the acidic compound is in the neutral form, the pH should be at least 2 units lower than the pK_a of the analyte. For the basic compounds, the pH must be larger than pK_a by 2 units **[49]**.

Addition of salt into the aquatic sample increases the ionic strength of the solution making the organic solutes less soluble. Salts commonly added are NaCl or Na_2SO_4. While sampling in the headspace of the sample, salt addition is possible because the fibre does not get damaged.

Effect of salt addition

Salt addition can force polar compounds to enter the vapour phase from the liquid by increasing the partition coefficients of organic compounds and decreasing their water solubility **[1]**. Salting can increase or decrease the amount extracted, depending on the compound and salt concentration, and the effect of salting on SPME has been

determined to date only by experiment. In general, the salting effect increases with increase of compound polarity as it increases the ionic strength of the solution **[10]**.

1.6. Scope

There is still great demand towards the development of new, solvent – free, fast and sensitive extraction methods. The main scope of the present work is to investigate the possibility of sampling semi – volatile analytes from the headspace of aqueous and soil samples using solid – phase microextraction under vacuum conditions. The new procedure was termed vacuum assisted headspace solid phase microextraction (Vac – HSSPME). The present work formulates for the first time a theoretical model demonstrating the pressure dependence of HSSPME sampling procedure under non equilibrium conditions where the K_H value may be used to predict the performance of Vac-HSSPME. The application of the proposed method is investigated on classes of semi – volatile organic pollutants (CPs, PAHs) and the automation possibility is gained by custom – made headspace gastight 22 mL vial.

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CHAPTER 2.

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

2.1. 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%.

2.2 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, Darouzes 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 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 albeit 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, 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 2.1).

Table 2.1. Main physicochemical properties of the chlorophenols studied (2,4-Dichlorophenol (DCP); 2,4,6-trichlorophenol (TrCP); 2,3,4,6-tetrachlorophenol (TeCP); pentachlorophenol (PCP)).

Compound	Molecular Weight	Vapor pressure 25 °C (mm Hg)	K_H (atm m^3mol^{-1})	pKa	Log Kow	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

2.3. 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

$$C_f^{\infty} V_f = \frac{K_f K_g V_s V_f}{K_f K_g V_f + K_g V_g + V_s} C_s^o \qquad (2.1)$$

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

$$C_s = C_s^o e^{-kt} \tag{2.2}$$

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

$$V_s \frac{dC_s}{dt} = -K_L A\,(C_s - C_i) \tag{2.3}$$

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. (2.3) yields Eq. (2.2) with evaporation rate constant (k) defined as **[14]**

$$k = \frac{K_L}{L} \tag{2.4}$$

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

$$K_L = \left[\frac{1}{k_L} + \frac{1}{K_H k_g} \right]^{-1} \tag{2.5}$$

where k_L and k_g are the liquid- and gas-film mass-transfer coefficients and K_H is the Henry's law 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 (ie., 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 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 ~5 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 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. $K_L \approx K_H k_g$). 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

$$k_g \propto D_g^m \tag{2.6}$$

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

$$D_g = \frac{0.001 \times T^{1.75} \sqrt{\frac{1}{M_{air}} + \frac{1}{M_C}}}{P\left[\left(\sum V_{air}\right)^{1/3} + \left(\sum V_C\right)^{1/3}\right]^2} \tag{2.7}$$

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. (2.7)) it is safe to conclude that reducing the total pressure of the system will increase D_g. Based on Eq. (2.6) 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 $K_L \approx K_H k_g$. 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.

2.4. Experimental section

2.4.1. 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 70000 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.

2.4.2. Vac-HSSPME Procedure

Figure 2.1 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 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 5-60 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.

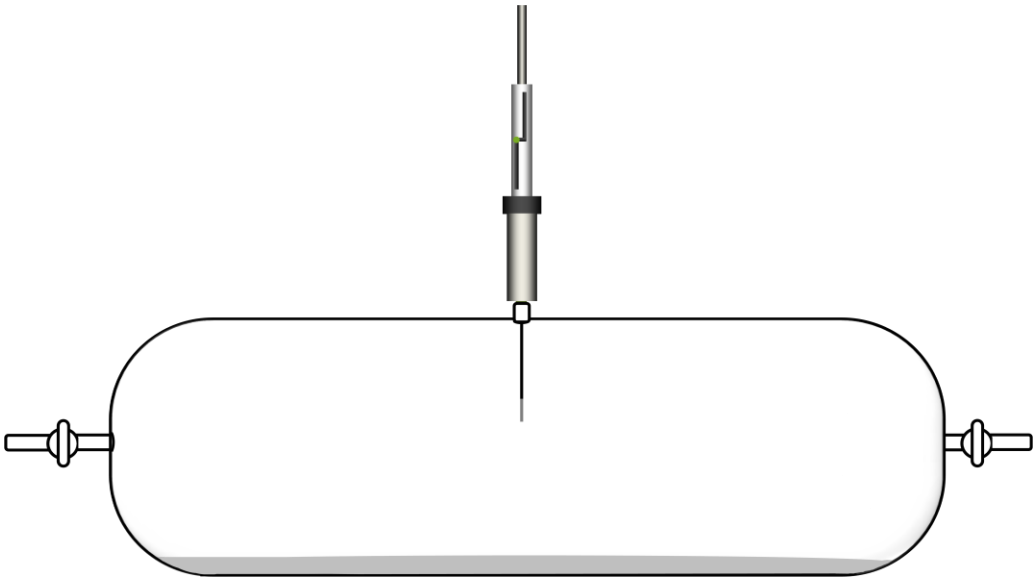


Figure 2.1. Schematic representation of the experimental setup used for Vac – HSSPME. For regular HSSPME, the same spiked aqueous sample was placed in the 1000 mL sampling chamber, the 22 or the 40 mL headspace glass vials (both vials were equipped with hollow caps and septum) and static HSSPME under atmospheric pressure was then performed with the rest of the experimental parameters set at the same values as those used for Vac-HSSPME.

2.4.3. 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 EquityTM-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.

2.5. Results and discussion

2.5.1. 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 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.

2.5.2. Comparison of Vac-HSSPME with regular HSSPME

Based on the K_H values of the model compounds used here (Table 2.1) evaporation rates were expected to be controlled by gas phase mass transfer resistance (i.e. $K_L \approx K_H k_g$) **[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 (Figure 2.2), for a short 10 min sampling at 25 °C, the amount of analyte extracted by the fiber when using Vac-HSSPME was 3.0 to 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 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 (Figure 2.2) 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.

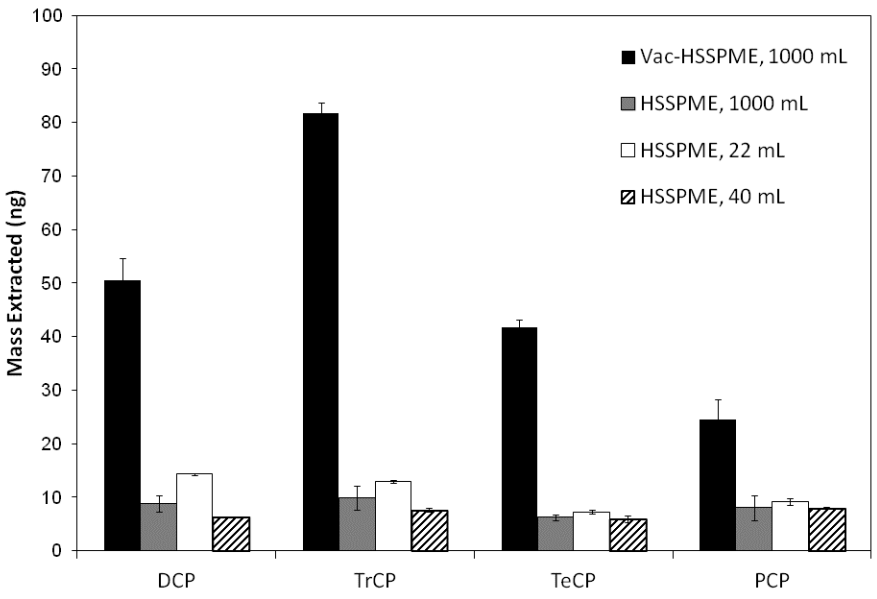


Figure 2.2. Comparison of extraction efficiencies obtained for the same sample with Vac-HSSPME using the 1000 mL sample container (Vac-HSSPME, 1000 mL) and HSSPME under atmospheric pressure using the 22 mL (HSSPME, 22 mL) and 40 mL (HSSPME, 40 mL) headspace vials and the 1000 mL sample container (HSSPME, 1000 mL). Other experimental parameters: 9 mL aqueous sample spiked at 100 μg L^{-1} with each chlorophenol; pH=2; 30 % NaCl (w:v); 25 °C sampling temperature; static HSSPME sampling for 10 min.

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 (Figure 2.2). 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.

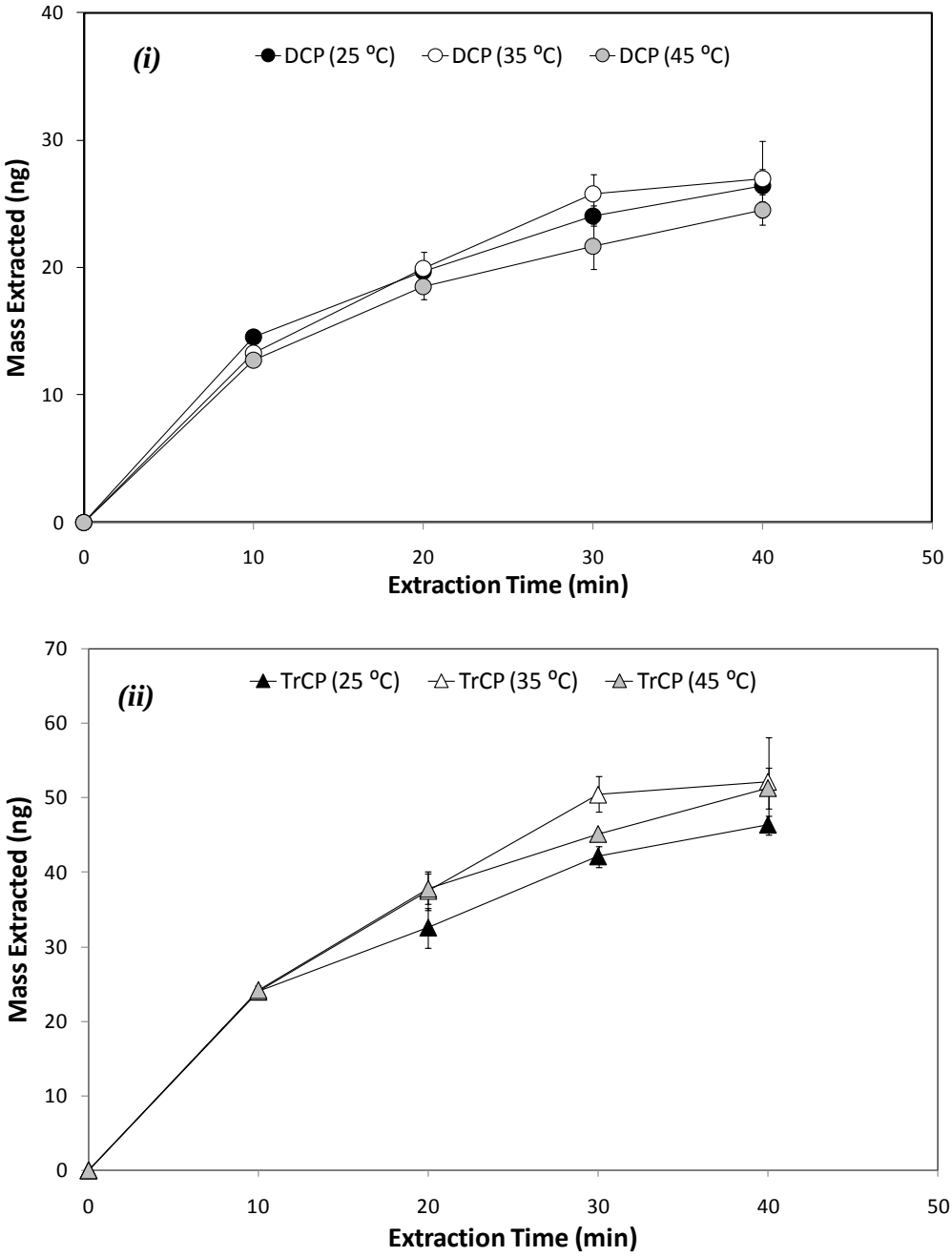
2.5.3. Investigations on the effect of temperature on Vac-HSSPME

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. Figure 2.3 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.

2.5.4. 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]**.


