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Parameters Affecting the Extraction of Polycyclic Aromatic Hydrocarbons with Pressurised Hot Water

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Academic Dissertation

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PREFACE

This thesis is based on experimental work carried out in the Laboratory of Analytical Chemistry of the Department of Chemistry, University of Helsinki, during the years 1998-2003.

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ABSTRACT

Pressurised hot water extraction (PHWE) exploits the unique temperature-dependent solvent properties of water. The use of harmful organic solvents is at the same time avoided. In addition to being an environmentally friendly extraction medium, water is cheap and easily available. The influence of different parameters on PHWE was examined in this work. The effects of temperature, pressure and extraction time have often been studied, but here the emphasis was on other parameters important for the extraction, most notably the dimensions of the extraction vessel and the stability and solubility of the analytes to be extracted.

First, pressurised hot water extraction (PHWE) was combined on-line with liquid chromatography–gas chromatography (LC-GC), and the system was applied to the extraction and analysis of polycyclic aromatic hydrocarbons (PAHs) in sediment. After the PAHs were extracted they were adsorbed into a solid-phase trap, which also worked as an LC column. The efficient on-line sample clean-up and concentration meant that GC with a flame ionisation detector (FID) could be applied for the final separation and detection. The method is of superior sensitivity compared with the traditional methods, and only a small 10 mg sample was required for analysis.

The commercial extraction vessels were replaced by laboratory-made stainless steel vessels because of some problems that arose. The performance of the laboratory-made vessels was comparable to that of the commercial ones. In an investigation of the effect of thermal desorption in PHWE, it was found that at lower temperatures (200ºC and 250ºC) the effect of thermal desorption is smaller than the effect of the solvating property of hot water. At 300ºC, however, thermal desorption is the main mechanism. With solid-phase trapping better recoveries were obtained when steam was used as extraction medium at 300ºC than when liquid water was used at the same temperature. In general, no clear trend was apparent for steam and liquid water. Sometimes the recoveries and repeatabilities were better with steam, sometimes with liquid water.

The effect of the geometry of the extraction vessel on recoveries was studied with five specially constructed extraction vessels. In addition to the extraction vessel geometry, the sediment packing style and the direction of water flow through the vessel were investigated. The results were studied statistically by one-way analysis of variance (ANOVA). The geometry of the vessel was found to have only minor effect on the recoveries, and the same was true of the sediment packing style and the direction of water flow through the vessel. These are good results because these parameters do not have to be carefully optimised before the start of extractions.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) were compared as trapping techniques for PHWE. In general, more optimisation is required in SPE than in LLE. LLE was also more robust than SPE and it provided better recoveries and repeatabilities than did SPE. Problems related to blocking of the Tenax trap and unrepeatable trapping of the analytes were encountered in SPE. Thus, although LLE is more labour intensive, it can be recommended over SPE.

The stabilities of the PAHs in aqueous solutions were measured using a batch-type reaction vessel. Degradation was observed at 300ºC even with the shortest heating time. Ketones and quinones and other oxidation products were observed. Although the conditions of the stability studies differed considerably from the extraction conditions in PHWE, the results indicate that the risk of analyte degradation must be taken into account, at least when PHWE is carried out in static mode.

The aqueous solubilities of acenaphthene, anthracene and pyrene were measured, first below and then above the melting point of the analytes. Measurements below the melting point were made to check that the equipment was working, and the results were compared with those obtained earlier with similar equipment. Good agreement was found between the measured and literature values. A new saturation cell was constructed for the solubility measurements above the melting point of the analytes because the flow-through saturation cell could not be used above the melting point. An exponential relationship was found between the solubilities measured for pyrene and anthracene and temperature.

Non-linear data analysis and self-organising maps were employed in the data analysis to obtain correlations between the parameters studied, recoveries and relative errors. The data analysis showed some clear relations in the data. For example, the larger relative errors in solid-phase collection than in liquid collection in PHWE and in thermal desorption than in pressurised hot water extraction were easily recognised and visualised.

LIST OF ORIGINAL PAPERS

The dissertation is based on the following five publications, hereafter referred to by their Roman numerals (**I-V**). In two of the publications, self-organising maps and non-linear data analysis were applied to elucidate correlations within the data (**II,III**).

I. Hyötyläinen, T., Andersson, T., Hartonen, K., Kuosmanen, K. and Riekkola, M.-L., reproduced with permission from *Anal. Chem. 72* (2000) 3070-3076. "Pressurized Hot Water Extraction Coupled On-line with LC-GC: Determination of Polyaromatic Hydrocarbons in Sediment". Copyright 2000 American Chemical Society.