(i)
DCP (25 °C)
DCP (35 °C)
DCP (45 °C)
Mass Extracted (ng)
Extraction Time (min)
0
10
20
30
40
50
(ii)
TrCP (25 °C)
TrCP (35 °C)
TrCP (45 °C)
Mass Extracted (ng)
Extraction Time (min)
0
10
20
30
40
50
60
70

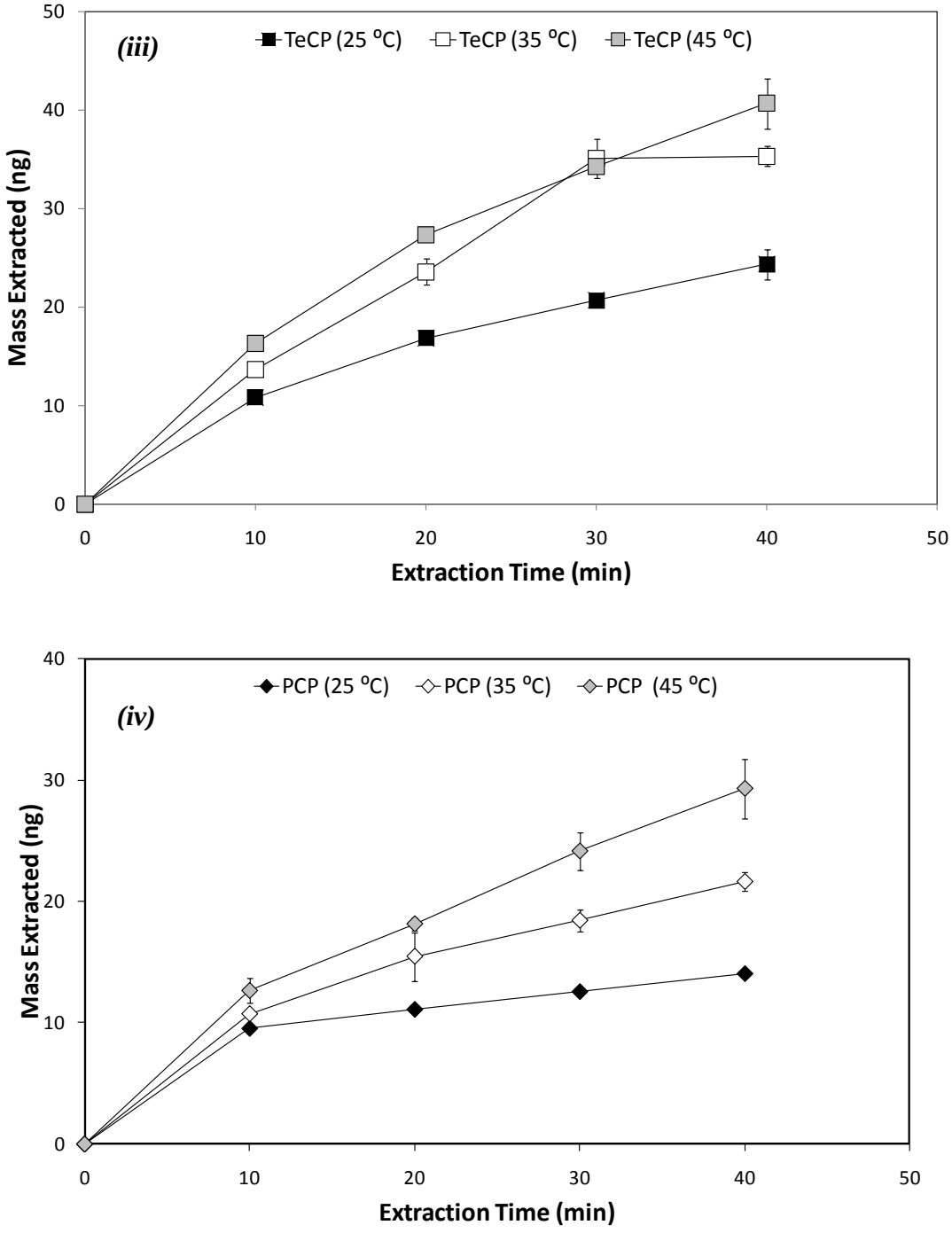
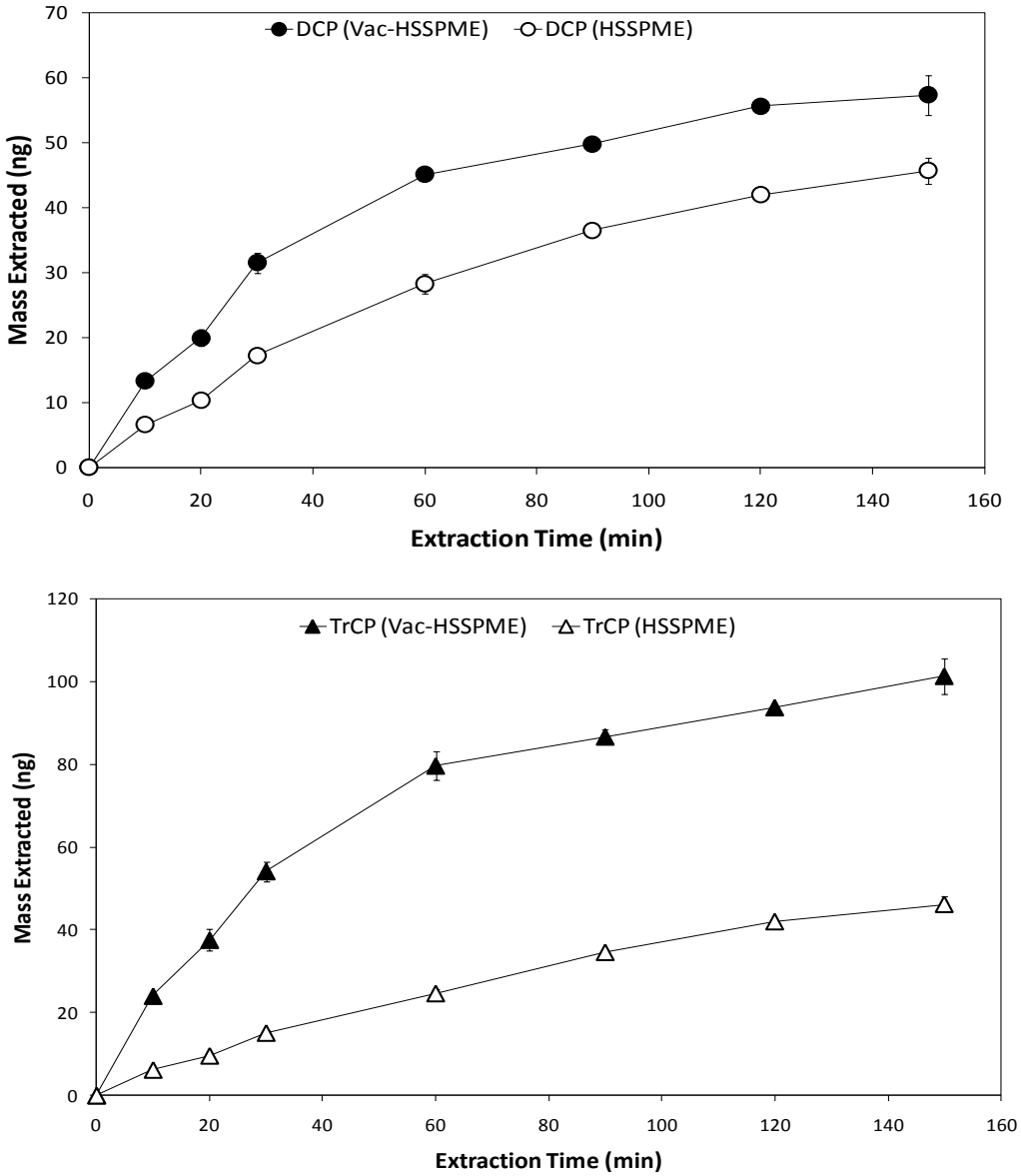


Figure 2.3. Extraction time profiles obtained with Vac-HSSPME at 25, 35 and 45 °C for (i) DCP, (ii) TrCP, (iii) TeCP and (iv) PCP. Other experimental parameters: 9 mL aqueous sample spiked at 20 μg L^{-1} with each chlorophenol; 1000 mL sample container; pH=2; 30 % NaCl (w:v); static HSSPME.

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 Figure 2.4. 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 (Figure 2.4). 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 2.1), 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 (Figure 2.4). 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 (Figure 2.4) and the Vac-HSSPME/HSSPME ratio was 4.0 and 3.8 after 10 and 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.



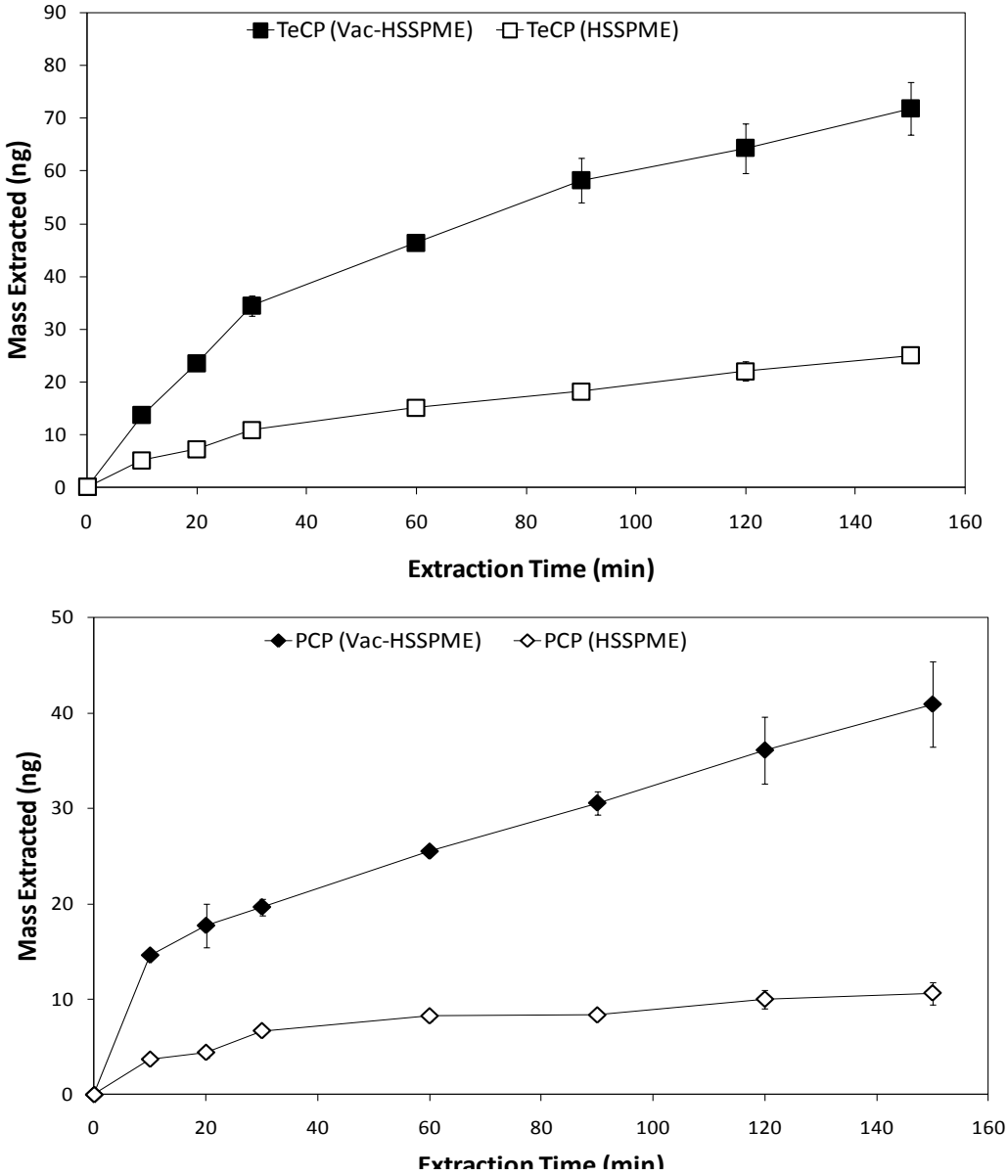


Figure 2.4. Extraction time profiles obtained with the 1000 mL sample container under reduced (Vac-HSSPME) and atmospheric (HSSPME) pressure conditions for (i) DCP, (ii) TrCP, (iii) TeCP and (iv) PCP. Other experimental parameters: 9 mL aqueous sample spiked at 20 μg L^{-1} with each chlorophenol; pH=2; 30 % NaCl (w:v); static HSSPME at 35 °C.

2.5.5. 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 μg L^{-1} for DCP, TrCP and TeCP and 0.250 to 10 μg 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 2.2). The repeatability of Vac-HSSPME method, expressed as relative standard deviation (RSD), was evaluated after extracting five consecutive aqueous samples spiked at 0.250 μg L^{-1} and the RSD values found ranged between 3.1 and 8.6 % (Table 2.2). The limits of detection (LOD) defined for a signal-to-noise of three (S/N=3) ranged between 0.018 and 0.111 μg L^{-1} for the static HSSPME approach used here (Table 2.2).

Table 2.2. 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 (μg L^{-1})	r^2	LOD (μg 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 μg L^{-1}; % RSD values given in parenthesis; n = 5.*

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 0.250 μg L^{-1} are given in Table 2.2 and the results showed that the matrix did not affect extraction.

2.6. 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 (eg. 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.

Acknowledgements

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CHAPTER 3.

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

3.1. Abstract

Nonequilibrium headspace solid-phase microextraction (HSSPME) sampling under vacuum conditions may dramatically improve extraction kinetics compared to regular HSSPME. 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, detection limits in the ppt level and precision varying between 1.8% and 8.4 %.

3.2. 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

$$n_f^\infty = C_f^\infty V_f = \frac{K_f K_g V_s V_f}{K_f K_g V_f + K_g V_g + V_s} \; C_s^o \tag{3.1}$$

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 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]**

$$V_s \frac{dC_s}{dt} = -K_L A\,(C_s - C_i) \tag{3.2}$$

where C_i (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 (m h^{-1}) is the overall mass transfer coefficient at the gas phase – sample interface related to the evaporation rate constant (k; h^{-1}) by $K_L = k\,L$ 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

$$K_L = \left[\frac{1}{k_L} + \frac{RT}{K_H k_g}\right]^{-1} \tag{3.3}$$

where k_L and k_g (m h^{-1}) are the liquid-film and gas-film mass-transfer coefficients respectively, K_H (atm m^3 mol^{-1}; 1 atm = 1.01 10^5 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 ($8.2 \cdot 10^{-5}$ 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.* $K_L \approx K_H \, k_g/R \, T$). 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.* $K_L \approx K_H \, k_g/R \, T$). 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.