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III. Andersson, T., Pihtsalmi, T., Hartonen, K., Hyötyläinen, T. and Riekkola, M.-L., reproduced with permission from *Anal. Bioanal. Chem. 376* (2003) 1081-1088. "Effect of Extraction Vessel Geometry and Flow Homogeneity on Recoveries of Polycyclic Aromatic Hydrocarbons in Pressurised Hot Water Extraction". Copyright 2003 Springer-Verlag.

IV. Andersson, T., Hartonen, K., Hyötyläinen, T. and Riekkola, M.-L., reproduced with permission from *Analyst 128* (2003) 150-155. "Stability of Polycyclic Aromatic Hydrocarbons in Pressurised Hot Water". Copyright 2003 The Royal Society of Chemistry.

V. Andersson, T.A., Hartonen, K.M. and Riekkola, M.-L., reproduced with permission from *J. Chem. Eng. Data 50* (2005) 1177-1183. "Solubility of Acenaphthene, Anthracene, and Pyrene in Water At 50°C to 300°C". Copyright 2005 American Chemical Society.

ABBREVIATIONS AND SYMBOLS

ASE	accelerated solvent extraction
BTEX	benzene, toluene, ethylbenzene and xylene
DMAE	dynamic microwave assisted extraction
DPTMDS	diphenyltetramethyldisilazane
EC-1	certified reference sediment
EI	electron impact ionisation
EPA	U.S. Environmental Protection Agency
FID	flame ionisation detector
GC	gas chromatography
HF	hollow fibre
HPLC	high performance liquid chromatography
i.d.	inner diameter
ISTD	internal standard
LC	liquid chromatography
LC–GC	on-line coupled liquid chromatography–gas chromatography
LLE	liquid−liquid extraction
LOQ	limit of quantification
MAE	microwave assisted extraction
MMLLE	microporous membrane liquid–liquid extraction
MS	mass spectrometry
NPLC	normal-phase liquid chromatography
o.d.	outer diameter
OTT	open tubular trap
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCSE	partially concurrent solvent evaporation
PEEK	poly(etheretherketone)
PHW	pressurised hot water
PHWE	pressurised hot water extraction
PHWO	pressurised hot water oxidation
PFE	pressurised fluid extraction
PLE	pressurised liquid extraction
RPLC	reversed-phase liquid chromatography
RSD	relative standard deviation
SCWO	supercritical water oxidation
SD	standard deviation
SETOC	International Sediment Exchange for Tests on Organic Contaminants
SFE	supercritical fluid extraction
SIM	selected ion monitoring
SOM	self-organising maps
SPE	solid-phase extraction
SPME	solid-phase microextraction
SVE	solvent vapour exit

SWC	superheated/subcritical water chromatography
Tenax	2,6-diphenyl-1,4-phenylene oxide polymer
TIC	total ion chromatogram
UV	ultra violet
c	concentration
d_c	critical density (kg m^{-3})
δ	solubility parameter (Hildebrand unit H, 1 H = 1 (cal cm^{-3})$^{1/2}$, 1 cal = 4.19 J)
EA	electron affinity (eV)
ε	relative permittivity (dielectric constant)
H_{exp}	experimental Henry's law constant (Pa * m^3 mol^{-1})
ΔH_f	enthalpy of formation (kJ mol^{-1})
log K_{ow}	octanol–water partition coefficient
μ	electric dipole moment (C m, 1 debye unit D = $3.33564*10^{-30}$ C m)
P	pressure (bar, 1 bar = 100 000 Pa)
P_c	critical pressure (bar)
log S	logarithm of solubility (g m^{-3})
T	temperature (ºC, 0ºC= 273.15 K)
T_c	critical temperature (ºC)
x_2	mole fraction solubility

1. INTRODUCTION

Extraction is an analytical procedure in which analytes are removed from a matrix and transferred to an extraction medium. The analytes are then analysed by an appropriate technique. Many factors affect the extraction and need to be optimised for maximal recoveries. Although extractions can be carried out off-line, on-line coupling with the final technique is becoming increasingly popular. The traditional extraction techniques, such as liquid–liquid extraction (LLE), Soxhlet extraction and sonication, are error-prone and often consume large quantities of harmful organic solvents. With the amount of hazardous waste needing to be decreased worldwide, the focus today is on automated and environmentally friendly techniques that utilise a minimal amount of organic solvent, or no organic solvent at all. Such techniques include solid-phase microextraction (SPME) and membrane extraction techniques for liquid samples and supercritical fluid extraction (SFE) and pressurised hot water extraction (PHWE) for solid samples.