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.

3.3. Materials and methods

3.3.1. Chemicals

The three polycyclic aromatic hydrocarbons (PAHs) compounds used here together with some of their physicochemical properties are listed in Table 3.1. They were all purchased from Sigma-Aldrich (Steinheim, Germany) and were each >98%. A stock solution containing 500 mg 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).

Table 3.1. Main physicochemical properties of Na; Ace; Fl; Phe; and Flu.

Compound	Molecular weight	Vapor pressure 25 °C (mm Hg)[a]	K_H (atm m^3 mol^{-1})[b]	$logK_{ow}$	Water solubility 25 °C (mg L^{-1})
Na	128.18	0.085	$4.4 \cdot 10^{-4}$	3.30	30
Fl	166.22	0.0006	$9.62 \cdot 10^{-5}$	4.18	1.992
Flu	202.26	$9.22 \cdot 10^{-6}$	$8.86 \cdot 10^{-6}$	5.16	0.265

[a] 1 mmHg = 133.322 Pa.
[b] 1 atm = $1.01 \cdot 10^5$ Pa.

3.3.2. Vac-HSSPME Procedure

Two custom-made glass sample containers having total volumes of 500 mL and 1000 mL were used as sampling chambers. Figure 3.1 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 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 3001 K, 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 3.1), 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.

3.3.3. 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.

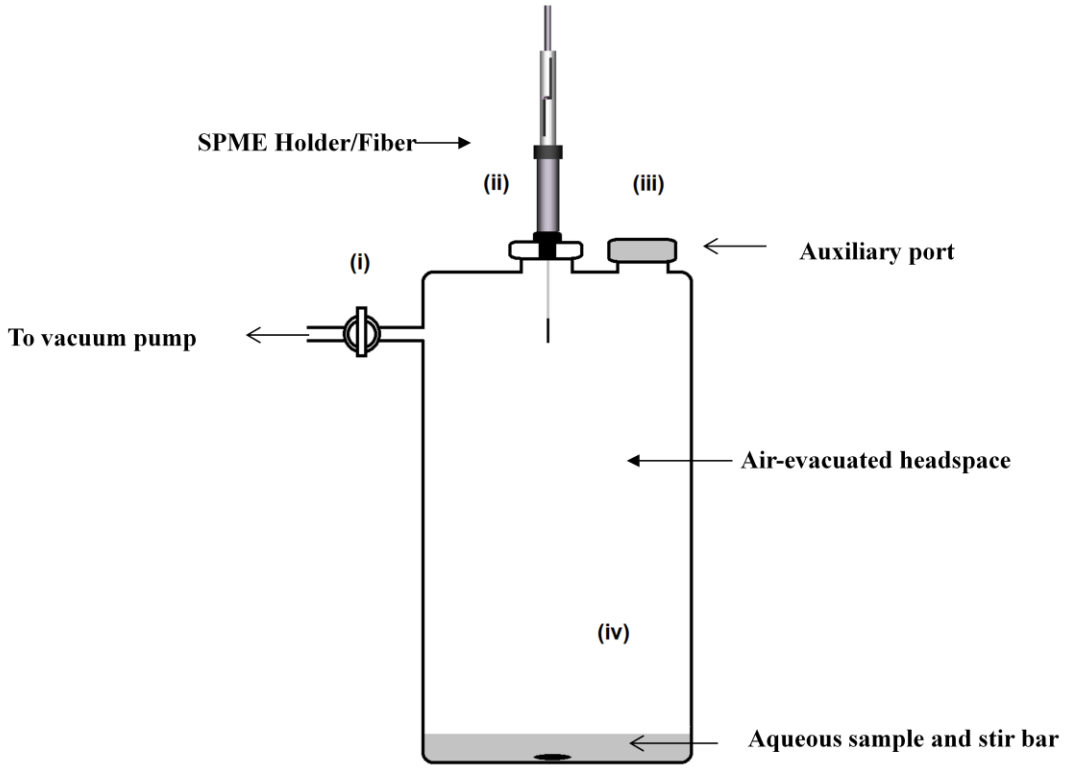

Figure 3.1. Experimental setup used for Vac-HSSPME (the 1000 mL sampling vessel is shown here). The custom-made apparatus was equipped with three gastight ports: (i) one high vacuum glass stopcock used for connecting to a vacuum pump and prepare the sampling chamber for microextraction (ii) one port 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.

3.4. Results and discussion

3.4.1. 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 $5 \cdot 10^{-3}$ 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: $1.2 \cdot 10^{-5}$ **[21,22]** or $1.6 \cdot 10^{-4}$ 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 3.1, 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.

Figures 3.2-3.4 show amongst others, the extraction time profiles obtained with HSSPME in the 500 mL sample container under vacuum and atmospheric pressure conditions. In particular, Figure 3.2 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.

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 (Figure 3.3). 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, Figure 3.4 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 (*eg.* 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.

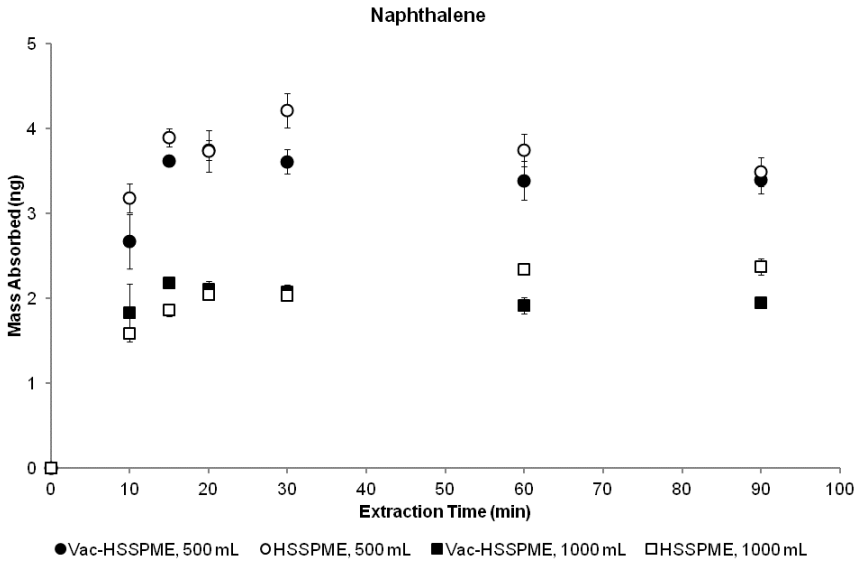

Figure 3.2. Extraction time profiles for naphthalene obtained with the 500 (circles) and 1000 mL (squares) sample containers under reduced (Vac-HSSPME; filled symbols) and atmospheric (HSSPME; open symbols) pressure conditions. Other experimental parameters: 10 mL aqueous sample spiked at 10 μg L^{-1}; 1000 rpm agitation speed; 25 °C sampling temperature.

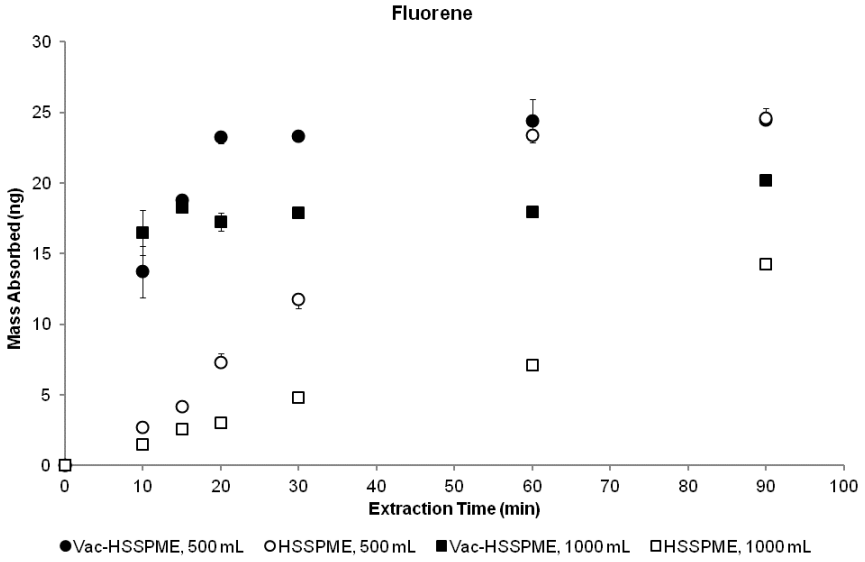

Figure 3.3. Extraction time profiles for fluorene obtained with the 500 (circles) and 1000 mL (squares) sample containers under reduced (Vac-HSSPME; filled symbols) and atmospheric (HSSPME; open symbols) pressure conditions. Other experimental parameters: 10 mL aqueous sample spiked at 10 μg L^{-1}; 1000 rpm agitation speed; 25 °C sampling temperature.

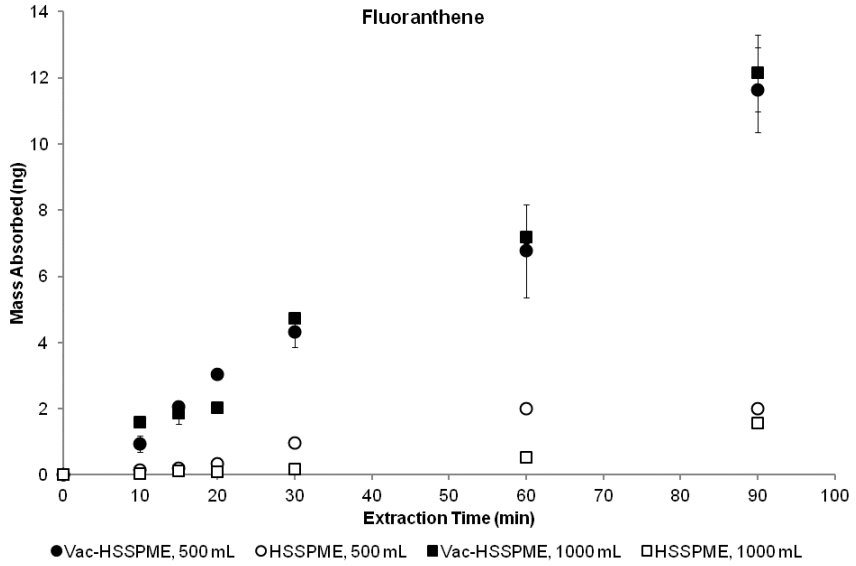

Figure 3.4. Extraction time profiles for fluoranthene obtained with the 500 (circles) and 1000 mL (squares) sample containers under reduced (Vac-HSSPME; filled symbols) and atmospheric (HSSPME; open symbols) pressure conditions. Other experimental parameters: 10 mL aqueous sample spiked at 10 μg L^{-1}; 1000 rpm agitation speed; 25 °C sampling temperature.

3.4.2. 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, $K_g V_g$, 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. (3.1), increasing the headspace volume may result in a loss of sensitivity given that the $K_g V_g$ term is present in the denominator. This is the case for volatile analytes whose K_g values are usually close to unity and the $K_g V_g$ 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 $K_g V_g$ 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 $K_g V_g$ 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.

Figures 3.2-3.4 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 μg L^{-1}) contained in a 500 or 1000 mL sampling vessel (yielding 490 and 990 mL headspace volumes respectively). As seen (Figure 3.2), 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 $K_g V_g$ term for this compound could not be neglected in Eq. (3.1) explaining thus the loss of sensitivity for the larger vessel.

For fluorene (Figure 3.3), 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 $K_g V_g$ term

that could not be neglected in Eq. (3.1). 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 Figure 3.4, 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 $K_g V_g$ 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 (naphathelene), the tested change in headspace volume affected HSSPME extraction

since the process was not affected by the total pressure conditions during both the nonequilibrium and equilibrium stages of the process.

3.4.3. 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 the headspace and reduce the diffusion time of solutes **[6,20]**. Figure 3.5 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 μg 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.

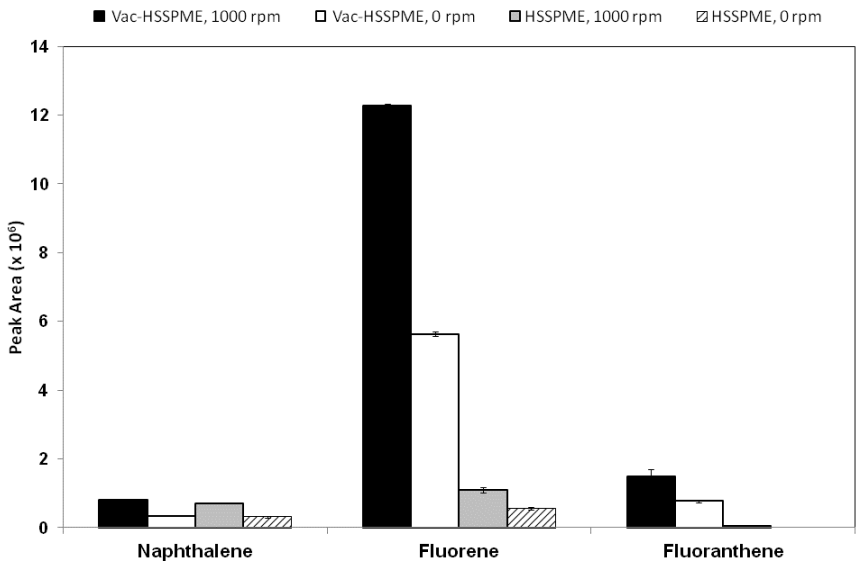

Figure 3.5. Effect of agitation: extraction efficiencies obtained with Vac-HSSPME under turbulent (Vac-HSSPME, 1000 rpm) and static (Vac-HSSPME, 0 rpm) conditions and regular HSSPME under turbulent (HSSPME, 1000 rpm) and static (HSSPME, 0 rpm) conditions. Other experimental parameters: 500 mL sample container; 10 mL aqueous sample spiked at 10 μg L^{-1} with each PAH; 25 °C sampling temperature; 10 min sampling time.