The use of hot water as an extraction medium has a long history, but the temperatures applied have usually been below 100ºC at atmospheric pressure. At these low temperatures only polar compounds are extracted, owing to the high relative permittivity of water at low temperatures. The use of supercritical water as an extraction solvent and as a reaction medium is increasing rapidly because it has both low relative permittivity (ε=1-25) and good solvating properties for relatively non-polar compounds. A serious drawback in the use of supercritical water is the requirement for high temperature (>374°C) and pressure (>221 bar). In addition, the use of supercritical water is limited by its potential reactivity and corrosivity. The stainless steel used in conventional extraction apparatus is easily corroded, and special and much more expensive materials (Hastelloy, Inconel) are needed. Fortunately, water at lower temperatures (150-300ºC) and pressures can be effectively applied in the extraction of low polarity organic compounds like PAHs and polychlorinated biphenyls (PCBs).

Pressurised hot water extraction (PHWE), also called subcritical water extraction or hot water extraction, has been applied in full scale since 1994. Both polar and non-polar analytes can be successfully extracted. Temperature is the key parameter in PHWE, because change in the temperature of water affects the relative permittivity and thereby the polarity and solvent properties. The polarity of the analytes to be extracted affects the choice of extraction temperature. At 100ºC or slightly above, the relative permittivity of water is high and polar analytes like phenols are extracted, while at higher temperatures, where water has a substantially lower relative permittivity, even low polarity analytes like PAHs and alkanes are extracted. Good selectivity is more easily obtained in PHWE than in many traditional extraction techniques with organic solvents as extraction medium, and thus cleaner extracts with fewer interfering compounds are obtained. On the other hand, increase in the extraction temperature increases the amount of matrix compounds, and at temperatures exceeding 300°C the amount of co-extracted matrix compounds may disturb the extraction. PHWE is environmentally friendly because no organic solvents are needed for the extraction. This feature alone makes PHWE an appealing extraction technique meeting today's requirements for "green chemistry".

The aim of the present work was to study the feasibility of the on-line combination of PHWE with LC-GC (**I**) and, after that, to explore in detail parameters affecting the performance of PHWE (**II-V**), with the emphasis on the extraction. Samples were sediment polluted with polycyclic aromatic hydrocarbons. In on-line coupled PHWE-LC-GC only drying and homogenisation were needed for the preparation of samples; no laborious manual sample clean-up, fractionation or concentration was required. And the whole analysis, including the extraction, could be done in a closed system with minimal contamination and errors. Although a drying step was not necessary, it facilitated the extraction. The effects of temperature, pressure, extraction time and flow-rate on the recoveries in pressurised hot water extraction have been extensively studied, but many other parameters have not been studied. Thus, further aims of this work were to explore the role of thermal desorption in PHWE (**II**) and the effect of the geometry of the extraction vessel on recoveries (**III**).

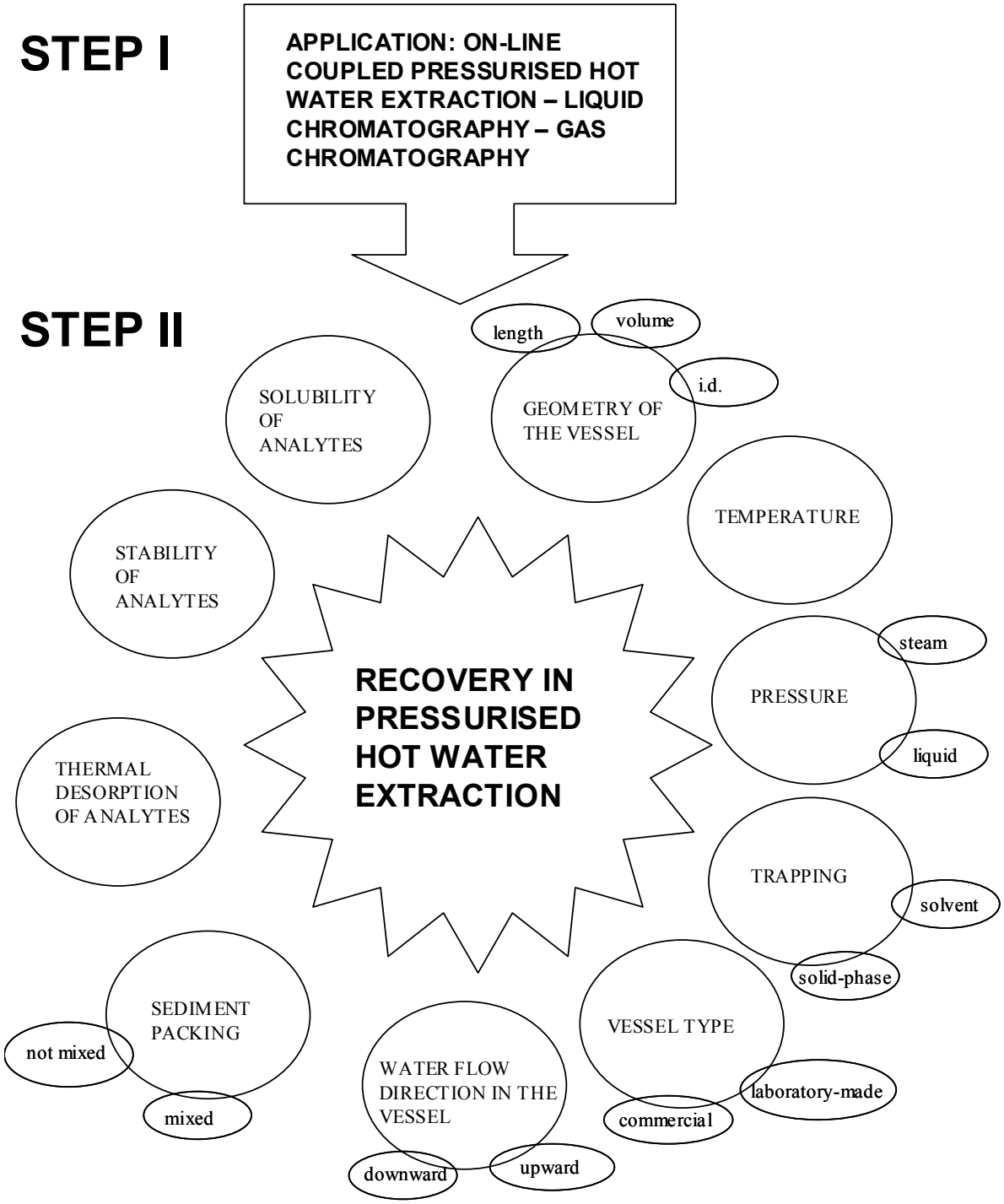
Analytes are exposed to high temperatures in pressurised hot water extraction. Degradation of some thermolabile analytes, mainly in food and plant matrices, has been observed, but also the degradation of compounds such as PAHs and dioxins, which are considered stable. Degradation is a particular risk in static PHWE, where the analytes are exposed to high temperatures for a longer time. In view of this, study was made of the possible degradation of PAHs at the temperatures applied in PHWE (**IV**). Finally, study was made of analyte solubility. Although the solubilities of many organic compounds are known at room temperature and slightly above, little solubility data exists for higher temperatures and especially above the melting point of analytes. However, the solubility of a compound is strongly dependent on temperature, and knowing the solubilities is important in understanding PHWE and industrial processes and reactions where hot water is involved. The aqueous solubilities of selected PAHs were measured at the temperatures typically applied in PHWE (**V**).

2. AIMS OF THE STUDY

More specifically the aims of the study were the following:

- To couple PHWE on-line to LC-GC and apply this system to the analysis of PAHs in sediment samples using flame ionisation detection (FID) (**I**)
- To extract PAHs in sediment samples using PHWE (**I-III**)
- To study the applicability of laboratory-made extraction vessels in PHWE (**II,III**)
- To investigate the thermal desorption of PAHs with nitrogen (**II**)
- To compare recoveries obtained with liquid water and steam in PHWE (**II,III**)
- To investigate the effect of the geometry of the extraction vessel on the recoveries (**III**)
- To compare the applicabilities of solid-phase (**II**) and liquid-phase (**III**) trapping in PHWE
- To study the stability of selected PAHs (**IV**)
- To measure the aqueous solubilities of selected PAHs both below and above the melting point of PAHs (**V**)
- To apply self-organising maps and non-linear data analysis to elucidate correlations within the data (**II,III**)

The aims of the study and the different parameters examined are described in the following scheme.



Chapters 3-8 provide the necessary background to the research. Chapter 3 looks at the analytes investigated, and Chapters 4-6 describe the relevant extraction and LC–GC techniques. The important question of analyte degradation is considered in Chapter 7 and the aqueous solubility of analytes in Chapter 8. Chapter 9 summarises the experimental details, and Chapter 10 the results presented in the five appended publications.