3.4.4. 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 μg L^{-1}. A sample size of 10 mL 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 μg L^{-1} and the RSD values found were 2.7, 1.8 and 8.4 % for 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 μg L^{-1} for naphthalene, fluorene and fluoranthene respectively.

3.5. 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 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.

Acknowledgements

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CHAPTER 4.

Downsizing vacuum-assisted headspace solid phase microextraction

4.1. Abstract

Recently, we proposed a new headspace solid-phase microextraction procedure (HSSPME), termed vacuum-assisted HSSPME (Vac-HSSPME), where headspace sampling of 10 mL aqueous sample volumes took place in 500 or 1000 mL sample containers under vacuum conditions. In the present study, we downsized the extraction device to a 22 mL modified sample vial and concluded that changes in the final total pressure of the pre-evacuated vial following sample introduction were sufficiently low to allow efficient Vac-HSSPME sampling. The downsized extraction device was used to extract five low molecular weight polycyclic aromatic hydrocarbons and several experimental parameters were controlled and optimized. For those compounds whose mass transfer resistance in the thin gas-film adjacent to the gas/sample interface controls evaporation rates, reducing the total pressure during HSSPME sampling dramatically enhanced extraction kinetics in the 22 mL modified vial. Humidity was found to affect the amount of naphthalene (intermediate K_H compound) extracted by the fiber at equilibrium as well as impair extraction of all analytes at elevated sampling temperatures. All the same, the high extraction efficiency and very good sensitivity achieved at room temperature and within short sampling times comprised the most important features of Vac-HSSPME in this downsized extraction device. Analytically, the developed method was found to yield linear calibration curves with limits of detection in the low ng L^{-1} level and relative standard deviations ranging between 3.1 and 6.4 %. Matrix was found not to affect extraction.

4.2. Introduction

More than two decades of research effort in Solid-Phase Microextraction (SPME) resulted in wide acceptance of this sample handling technique and in growing interest of both analysts and manufacturers. The initially developed "fibre-SPME" format continues to be the most common form of the technique for sampling directly the sample matrix or the headspace above it **[1,2]**. Direct and headspace SPME techniques

are nowadays considered mature sample preparation methods suitable for use in routine and/or automated analysis by specialists and non-specialists alike.

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

Hitherto, Vac-HSSPME was investigated in large sampling vessels. Although the effect of reduced pressure conditions was found to dominate over any effect of headspace volume on the extraction kinetics of low volatility compounds **[4]**, manipulation of the

500 and 1000 mL containers may be cumbersome to routine users. Downsizing Vac-HSSPME will enable practical and effortless application of the method to routine analysis as well as substantially increase the automation potential of the method. This is particularly important to environmental laboratories aiming at reducing analyst time both for routine analysis and method development, faster sample throughput and greater reproducibility **[2,8]**. In addition, for those analytes that reach equilibrium within a reasonable amount of time, reducing the size of the sample container and accordingly the volume of the headspace will also increase the final amount of analyte extracted by the fiber as predicted by the theory **[9]**.

The present work reports for the first time the performance of Vac-HSSPME in a 22 mL modified headspace sample vial. Five low molecular weight polycyclic aromatic hydrocarbons (PAHs) compounds were used as model compounds (Table 4.1). Parameters such as sample volume, agitation speed, extraction time and temperature were controlled and optimized. Comparison of the results with those obtained with regular HSSPME and our previous findings, revealed some new and important insights on the Vac-HSSPME procedure. Finally the performance of the resulting method was assessed and matrix effects upon extraction were evaluated by analyzing spiked tap water as well as effluent water sample taken from a municipal wastewater treatment plant.

4.3. Materials and methods

4.3.1. Chemicals and Reagents

The five PAHs selected for investigation were all purchased from Sigma-Aldrich (Steinheim, Germany) and were each >98% in purity. A stock solution, containing 500 mg L^{-1} of each target analyte in acetonitrile (pesticide-grade; Merck, Darmstadt, Germany), was used daily for the preparation of the spiked aqueous solutions and was stored in the dark at 4 °C when not in use. Deionized water used for sample preparation was prepared on a water purification system (Barnstead EASYpure II) supplied by

Thermo Scientific (Dubuque, USA). Recovery studies were carried out using tap water from the drinking water distribution network of Chania (Crete, Greece). Secondary treated wastewater effluent samples from the municipal wastewater treatment plant of Chania, serving approximately 70000 inhabitants, were collected the day before being used and stored in glass bottles in the dark at 4 °C. HSSPME sampling of the unspiked real samples under both reduced and atmospheric conditions ensured that the samples were free of the target analytes.

4.3.2. Vac-HSSPME Procedure

The custom-made gastight sample container used for extraction had a final volume of 22 mL and was built from a 20 mL headspace rounded bottom glass vial (O.D. 22.5 mm x H. 75.5 mm) further modified to accommodate on the top part two gastight ports: one high vacuum glass stopcock and one glass port equipped with a half-hole cylindrical Thermogreen septum (Supelco, Bellefonte, PA) compatible with the needle of the SPME. For Vac-HSSPME, the modified headspace vial containing a cylindrical Teflon-coated magnetic stir bar (9 mm × 3 mm) was air-evacuated after connecting the high vacuum stopcock with the vacuum pump (7 mbar ultimate vacuum without gas ballast; Vacuubrand GmbH & Co. KG, Model MZ 2C NT, Wertheim, Germany). Upon air evacuation, the glass stopcock was closed and the vacuum pump was disconnected. Unless otherwise stated in the text, a 7 mL spiked aqueous solution was then introduced into the vial through the Thermogreen septum with the help of a 10 mL gastight syringe (SGE, Australia). The modified vial containing the sample and stir bar was then mounted on top of a stir plate (Heidolph, MR 3001 K, Germany). Agitation at 1400 rpm was then applied and target analytes in the aqueous solution were left to equilibrate with the headspace for 10 min. Upon sample equilibration, the needle of the SPME fiber/holder assembly (Supelco, Bellefonte, PA) was introduced into the sampling chamber by piercing the Thermogreen septum and HSSPME sampling was performed for a preset period of time (typically 30 min). Based on previous reports the 100-μm PDMS SPME fiber (Supelco, Bellefonte, PA) was used for extraction **[10,11]**.

Unless otherwise mentioned in the text, extraction was performed at 25 °C and 1400 rpm agitation speed. When microextraction sampling was completed, the PDMS fiber was retracted and the SPME device was transferred to a gas chromatographer – ion trap mass spectrometer (GC-MS-IT) for analysis. The pressure inside the modified vial was then equilibrated with atmospheric and the apparatus was emptied, washed and used for the next microextraction sampling. A schematic representation of the extraction procedure used for Vac-HSSPME is given in Fig. 4.1. The Thermogreen septum was replaced daily to avoid pressure loss due to septum damage. All extractions were run at least in duplicates.

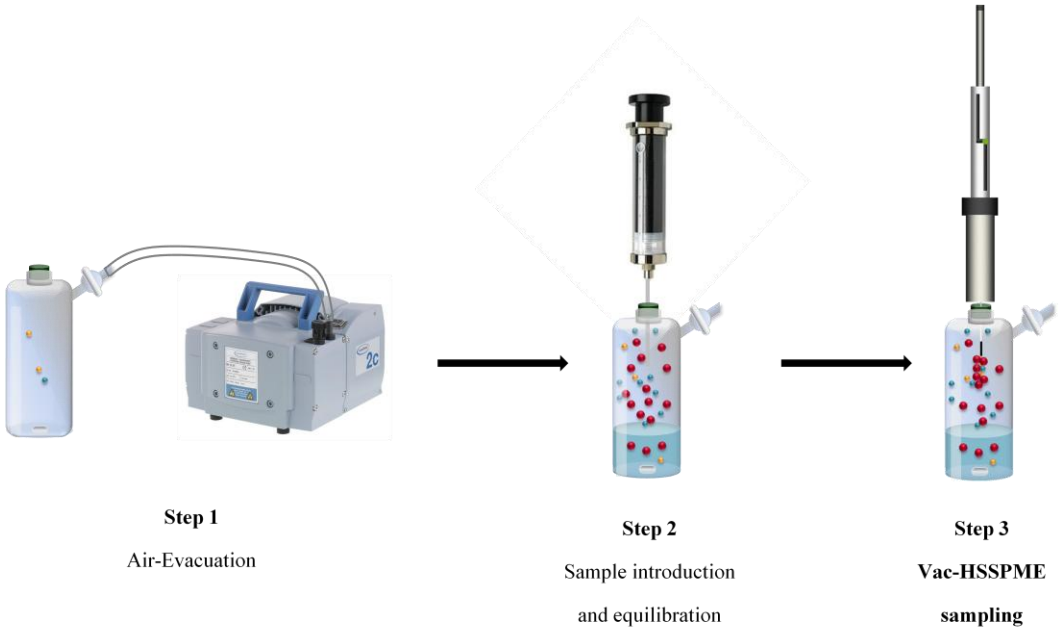


Figure 4.1. Schematic representation of the extraction procedure used for Vac-HSSPME: (i) air evacuation of the modified sample vial after connecting the high vacuum stopcock with the vacuum pump, (ii) the glass stopcock was closed, the vacuum pump was disconnected and the aqueous sample was introduced through the port equipped with a septum; the aqueous solution was then left to equilibrate with the headspace for 10 min, and (iii) upon sample equilibration HSSPME sampling was performed for a preset period of time. In this simplified representation air (yellow spheres), water (blue spheres) and analyte (red spheres) molecules are also illustrated.

4.3.3. GC-MS-IT Analysis

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

4.4. Results and discussion

During the present investigations five low molecular weight PAHs compounds were used as model compounds since they are environmentally significant and cover the range from intermediate to low volatility compounds. Based on the K_H values of the target analytes (Table 4.1), naphthalene (Na) represents the case of an intermediate K_H compound, acenaphthene (Ace), fluorene (Fl) and phenanthrene (Phe) lies on the border between intermediate and low K_H compounds and fluoranthene (Flu) represents the low K_H class of compounds.

Table 4.1. Main physicochemical properties of the five PAHs compounds investigated here.

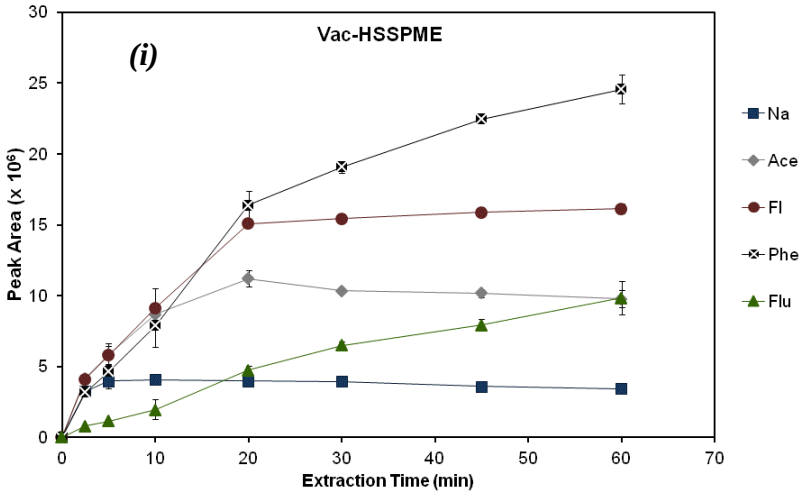
Compound	Molecular weight	Vapor pressure 25 °C (mm Hg)[a]	K_H (atm m^3 mol^{-1})[b]	$logK_{ow}$	Water solubility 25 °C (mg L^{-1})
Na	128.18	0.085	$4.4 \cdot 10^{-4}$	3.30	30
Ace	152.21	0.00215	$1.84 \cdot 10^{-4}$	3.92	4
Fl	166.22	0.0006	$9.62 \cdot 10^{-5}$	4.18	1.992
Phe	178.23	0.000121	$4.23 \cdot 10^{-5}$	4.46	1.6
Flu	202.26	$9.22 \cdot 10^{-6}$	$8.86 \cdot 10^{-6}$	5.16	0.265

[a] 1 mmHg = 133.322 Pa.
[b] 1 atm = $1.01 \cdot 10^5$ Pa.