3. POLYCYCLIC AROMATIC HYDROCARBONS

The analytes investigated in this work were polycyclic aromatic hydrocarbons (PAHs). Also known as polynuclear aromatic hydrocarbons and polyarenes, PAHs are harmful compounds and many of them are toxic, carcinogenic and mutagenic [1]. Many aspects of the properties, extraction, analysis and distribution of PAHs in different matrices have been studied [2]. PAHs contain two or more benzenoid groups and are formed as a result of incomplete combustion in both natural and anthropogenic processes. Natural sources include volcanoes and forest fires, while typical anthropogenic sources are wood burning, automobile exhaust, industrial power generators and incinerators [3]. The main sources of PAHs are industry and traffic emissions. PAHs are present everywhere in the environment — in water, air and soil and also in humans. The fate of PAHs in the environment is primarily controlled by their physicochemical properties. Understanding of their transport, partitioning and transformation processes in the environment is of fundamental importance. PAHs are highly lipophilic compounds owing to their non-polarity, and they tend to accumulate in the lipid tissues of living organisms, such as fish and humans. Although the levels of PAHs are usually low, their bioaccumulation can lead to concentration levels that are toxic for living organisms. PAHs require metabolic activation to exert carcinogenic or mutagenic effects. In general, they are resistant to degradation and thus are persistent in the environment, but they can also undergo chemical and photochemical transformation to other compounds some of them more harmful than the parent compounds. Several hetero-PAHs, especially certain nitrogen containing PAHs, are toxic, mutagenic and carcinogenic [1]. Nitrogen-containing PAHs may be formed in some conditions, where the PAHs react with nitric acid.

PAHs can be classified as alternant and nonalternant. Alternant PAHs contain only six-membered rings, while non-alternant PAHs contain both six- and odd-membered rings [3]. PAHs are also non-planar and planar PAHs. Non-planar PAHs are more carcinogenic and mutagenic, while planar PAHs are more stable, less reactive and biologically less toxic. Benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene and dibenzo[a,h]anthracene are carcinogenic. PAHs containing more than three rings are by far the most potent carcinogens [1] and they are easily adsorbed on particles and dust in the atmosphere. Because PAHs are widespread in the environment and many of them are highly toxic, the U.S. Environmental Protection Agency (EPA) has classified 16 of them as priority pollutants. Monitoring of PAHs is therefore important.

PAHs are often determined by a chromatographic technique because they are a large group of compounds with similar characteristics [4]. Since they are easily adsorbed to the surfaces during sampling and storage, this has to be taken into account in their analysis. Some PAHs are sensitive to light and this, too, must be considered.

Usually, analytical methods have focused on the determination of total PAH concentrations. However, when organic compounds age in the soil they become progressively less bioavailable and this immobility makes them less harmful to the environment. For this reason it is, in fact, more important to know the fraction of PAH or other contaminant that is available to organisms and transport processes, because only this fraction is relevant [5,6]. If

total instead of bioavailable concentrations of PAHs are measured, the risk from environmental pollutants is easily overestimated [7]. Hydrophobic pollutants like PAHs are less bioavailable in soils where the organic content is high, as the soil organic material acts as a sink for hydrophobic pollutants. The major problem in estimating the reduction of bioavailability caused by aging is that bioavailability differs for the same compound in different soils and for different compounds in the same soil.

4. EXTRACTION

Extraction always involves a chemical mass transfer from one phase to another. The principles of extraction are used to advantage in everyday life, in as simple a task as making coffee, for example. Extractions in analytical chemistry can be classified as exhaustive or non-exhaustive. LLE, SPE, SFE and Soxhlet extraction are exhaustive, or quantitative techniques, whereas many membrane techniques, headspace extraction and SPME are non-exhaustive, and recoveries are less than quantitative [8]. Extractions can be carried out in dynamic or static mode.

The ideal extraction system should be automatic, rapid, quantitative, precise, simple, economical, non-hazardous to both operator and environment, selective, analyte concentrating and nondegrading of the analytes [9]. Quantitativity and selectivity are not easily obtained at the same time and often a compromise must be found, especially if several analytes are to be determined simultaneously. Thus, the extraction system has to fulfil several requirements, and it depends on the analytes and the application which extraction technique is best suited for the purpose.

4.1. Extraction of environmental solid samples

Organic pollutants, like PAHs, have traditionally been extracted from environmental solids with organic solvents, with or without heating. Sample preparation is usually needed before extraction. Desorption, diffusion and solvation of the analytes from the matrix to the extractant need to be as easy as possible and, accordingly, the solid sample is usually ground and homogenised before the extraction. Depending on the technique, the sample to be extracted may also need to be dried. A dry sample is more homogeneous and more easily quantitated than a wet sample. Drying is not needed in PHWE because water is applied as extractant.

The first stage in any extraction is to remove the analyte from the matrix. The stages related to the removal of an analyte from a solid sample matrix are illustrated in Figure 1 [10]. Since not all analytes are located in the particle core, not all stages presented in Figure 1 are required in their extraction. The interactions between the analyte and the matrix are destroyed in the extraction process and new interactions between the analyte and the extractant take their place. The matrix affects the extraction, and optimisation of the extraction must be done according to matrix type. After the analyte has diffused into the bulk of the extractant, the

extraction proceeds similarly to chromatographic elution, but with the important difference that the matrix (i.e. the column packing) is well characterized in chromatography, whereas in extraction it is largely unknown. The physicochemical properties of the matrix have an effect on the extraction, and the extraction may need to be optimised separately for each matrix. The extracting phase applied to release the analytes from the matrix can be liquid, gas or supercritical fluid, with or without modifiers. External energy is often applied to facilitate the extraction. Thermal or electrical energy or ultrasound is in common use.

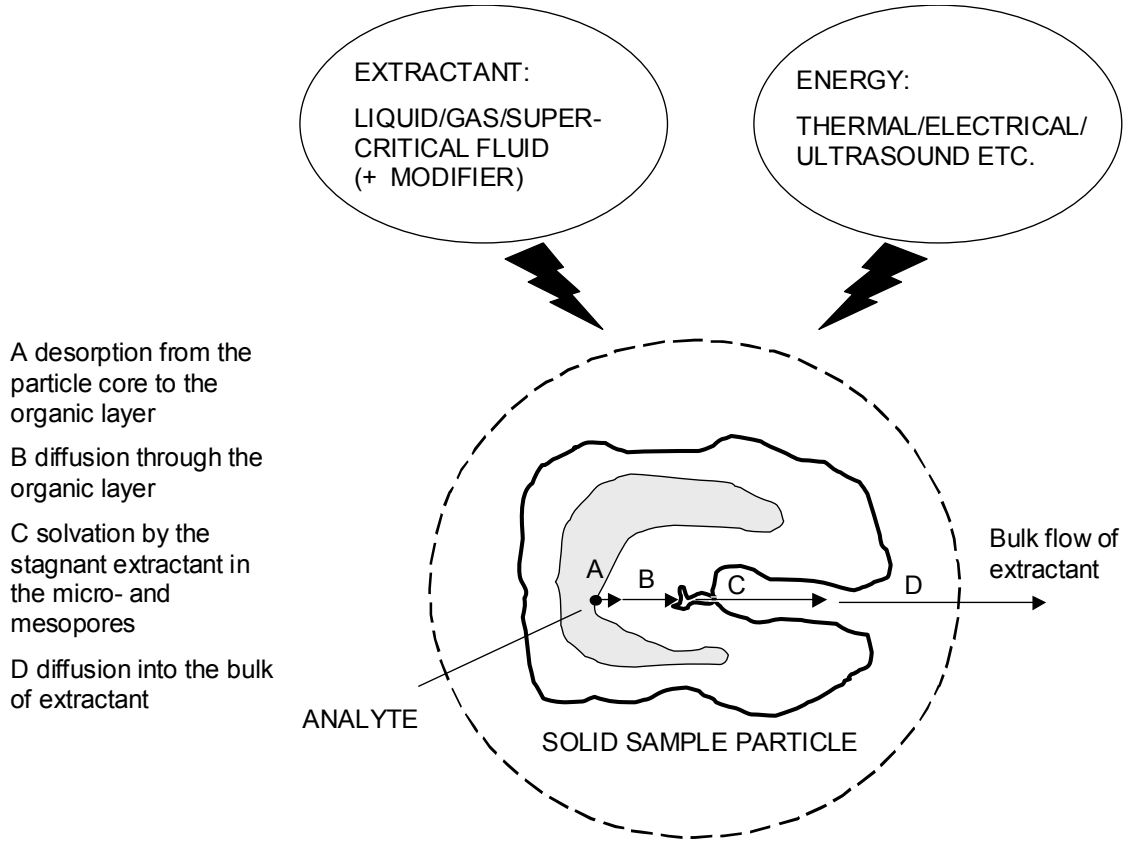


Figure 1. Stages in the extraction of a solid sample [10].

Soils and sediments, which are not the same, are the most commonly extracted environmental samples [11]. According to definition, soil is a variable mixture of minerals, organic matter and water, capable of supporting plant life on the Earth´s crust. Most of the soil consists of inorganic matter, and about 5% of soil is organic matter. The most important organic compound in soil is humus, which binds metals and, owing to its acid-base character, acts as a buffer in soil. Sediment in turn, is the relatively finely divided matter covering the bottoms of rivers, streams, lakes, reservoirs, bays, estuaries, and oceans. The composition of sediment can vary from pure inorganic mineral matter to predominantly organic matter. In this work, sediment samples were extracted and analysed.