4.4.1. Effect of extraction time

Fig. 4.2 shows the extraction time profiles obtained in the 22 mL sample container under vacuum and atmospheric pressure conditions. As seen, Vac-HSSPME dramatically improved extraction kinetics compared to regular HSSPME for almost all compounds investigated here. This is emphatically visible in the case of Ace and Fl, where Vac-HSSPME extraction time profiles clearly showed the two-stage nature of the HSSPME process (~20 min equilibration time). On the contrary, regular HSSPME of Ace and Fl was still far from equilibrium even after sampling the headspace for 60 min (Fig. 4.2). For these two compounds, reducing the total pressure of the system resulted in an indubitable transition from slow to fast equilibration. A 20 min Vac-HSSPME equilibration time was also recorded for Fl in the 500 and 1000 mL sample containers (Na, Fl and Flu were the three model compounds included in our previous investigations) **[4]**, demonstrating once again that Vac-HSSPME extraction kinetics are independent of the headspace volume since evaporation rates dramatically increase under reduced pressure conditions and the sample responds much faster to the concentration drops in the headspace. For Phe, equilibrium was not attained even after sampling the headspace for 60 min under reduced pressure conditions (Fig. 4.2).

Nevertheless, the positive effect of reduced pressure sampling conditions remained important throughout the sampling times tested as evidenced by the Vac-HSSPME/HSSPME peak area ratios obtained at each sampling time point (eg. 12 and 8 at 30 and 60 min respectively). Similar conclusions can be reached for Flu, the most hydrophobic and least volatile compound investigated here. With a K_H value well below the reported threshold values for low K_H compounds, gas-phase resistance controlled evaporation rate and HSSPME sampling under reduced pressure conditions dramatically enhanced extraction kinetics when compared to regular HSSPME. As expected **[4]**, for Flu equilibrium was not attained under both pressure conditions after sampling the headspace for 60 min (Fig. 4.2). Nevertheless, the positive effect of reduced pressure sampling conditions remained markedly important and even after sampling the headspace for 60 min the amount of Flu extracted with Vac-HSSPME was ~33 times larger compared to that with regular HSSPME.



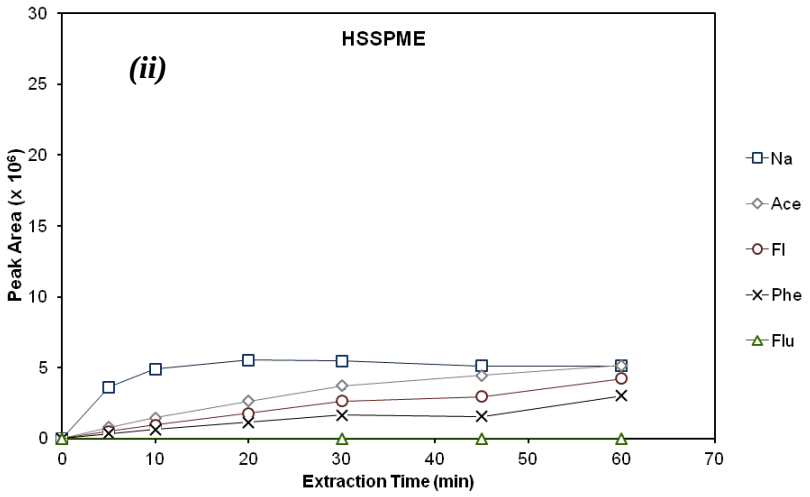


Figure 4.2. Extraction time profiles for all target PAHs obtained in the 22 mL modified sample vial (i) under reduced (Vac-HSSPME; filled symbols) and (ii) atmospheric (HSSPME; open symbols) pressure conditions. Other experimental conditions: 7 mL aqueous sample spiked at 5 μg L^{-1}; 1400 rpm agitation speed; 25 °C sampling temperature. Some error bars are too small to be visible as compared with the physical size of the symbol.

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

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

4.4.2. Effect of sample volume

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

$$P = \Sigma Pi + Pw + Pvac \quad (4.1)$$

where ΣP_i are the sum of the analytes' partial pressures, P_w is the partial pressure of water and P_{vac} is the final pressure after most of the air has been removed from the sampling chamber and the aqueous sample has been introduced. The value of P_{vac} is

directly related to the pressure attained upon air-evacuation of the sampling vessel, P_{evac}, through the ideal gas law,

$$P_{vac} = P_{evac}\left(1+\frac{V_s}{V_g}\right) \tag{4.2}$$

with V_s and V_g denoting the volumes of the sample and headspace respectively. The lowest value P_{evac} can attain is the ultimate pressure limit of the vacuum pump used (in our case 7 mbar; 1 mbar = 100 Pa). Based on this value and the vapor pressure values of the target analytes (Table 4.1), it can be safely assumed that for a low sample to headspace volume ratio the final total pressure in the gas phase upon sample equilibration will be ultimately slightly higher than that of pure water and less than 40 mbar in total at 25 °C. The markedly small sample to headspace volume ratio achieved with the 500 and 1000 mL vessels used in our previous studies could meet this criterion and changes in pressure upon sample introduction and equilibration were not expected to be significant **[3,4]**. During the present investigations, the use of a 22 mL vial resulted in a sample to headspace volume ratio that could no longer be neglected. Nevertheless, as long as P_{evac} is sufficiently low, changes in the final total pressure, P, upon different sample volumes introduction will not be significant. For example, introducing a 7 mL aqueous sample in the pre-evacuated 22 mL vial should result in an approximate 1.5-fold increment in P_{vac} leading to minor changes in the final total pressure despite the substantial increase in sample to headspace volume ratio.

To demonstrate the above assumption the effect of aqueous sample volume on Vac- and regular HSSPME was investigated within the range from 3 to 13 mL after a 30 min sampling time at 25 °C and the results are given in Fig. 4.3. As seen, with the exception of Na, HSSPME sampling under reduced pressure conditions enhanced extraction kinetics for each sample volume when compared to regular HSSPME. Hence, pressure changes induced for sample to headspace volume ratios commonly used in HSSPME are sufficiently low to allow efficient Vac-HSSPME sampling. In fact, even after

introducing 13 mL of the aqueous sample the Vac-HSSPME/ HSSPME peak area ratio still remained important (2.2, 4.5, 9.6 and 22 for Ace, Fl, Phe and Flu respectively).

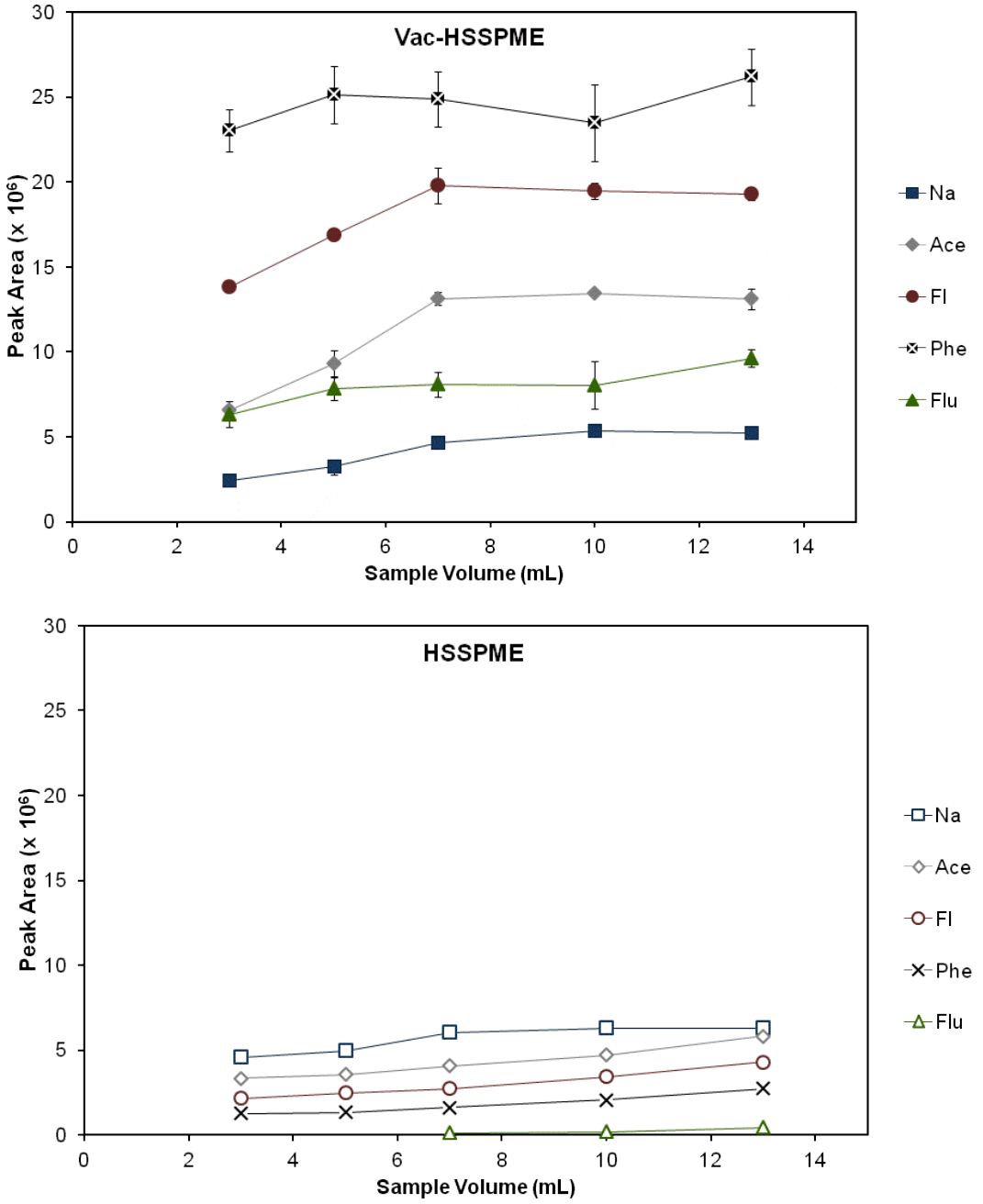


Figure 4.3. Effect of sample volume on the extraction of PAHs obtained in the 22 mL modified sample vial (i) under reduced (Vac-HSSPME; filled symbols) and (ii) atmospheric (HSSPME; open symbols) pressure conditions. Other experimental conditions: aqueous samples spiked at 5 μg L^{-1}; 30 min sampling time; 1400 rpm agitation speed; 25 °C sampling temperature.

A closer look on Fig. 4.3 shows that for Vac-HSSPME the amount of extracted analyte gradually increased for sample volumes up to 7 mL and then remained, to some extent, constant. In general, the flat part of the curves does not necessarily mean saturated absorption, especially if the analyte's concentration is low. When the concentration change after absorption is no longer significant, SPME absorption is practically independent of sample volume **[21]**. It therefore appears that for Vac-HSSPME, the presence of an air evacuated headspace accelerated extraction kinetics and curves leveled off for almost all analytes. On the other hand for regular HSSPME, with the exception of Na, the amount of analyte extracted by the fiber increased with increased sample size throughout the volumes tested. This is consistent with the fact that for the less volatile analytes, higher sensitivities (i.e. shorter equilibration times) can be obtained during regular HSSPME by increasing the aqueous phase volume, because for these analytes reducing the headspace volume increases the concentration gradient in the headspace and it takes less time to diffuse through the headspace **[9,22]**. As expected for the more volatile Na, the amount absorbed by the fiber increased with regular HSSPME for water volumes up to 7 mL and then SPME adsorption became practically independent of the sample volume **[22]**. Based on the present findings it was decided to use a 7 mL sample volume for all subsequent experiments.

4.4.3. Effect of agitation

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

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

4.4.4. Effect of temperature

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

Combining the effects of temperature and reduced pressure in Vac-HSSPME, was expected to enhance even further the kinetics of the extraction up to a certain temperature above which the effect of temperature would dominate and basically control the extraction. The reason for this is that the vapor pressure of water, P_w, increases exponentially with temperature leading to a considerable increment in the final total pressure, P, according to Eq. (4.1), when heating the sample.

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

$$y_i = \frac{P_i}{P} = \frac{P_i}{P_i + P_w + P_{air}} \tag{4.3}$$

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

$$E_i = \frac{y_{i,vac}}{y_i} = \frac{P_i + P_w + P_{air}}{P_i + P_w + P_{vac}} \tag{4.4}$$

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

In our previous report we were able to investigate the effect of temperature on Vac-HSSPME over a small temperature range (from 25 to 45 °C) due to limitations of the experimental setup and a positive effect of temperature was reported for the less volatile chlorophenol compounds **[3]**. During the present investigations, the use of a 22 mL modified vial allowed us to examine sampling temperatures from 25 to 60 °C. For comparison, HSSPME extractions were performed under both vacuum and atmospheric pressure conditions and the results are given in Fig. 4.4. As seen, for a 30 min Vac-

HSSPE sampling, heating the sample gradually decreased mass loading of the more volatile Na, Ace and Fl (all expected to be at equilibrium) until the point (60 °C) where extraction was found to be practically impaired. On the other hand, for Phe and Flu, increasing the temperature from 25 to 40 °C improved extraction; yet a further increase to 60 °C drastically restricted extraction (Fig. 4.4). As mentioned earlier, during Vac-HSSPME the water vapor in the headspace is close to saturation. Increasing the temperature greatly increases humidity and challenges even more the fiber. Since more water molecules are available to partition with the PDMS fiber **[12]** the fiber's characteristics are changed, thus impairing mass loading of the analytes **[13,20]**.

As expected for regular HSSPME, heating the sample improved extraction (Fig. 4.4). However, when sampling under atmospheric pressure conditions, a 60 °C sample temperature is necessary in order to reach the maximum peak area values attained with Vac-HSSPME (at 25 or 40 °C and depending on the analyte). It should be emphasized however that regardless of the adverse effect of higher temperatures on Vac-HSSPME, one of the most important features of Vac-HSSPME is that high extraction efficiency and very good sensitivity can be achieved under mild extraction conditions and that includes extraction at room temperature. In cases where higher sensitivity is needed then fine tuning of the Vac-HSSPME method can be achieved by simply increasing the sampling time. Based on the above discussion it was decided to use a 25 °C as sampling temperature.