Extraction of solid samples is never straightforward. As an example of this, laboratories have obtained widely varying results for PAHs in the certified reference sediment EC-1 because of the systematic errors between the laboratories [12]. Despite the different values, the mean value between the laboratories was nevertheless a good estimate of the true value.

4.2. Extractions with water; physicochemical properties of water

With the current emphasis on environmentally friendly chemistry, water is a highly attractive extractant. Water has many unique properties, and these properties are temperature and pressure dependent [13,14]. Strong hydrogen bonds join water molecules at ambient conditions, but unlike other hydrogen-bonding liquids, the hydrogen bonds in water form a three-dimensional network. This is the source of some of the unique characteristics of water. At ambient conditions the relative permittivity of water is high, as is its viscosity and the ability to solvate ionic and polar compounds. When the temperature is increased, the relative permittivity and viscosity of water drop, and the three-dimensional network of hydrogen bonds gradually breaks up. Ionic and polar compounds are no longer soluble in water, and non-polar compounds become soluble instead. The concentrations of H^+ and OH^- ions are much higher in the vicinity of the critical point of water than at ambient conditions due to the dissociation of water, and this enables different reactions because the dissolved ions can freely form ion-pairs. Just below the critical point of water [T_c= 374.0°C, P_c= 220.6 bar (1 bar = 10^5 Pa) and d_c= 322 kg/m^3], the relative permittivity of steam is close to unity, and that of liquid water can reach a value below ten depending on the pressure. It needs to be emphasized that, above the critical point of water, the dissociation constant of water decreases rapidly diminishing markedly the amount of ionic species.

The changes in relative permittivity, viscosity, fugacity, density, internal energy and the solubility parameter of water as a function of temperature at selected pressures are depicted in Figure 2 a-f. The changes tend to be sharper at low pressures and smoother at high pressures. The most abrupt change in the parameters is related to a change in state, from steam to liquid water and from liquid water to supercritical fluid, or vice versa. The relative permittivity of water decreases dramatically as the temperature is increased as is illustrated in Figure 2 a. Viscosity (Figure 2 b) and density (Figure 2 d) decrease as the temperature increases. At constant temperature, increase in pressure is accompanied by increased viscosity and density. Fugacity (Figure 2 c) is a measure of the tendency of a substance to escape by some chemical process from the phase in which it exists. Usually the term fugacity is applied to gases, and it reflects the tendency of a gas to expand or escape. Fugacity assumes high values at high temperature and pressure. Internal energy (Figure 2 e) refers to the total energy of all the particles in a sample, and it increases with temperature and decreases with pressure. The solubility parameter (Figure 2 f), which is a measure of the intermolecular forces in a pure substance, decreases as a function of temperature. Solvents with similar solubility parameters are miscible.