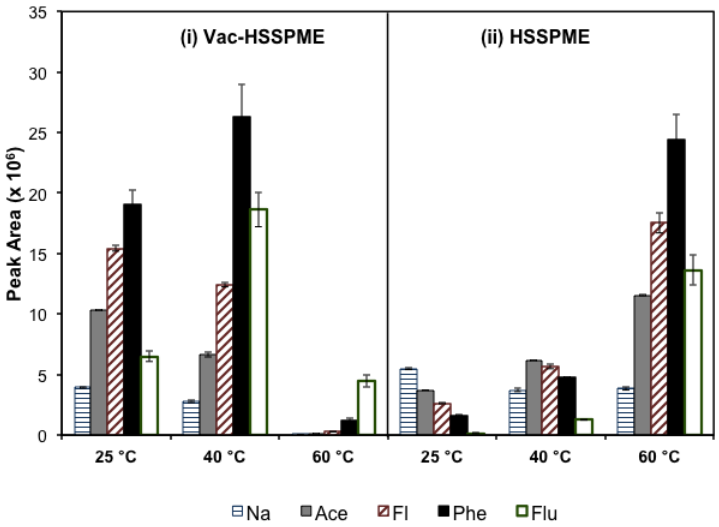


Figure 4 4. Effect of temperature on the extraction of PAHs obtained in the 22 mL modified sample vial (i) under reduced (Vac-HSSPME) and (ii) atmospheric (HSSPME) pressure conditions. Other experimental conditions: 7 mL aqueous samples spiked at 5 μg L^{-1}; 30 min sampling time; 1400 rpm agitation speed; 25 °C sampling temperature.

4.4.5. Validation of the method

The main analytical parameters of merit were determined for the newly proposed extraction approach. The analytical curve was constructed by extracting for 30 min at 25 °C the headspace of 7 ml aqueous solutions stirred at 1400 rpm and spiked with all target analytes using five concentration levels ranging from 0.1 to 10 μg L^{-1}. The calculated calibration curves gave a high level of linearity for all target analytes with correlation coefficients (r^2) ranging between 0.9929 and 0.9997. The repeatability of the proposed method, expressed as relative standard deviation (RSD), was evaluated by extracting five consecutive aqueous samples spiked at 0.25 μg L^{-1} with each target analyte and was found to range between 3.1 and 6.4 % (Table 4.2). The limits of detection (LODs) were also determined and were found to be in the low ng L^{-1} level (Table 4.2) and, as expected, they were better than those reported in the 500 mL sample container **[4]**. Analyte recoveries from tap and secondary treated wastewater effluent samples spiked at 1 μg L^{-1} ranged between 102 – 106 % and 99 - 104 % respectively (Table 4.2), relative to the amount extracted from pure water samples, demonstrating that matrix did not affect Vac-HSSPME extraction.

Table 4.2. 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[a] (% RSD)	Relative Recoveries	
					Tap[b]	WW effluent[b]
Na	0.2 – 10	0.9960	0.027	5.2	105 (1.1)	99 (1.8)
Ace	0.1 – 10	0.9993	0.013	3.6	102 (4.3)	99 (3.9)
Fl	0.1 – 10	0.9997	0.015	1.3	106 (2.5)	101 (1.3)
Phe	0.1 – 10	0.9979	0.014	5.4	105 (3.1)	104 (3.1)
Flu	0.2 – 10	0.9929	0.021	5.8	104 (3.7)	97 (4.0)

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

Regular HSSPME sampling of PAHs from water samples has been investigated on several occasions **[25]**. To the best of our knowledge, the majority of these reports discuss and compare the effect of different extraction parameters on headspace and direct immersion HSSPME sampling modes **[9,10,25]** and only few of them present the analytical performance of developed HSSPME procedures **[26,27]**. Table 4.3 summarizes LODs and the main experimental conditions under which they were obtained for Vac-HSSPME and previously reported regular HSSPME methods. Since each method uses different fibers, sample volumes, analytical instrumentations and ionic strength, care should be taken when comparing their analytical performances. Nevertheless, with Vac-HSSPME very good sensitivity is achieved whilst extracting small sample volumes for short sampling times, at room temperature and without adding salt to the water samples.

Table 4.3. Summary of Vac-HSSPME and other published HSSPME procedures used for the determination of PAHs in water samples.

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

[a] Polyacrylate 85 μm.
[b] Porous Layer of Activated Charcoal; laboratory made.

4.5. Conclusions

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

4.6. References

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CHAPTER 5.

Vacuum-Assisted Headspace Solid Phase Microextraction of Polycyclic Aromatic Hydrocarbons from Soil Samples

5.1. Introduction

In general, the association of nonpolar organic compounds to soil organic matter is attributable to "hydrophobic bonding". This type of bonding is due to the combination of short-range van der Waals forces and a "thermodynamic gradient" driving nonpolar organics of low solubility out of solution, since the interactions between these compounds and natural organic matter are energetically preferred to compound/water or compound/compound interactions **[1]**. For neutral PAH molecules it is believed that binding onto soil material is dominated by van der Waals type interactions **[1]** and occurs through an initial fast adsorption process thought to reflect rapid adsorption of the hydrophobic pollutants onto hydrophobic areas of soil surfaces, followed by a slow adsorption process assumed to be based on migration of the hydrophobic contaminants to less accessible sites within the soil matrix **[2]**. Likewise, desorption kinetics of hydrophobic organic compounds in soils and sediments have been proved to occur in two distinct stages: a rapid release (on the order of minutes to hours) followed by a slow release (in the order of weeks to years) **[3]**. Most researchers attribute slow kinetics of desorption by diffusion limitations. For the sorbed molecules to leave an aggregated and porous geosorbent particle, they may need to pass through penetrable solid phases (matrix diffusion), pores within the particle (pore diffusion), the relatively immobile liquid "film" extending from the solid surface (film diffusion) and the bulk liquid **[4]**. This is almost certainly true because sorbing molecules are subject to diffusive constraints throughout almost the entire sorption/desorption time course because of the porous nature of particles. For the sorbed molecules to leave an aggregated and porous geosorbent particle, they may need to pass through penetrable solid phases (matrix diffusion), pores within the particle (pore diffusion), the relatively immobile liquid "film" extending from the solid surface (film diffusion) and the bulk liquid **[4]**. The low apparent activation energy of desorption, which approximately indicates the mechanism of diffusion, of pyrene from aged sand samples, was previously assigned to the non-porous structure of sand particles and the absence of sorbent organic matter

(SOM) in sand matrix, which means that the two important rate-limiting steps in desorption process i.e. retarded diffusion in narrow pores and diffusion through hard SOM are not significant when dealing with sand matrices. The interactions between the PAH molecules and the inorganic mineral sorption sites on sand particle surface is of dipole–dipole-induced type which are low energy, however the greater the polarizability of the PAH molecules the stronger are the temporary van der Waals interactions between the molecules and the surface of sand particles **[3]**.

Headspace solid phase microextraction (HSSPME) can be used to extract target analytes from very complex matrices, such as sludge, wastewater, and soil, since the fiber is not in direct contact with the matrix **[5]**. Depending on the physico-chemical properties of organic contaminants and the characteristics of the soil matrix, HSSPME analysis of soil samples can be hindered by the low recovery of the target analytes **[6]**. Accordingly, for volatile organic contaminants SPME sampling of the headspace above the soil sample can be very efficient, yet for the less volatile compounds or compounds involved in strong interactions with the soil, the concentration of the analytes in the headspace is expected to be low and a small amount of analytes is expected to be sorbed by the fiber coating when exposed to the headspace. The latter should account for the slow development of SPME procedures for organic contaminants determination in soil samples **[7]**.

There are different ways to facilitate and/or enhance the release of analytes from their matrix such adding modifiers to the soil sample before extraction and/or as heating the soil sample **[8]**. Regarding the latter, problems are commonly encountered given that increased temperatures were also found to decrease distribution coefficients of the analytes between the extraction phase and the sample matrix, potentially resulting in a lower extracted amount under equilibrium. To prevent loss of sensitivity, the coating can be cooled simultaneously with sample heating leading to the development of some dedicated instrumentation the so-called internally cooled or low temperature SPME devices, which were recently miniaturized and automated **[3,5,9]**.

In the past a device referred to as a field vacuum extractor was used to create an enclosed volume over surfaces using a small disposable chamber, reduce the pressure, and then sample the headspace using a SPME fiber **[10]**. Although, the device was successfully used to collect organophosphorus compounds from glass surfaces, it is only functional for sampling surfaces.

We recently reported a new method whereby HSSPME sampling is taking place under reduced pressure conditions and the resulting procedure was termed vacuum-assisted headspace solid phase microextraction (Vac-HSSPME) **[11-13]**. In this article, we report on the use of Vac-HSSPME for the direct extraction of PAHs from solid matrices. The method was developed and evaluated for the quantitative extraction of polycyclic aromatic hydrocarbons (PAHs) from solid matrices. Overall, the proposed Vac-HSSPME method was found to improve the efficiency of the release of analytes from the matrix, facilitating the mass transfer into the headspace even when sampling under mild temperatures.

5.2. Materials and methods

5.2.1. Chemicals and Reagents

The PAHs used in these studies together with some of their physico-chemical properties are listed in Table 5.1. They were purchased from Sigma-Aldrich (Steinheim, Germany) at a purity of >98%. A 10 mg L^{-1} acetonitrile solution containing all target analyte, was used daily for the preparation of the spiked soil samples and was stored in the dark at 4 °C when not in use. Anthracene-d_{10}, was purchased from Supelco (Bellefonte, PA, USA) and was used as the internal standard. Deionized water used for sample preparation was prepared on a water purification system (Barnstead EASYpure II) supplied by Thermo Scientific (Dubuque, USA). All solvents (pesticide grade) and sodium chloride (>99.5%) were obtained from Merck (Darmstadt, Germany).

The sandy soil used here was obtained from Agios Onoufrios (Chania, Crete, Greece). Soils were first dried at 50 °C, then sieved with a 2 mm i.d. mesh to remove coarse

particles and debris and finally stored in a desiccator. HSSPME sampling of unspiked soil samples under both reduced and atmospheric conditions ensured that they were free of the target analytes. All spiked sand samples were freshly prepared according to the following procedure: a certain amount of soil (2 g unless otherwise stated in the text) was prepared by spiking appropriate amounts of diluted working standard solutions to the sand to get the preset final concentrations given in the text. The mixture was then left to equilibrate and then the solvent to fully evaporate. For calibration studies, sand samples were also spiked at 100 ng g^{-1} with the internal standard.

Table 5.1. Main physicochemical properties of the five PAHs compounds investigated here.

Compound	Molecular weight	Vapor pressure 25 °C (mm Hg)[a]	K_H (atm m^3 mol^{-1})[b]	$logK_{ow}$	Water solubility 25 °C (mg L^{-1})
Na	128.18	0.085	$4.4 \cdot 10^{-4}$	3.30	30
Ace	152.21	0.00215	$1.84 \cdot 10^{-4}$	3.92	4
Fl	166.22	0.0006	$9.62 \cdot 10^{-5}$	4.18	1.992
Phe	178.23	0.000121	$4.23 \cdot 10^{-5}$	4.46	1.6
Flu	202.26	$9.22 \cdot 10^{-6}$	$8.86 \cdot 10^{-6}$	5.16	0.265

[a] 1 mmHg = 133.322 Pa.
[b] 1 atm = $1.01 \cdot 10^5$ Pa.

5.2.2. Vac-HSSPME Procedure

A 40 mL custom – made gastight sample container, equipped with a high vacuum glass stopcock, an auxiliary gastight port equipped with a black propylene open – hole cap and septum and a glass port equipped with a half-hole cylindrical Thermogreen septum (Supelco, Bellefonte, PA) compatible with the needle of the SPME, was used in the present studies. For Vac-HSSPME, 2 g of spiked soil sample were introduced in the sample container together with a cylindrical Teflon-coated magnetic stir bar. The

extraction device was then was air-evacuated after connecting the high vacuum stopcock with the vacuum pump (7 mbar ultimate vacuum without gas ballast; Vacuubrand GmbH & Co. KG, Model MZ 2C NT, Wertheim, Germany). Upon air evacuation, the glass stopcock was closed, the vacuum pump was disconnected and 2 mL of deionized water were then introduced into the sample container through the Thermogreen septum with the help of a 10 mL gastight syringe (SGE, Australia). Based on previous investigations the water/soil ratio was kept at a minimum thus avoiding dilution of the target analytes in the liquid phase and consequently decrease in responses of PAHs **[14]**. It should be mentioned here that our preliminary studies confirmed that extraction was the same when the sample container was evacuated before or after water addition and that, after adding water, a 10 min agitation at 1400 rpm was sufficient to equilibrate with the headspace. Upon sample equilibration with the headspace, the needle of the SPME fiber/holder assembly (Supelco, Bellefonte, PA) was introduced into the sampling chamber by piercing the Thermogreen septum and HSSPME sampling was performed for a preset period of time (typically 30 min). The 100-µm PDMS SPME fiber (Supelco, Bellefonte, PA) was used for all extractions. When microextraction sampling was completed, the PDMS fiber was retracted and the SPME device was transferred to a gas chromatographer – mass spectrometer (GC-MS) for analysis. The pressure inside the modified vial was then equilibrated with atmospheric and the apparatus was emptied, washed and used for the next microextraction sampling. The Thermogreen septum was replaced daily to avoid pressure loss due to septum damage. All extractions were run at least in duplicates.

5.2.3. 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 270 °C, with the purge flow closed for 5 min. Helium (>99.999% pure) was used as the carrier gas at a 1.0 mL min^{-1} flow-rate. Separation was performed on a 30 m × 0.25 mm × 0.25 µm EquityTM-5 capillary column (Supelco, Bellefonte, PA). The column oven was programmed as follows: 75 °C

for 2 min, programmed to 150 °C at a rate of 25 °C min^{-1} and then held for 2 min, increased to 240 °C at a rate of 10 °C min^{-1} and then held for 2 min resulting at a total analysis time of 21 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 = 50–250. Analytes were quantified using a five-point external calibration curve obtained by analyzing mixtures of PAHs standards.

5.3. Results and discussion

5.3.1. Effect of extraction temperature and modifiers

In general, heating the sample to an elevated temperature provides energy for analyte molecules to overcome energy barriers which tie them to the matrix, enhances the mass transfer process, and increases the vapor pressure of the analyte **[9]**. The higher headspace concentrations and shorter equilibration times achieved when heating the sample may thus improve the sensitivity of HSSPME. However, while heating the sample is advantageous for releasing analytes from their matrix, it can adversely affect absorption of analytes by the fiber coating. This is because the absorption of analytes by the fiber is an exothermic process and at higher temperatures the distribution coefficients between the fiber and headspace decrease, ergo favoring the headspace phase **[9,14]**. There is usually an optimum temperature for HSSPME sampling, which, however, is often quite low, limiting thus the success of thermal desorption **[9]**.

According to the theoretical model we formulated in the past extraction kinetics for HSSPME may be further enhanced when combining the effects of temperature and reduced pressure **[11]**. However, whilst studying Vac-HSSPME sampling of aqueous samples we could not provide experimental confirmation of this theoretical prediction since high temperatures had an adverse effect on Vac-HSSPME **[13]**. It was assumed that increasing the temperature of the spiked aqueous samples greatly increased humidity in the headspace, thus affecting the fiber's characteristics and impairing mass loading of the target analytes **[13,15,16]**.

During the present investigations, the effect of temperature on Vac-HSSPME was initially studied using dry sandy soil samples. Figure 5.1 shows the results obtained after extracting the headspace of sand samples spiked at 200 ng g^{-1} with each target analyte at temperatures ranging from 25 to 100 °C. For comparison, extractions were performed under both vacuum and atmospheric pressure conditions. As seen, for almost all target analytes and at each sampling temperature, Vac-HSSPME resulted in higher sensitivity compared to regular HSSPME. During this set of experiments the absence of a substantial amount of water molecules in the headspace allowed recording the net effect of heating the sample at reduced pressure conditions on HSSPME sampling. This is an important finding, demonstrating that in Vac-HSSPME temperature and reduced pressure can be effectively combined to further enhance extraction kinetics.

A closer examination of the effect of temperature on Vac-HSSPME in Figure 5.1 reveals the optimum temperatures recorded. Accordingly, for Ace, Fl and Phe increasing the temperature up to 60 °C initially improved Vac-HSSPME and a further increase decreased mass loading. For Flu, heating the spiked sand sample up to 40 °C improved Vac-HSSPME recovery and after that the amount of Flu extracted by the fiber remained somewhat constant. This is an indication that thermal desorption had only limited success for the sandy matrix, and temperature could not fully release Flu to the headspace **[9]**. In the case of the more volatile compound examined here, Na, temperatures greater than 25 °C decreased mass loading. According to a previous report, the PDMS coating is not a perfect zero sink for Na **[17]** and the analyte is re-released to the surrounding gas phase when heating the sample above room temperature. Regarding the set of extractions run under regular pressure conditions it was necessary to heat the spiked sand samples at elevated temperatures to provide the necessary energy for analyte molecules to overcome energy barriers which tie them to the matrix **[9]**. For example, in the case of Flu, the least volatile compound studied here, heating the sample at temperatures above 60 °C was necessary for detecting the analyte

and the peak areas obtained above this temperature point were 3 to 4 times smaller than those obtained with Vac-HSSPME. Overall, the optimum sampling temperature recorded under normal pressure conditions was approximately 80 °C for almost all analytes studied here. However, Figure 5.1 clearly shows that at this sampling temperature analytes were only partially desorbed from the sample matrix when compared to Vac-HSSPME.

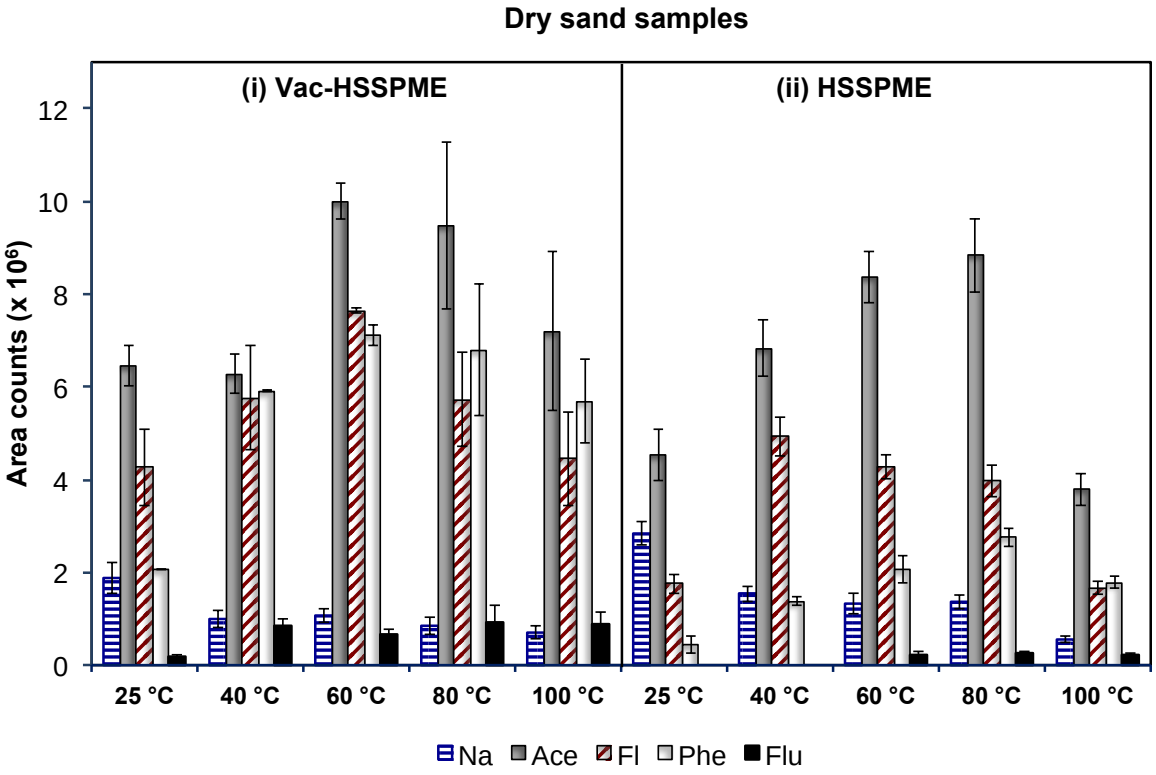

Figure 5.1. Effect of sampling temperature on the extraction of PAHs from dry sandy soil samples under (i) reduced (Vac-HSSPME) and (ii) atmospheric (HSSPME) pressure conditions. Other experimental conditions: 2 g sandy soil samples spiked at 200 ng g^{-1}; 30 min sampling time; 1400 rpm agitation speed; 40 mL sample container. Some error bars are too small to be visible as compared with the physical size of the symbol.

Several past studies reported that modifying the matrix, such as adding water to the soil sample, is a simple approach to promote the release of organic compounds into the headspace and as such improve sensitivity of the method **[8,9,14,18,19]**. In a subsequent set of experiments, the effect of water on extraction was investigated. HSSPME

sampling of 2 g of sand spiked at 200 ng g^{-1} with each target analyte in the presence (2 mL) or absence (0 mL) of water was performed under vacuum and regular pressure conditions. The results, summarized in Figure 5.2, clearly show that the presence of water dramatically increased the amount of analytes extracted by the fiber under both sampling pressure conditions. In fact, the percentage improvement in peak areas was similar for each target analyte most possibly indicating that the net effect of adding water to the system was the same whether working under reduced or regular pressure conditions. Nevertheless, with the exception of Na, sampling the headspace of a wet sand sample whilst using Vac-HSSPME resulted in a higher sensitivity when compared to regular HSSPME.

Based on the above results, it was decided to further investigate the effect of modifiers on Vac-HSSPME by adding water containing 5 or 10 % w/v NaCl to 2 g of spiked sandy soil samples prior to Vac-HSSPME sampling. The results (not shown here) revealed that for almost all analytes tested here, Vac-HSSPME was hardly dependent on the salt concentrations tested. The only exception was for Flu, the most hydrophobic analyte studied here, where sensitivity decreased with increasing amounts of NaCl (30 and 50% peak area decrease for 5 and 10% w:v NaCl content respectively when compared to the peak area obtained in the absence of salt). In general, electrolytes, such as salts, can change the compressibility of water, mainly because of the enhancement in the arrangement of the water molecules. In the presence of salts, hydrophobic compounds are "squeezed out" from the saline solution where water is more ordered and compressible thus decreasing their solubilities (salting out) **[20-22]**. The theory of the salt predicts an increase of the soil-water partition coefficient with increasing ionic strength for hydrophobic organic pollutants. Indeed, a previous report dealing with the effect of salting out on the desorption-resistance of PAHs in coastal sediment suggested that the distribution of naphthalene, phenanthrene and pyrene in sediment was affected by salinity and that the "salting out effect" contributed to the increase in the sorbed amount as well as desorption-resistance **[22]**. During headspace analysis of soil water

systems it is reasonable to assume that an increase in soil-water partition coefficient will reduce the amount of analyte present in the headspace available for extraction. Moreover, the salting out effect is more pronounced for polar analytes than non-polar ones **[18]** and for non-polar compounds like PAHs, the effect was found to depend on the degree of hydrophobic properties of the compounds **[20]**. The latter accounts for the fact that the presence of salt affected the extraction of the more hydrophobic Flu.

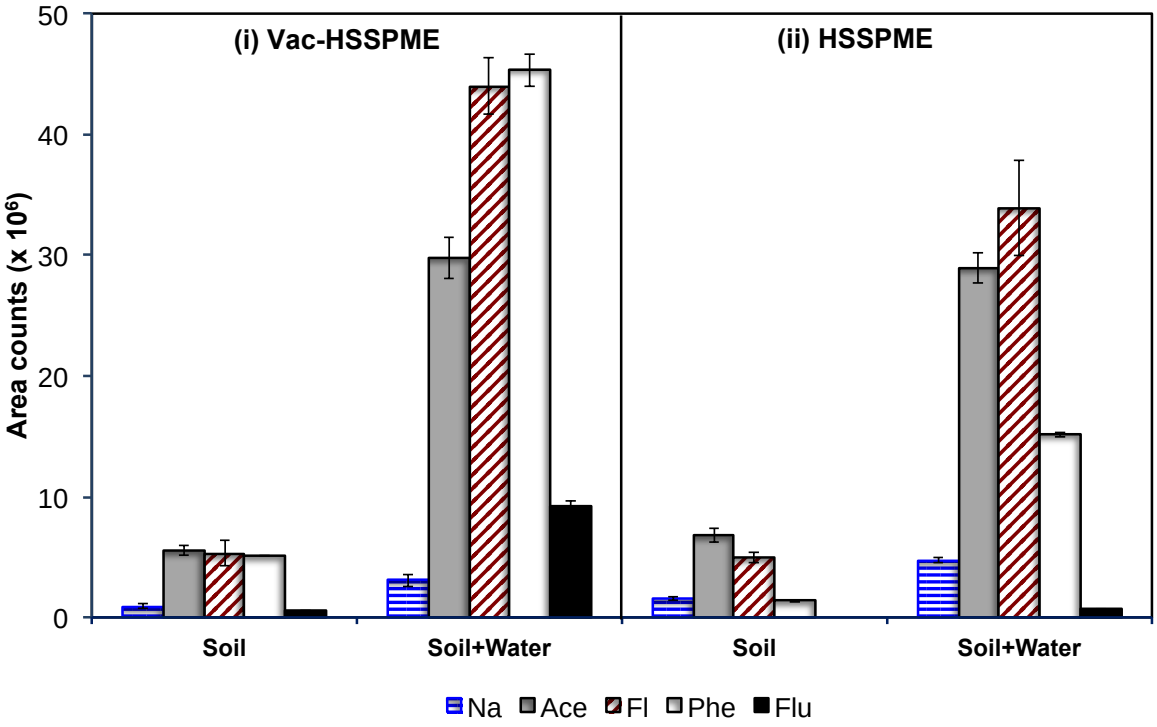

Figure 5.2. Responses obtained with HSSPME sampling in the absence (soil) and presence (soil+water) of 2 mL water under (i) reduced (Vac-HSSPME) and (ii) atmospheric (HSSPME) pressure conditions. Other experimental conditions: 2 g sandy soil samples spiked at 200 ng g^{-1}; 30 min sampling time; 25 °C sampling temperature; 1400 rpm agitation speed; 40 mL sample container. Some error bars are too small to be visible as compared with the physical size of the symbol.

Based on the positive effect of water alone on extraction, it was then decided to repeat the experiments dealing with the effect of temperature on Vac-HSSPME. This time 2 mL of water were added to the 2 g of spiked sand (wet sand samples) and extractions were

performed at sampling temperatures ranging from 25 to 80 °C. For comparison, HSSPME extractions were performed under both vacuum and atmospheric pressure conditions and the results are given in Figure 5.3. As seen, in the presence of 2 mL of water, increasing the sampling temperature had an adverse effect on Vac-HSSPME. In particular, heating the sample gradually decreased mass loading of the more volatile Na and Ace up to the point (approximately 60 °C) where Vac-HSSPME was found to be practically impaired. On the other hand, for Phe, increasing the temperature from 25 to 40 °C improved extraction; yet a further increase to 60 °C drastically restricted extraction. A similar trend was observed in the case of Flu where the optimum temperature recorded was 60 °C. During Vac-HSSPME the water vapor in the headspace is close to saturation. Increasing the sampling temperature increases the amount of water molecules available to partition with the PDMS fiber challenging mass loading of the analytes **[13,15,16]**. As expected **[8,23]**, for regular HSSPME, heating the sample improved extraction (Fig. 3) for all target analytes and with the exception of Na the optimum sampling temperature recorded when sampling under atmospheric pressure conditions was 60 °C. Nevertheless, maximum peak area values attained for almost all analytes with Vac-HSSPME (40 or 60 °C depending on the analyte) were higher than those recorded with regular HSSPME. The present findings are in agreement with our previous investigations dealing with Vac-HSSPME of aqueous samples where higher sampling temperatures were found to have an adverse effect on Vac-HSSPME. It should be mentioned here, that in the case of soil samples, the impact of heating the sample on mass loading was not so important most probably because of the lower headspace water content. Overall, high extraction efficiency and very good sensitivity under mild extraction conditions could be achieved with Vac-HSSPME and this is one of the most important features of the proposed method. Based on the above, 40 °C was selected as the optimum temperature for extraction.

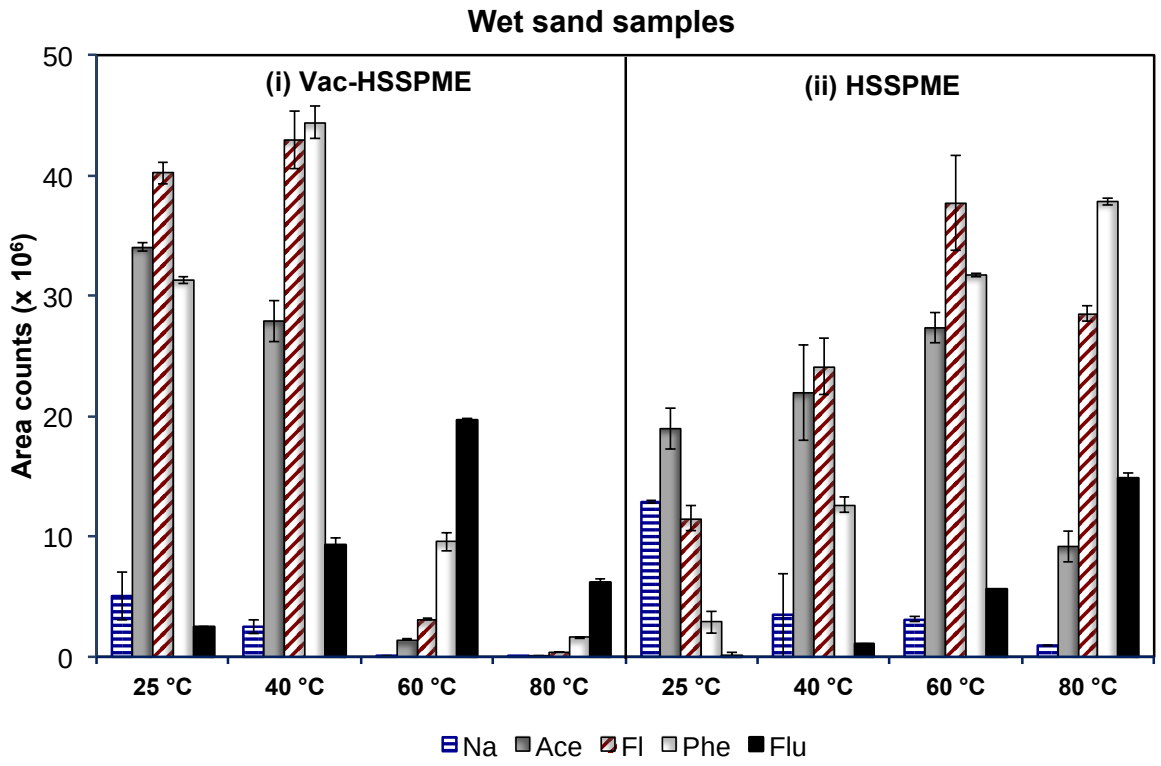

Figure 5.3. Effect of sampling temperature on the extraction of PAHs from wet sandy soil samples under (i) reduced (Vac-HSSPME) and (ii) atmospheric (HSSPME) pressure conditions. Other experimental conditions: 2 g sandy soil samples spiked at 200 ng g^{-1}; 2 mL of water; 30 min sampling time; 1400 rpm agitation speed; 40 mL sample container. Some error bars are too small to be visible as compared with the physical size of the symbol.

5.3.2. Effect of sampling time

Finally, the effect of sampling time upon Vac-HSSPME was investigated and the results are depicted in Figure 5.4. As can be seen, Na reaches equilibrium within the first 10 min. In the case of Ace and Fl, the extracted amounts increased with increasing extraction time, at a constant temperature, and reached a plateau when equilibrium was established (approximately 30 min of extraction). For Phe and Flu, the latter being the most hydrophobic and least volatile compound investigated here, equilibrium could not be attained even after sampling the headspace for 60 min. A 30 min was chosen as

optimum extraction time, providing sufficient extracted amounts within a relatively short assay time.

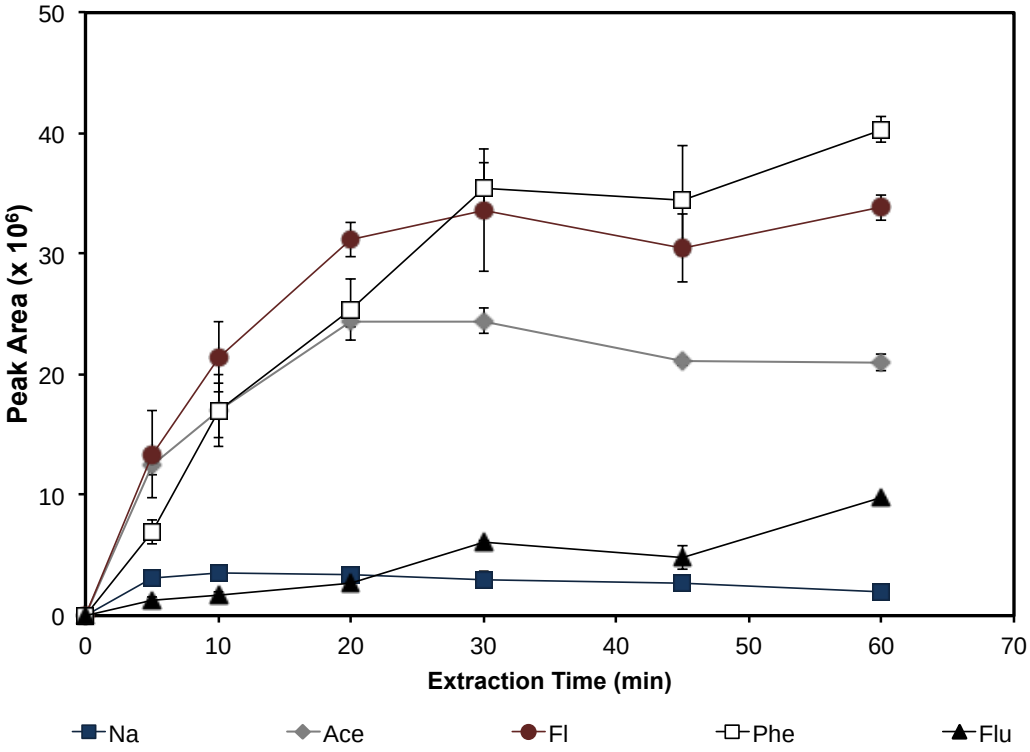

Figure 5.4. Extraction time profiles for all target PAHs obtained with Vac-HSSPME. Other experimental conditions: 2 g sandy soil samples spiked at 200 ng g^{-1}; 2 mL of water; 1400 rpm agitation speed; 40 mL sample container; 40 °C sampling temperature. Some error bars are too small to be visible as compared with the physical size of the symbol.

5.3.3. Validation of the method

The performance of the proposed method was evaluated using five concentration levels ranging from 1 to 400 ng g^{-1}. For all quantification experiments, sandy soils were also spiked with the internal standard. Target analytes were extracted using Vac-HSSPME under the optimized experimental conditions (2 g spiked sandy soil samples; 2 mL of deionized water, 30 min sampling time; 1400 rpm agitation speed; 40 °C sampling temperature). With the exception of Flu, the calculated calibration curves gave a high level of linearity (Table 5.2). The repeatability of the proposed method, expressed as

relative standard deviation (RSD), was evaluated by extracting five consecutive sand samples spiked at 100 ng g^{-1} with each target analyte and was found to range between 4.3 and 10 % (Table 5.2). The estimated limits of detection (LODs) for signal to noise ratio (S/N) equal to three were in the low ng g^{-1} ranging between 0.003 and 0.233 ng g^{-1}.

Table 5.2. Linearity, detection limits and repeatability obtained for PAHs with Vac-HSSPME.

Compound	Conc. Range (ng g^{-1})	r^2	LODs (ng g^{-1})	Repeatability [a] (% RSD)
Na	1 – 400	0.9975	0.029	10
Ace	1 – 400	0.9976	0.003	6.8
Fl	1 – 400	0.9954	0.056	4.3
Phe	1 – 400	0.9900	0.013	4.7
Flu	1 – 400	0.9478	0.233	8.4

[a] Spiking level 100 ng g^{-1}; n=5.

5.4. Conclusions

In this study, Vac-HSSPME was successfully applied to the analysis of sandy soil samples. Heating sand samples in the presence of a small amount of water was initially found to enhance extraction kinetics up to a certain temperature above which mass loading of the target analytes was found to be impaired due to the presence of water molecules in the headspace available to partition with the SPME fiber. On the other hand, heating the dry sandy soil samples during Vac-HSSPME was found to further enhance extraction kinetics. Nevertheless, the presence of water during Vac-HSSPME was found to be essential since it substantially promoted the release of organic compounds into the headspace and as such improved sensitivity of the Vac-HSSPME method. The results obtained with the proposed Vac-HSSPME method confirmed that

very good sensitivity and precision could be attained within short sampling times and under mild sampling temperatures, highlighting once again the most important advantages of using Vac-HSSPME.

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CHAPTER 6.

Conclusions-Future work

6.1. Conclusions

The present thesis investigated a new HSSPME procedure carried out under reduced pressure conditions, termed vacuum-assisted HSSPME (Vac-HSSPME). The proposed procedure ensured reproducible conditions for HSSPME and excluded the possibility of analyte losses. 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 in semivolatiles, whose evaporation rates are controlled by mass transfer resistance in the thin gas film adjacent to the sample/headspace interface.

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 (eg. lower temperatures). 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 were 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.

Moreover, the automation of Vac-HSSPME has been made possible by downsizing the sampling containers. To overcome problems associated with the small openings and the

orientation of the commercially available gas sampling chambers, custom-made glass sample containers having total volumes of 1000, 500, 40 and finally 22 mL were used as sampling chambers. The 22 and 40 mL modified sample vials offer ease in handling and significant analytical performance. This is the first work indicative of the automation potential, of such an efficient methodology, destined for routine laboratories.

Furthermore, Vac-HSSPME was successfully applied to the analysis of sandy soil samples. It was demonstrated that heating the dry sandy soil samples during Vac-HSSPME further enhanced extraction kinetics, while the presence of water during Vac-HSSPME was found to be essential and improved the sensitivity of the proposed method. Heating sand samples in the presence of a small amount of water enhanced extraction kinetics up to a certain temperature above which the presence of water molecules in the headspace was found to impair the partitioning of the target analytes with the SPME fiber. Overall, with Vac-HSSPME confirmed very good sensitivity and precision could be attained within short sampling times and under mild sampling temperatures.

6.2. Future work

The idea of down – porting the Vac – HSSPME device into a specially constructed gastight cap should be investigated in the future. This custom – made cap will offer the simplicity of one port – instead of three – for the introduction of sample matrixes (liquid and soil), facilitates in sample agitation, heating, and overall operation. The cost of construction of such a cap is expected to be very low and should fit to commercially available glass vials thus allowing to be used for a numerous routine extraction experiments.

Furthermore, the application of Vac – HSSPME on new classes of organic pollutants (PolyChlorinated Biphenyls, PCBs and Organophosphorous Pesticides, OPs) and more complex matrices (olive oil, honey) should be investigated. These matrices are highly

nutricious fatty food products and can be source of toxic substances, such as pesticide residues.

Finally, the effect of reduced pressure conditions on headspace LPME, should be investigated. To this end, sampling chamber should be redesigned to his will be possible. Overall, the capabilities and potential applications of this new, cost-effective and easy-to-use HSSPME approach need to be further explored.

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