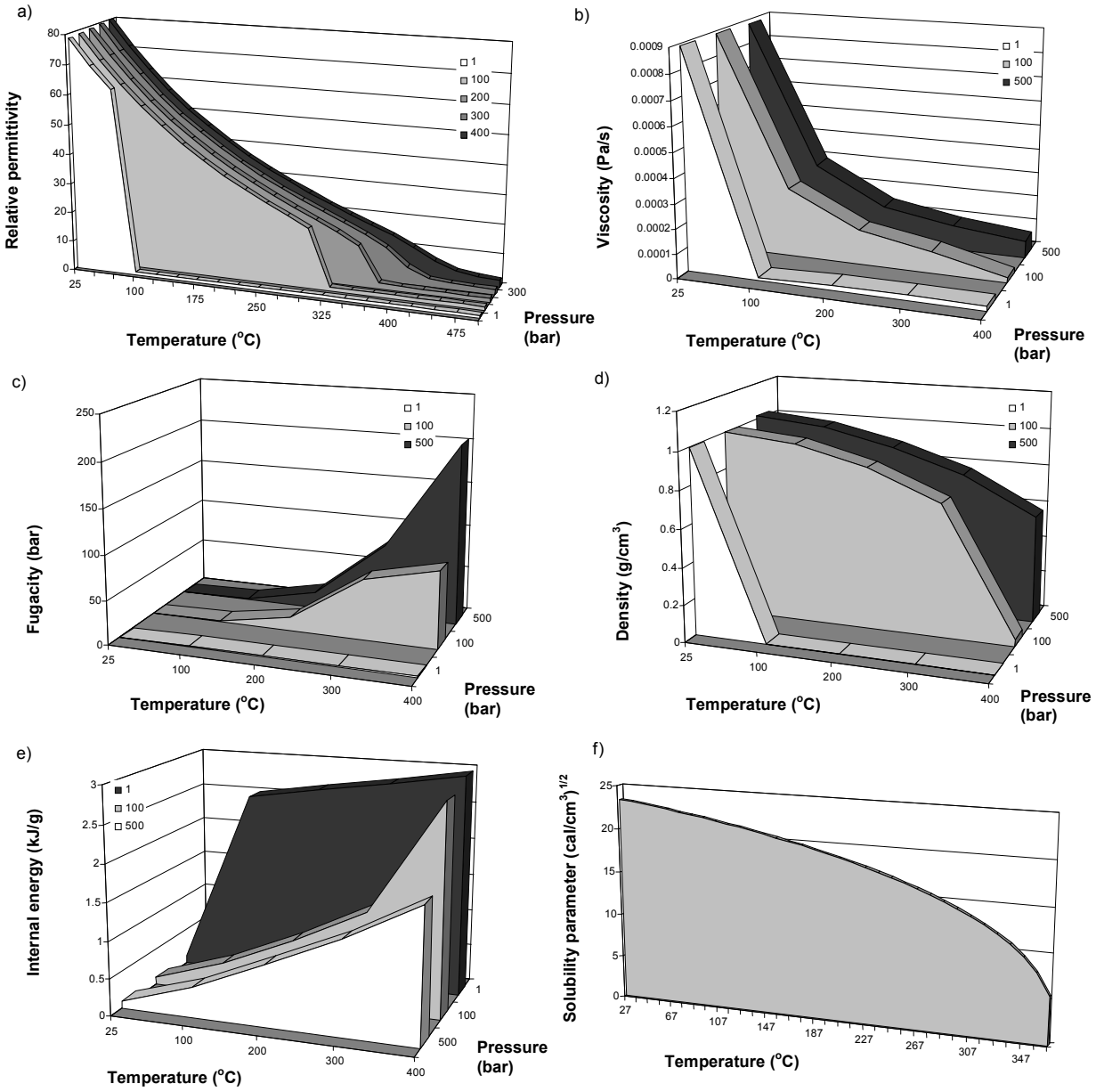


Figure 2. Properties of water as a function of temperature (ºC) at selected pressures (bar). a) Relative permittivity, b) viscosity, c) fugacity, d) density, e) internal energy and f) solubility parameter.

Ionic, hydrogen bonding, dipole-dipole, induction and dispersion forces are important when water is used as extractant. The presence of these forces between different types of molecules is noted in Table 1.

Table 1. Intermolecular forces with water as solvent.

Force	Type of atom/ molecule/ compound	Temperature dependence of the interaction
Ionic	Ionic compounds	Small
Hydrogen bonding	Polar molecules, where nitrogen, oxygen or fluorine atoms are linked by a hydrogen atom	Great
Dipole-dipole	Polar molecules with permanent dipole moments	Great
Induction	Non-polar compounds	Small
Dispersion (London force)	All atoms and molecules	Small

These physical parameters (Figure 2) and intermolecular forces (Table 1) have an effect on solvation, a process in which the solvent molecules form a layer around the solute molecules. The chemical similarity between solute and solvent facilitates this solvation process. The factors that are most significant in solvation are dipole moments (μ), polarities (α), capability to form hydrogen bonds, and sizes of the molecules [15]. The solubility parameter, which can be calculated from the other parameters, is another important indicator of chemical behaviour. The solvation of an analyte is essential in all extractions, for if the analyte is not solvated in the extraction fluid it will not be extracted.

5. PRESSURISED HOT WATER EXTRACTION

The principles of green chemistry are critically important today, and pressurised hot water extraction, as a "solventless" extraction technique fulfils the criteria of green chemistry well. According to definition, green chemistry involves approaches that reduce or eliminate the use or generation of hazardous substances in the design, manufacture and application of chemical products [16]. The first principle of green chemistry is that it is better to prevent waste than to treat or clean it up after it is formed.

Pressurised hot water extraction, also called subcritical water extraction (SWE), hot water extraction, superheated water extraction, high-temperature water extraction or hot liquid water extraction, was of interest in this work as an environmentally friendly, green technique [17,18,19]. Water is cheap, non-toxic, readily available and easily disposed of, and its properties can be tuned simply by adjusting the temperature of the water. The temperature in pressurised hot water extraction varies between the boiling point of water (100°C) and the critical temperature of water (374.0°C), although the term PHWE and its synonyms have sometimes been erroneously applied to extractions below 100°C as well. The pressure is usually kept high enough that water exists in the liquid state. The pressure needed to keep the water in liquid state is only 5 bar at 150°C and 86 bar at 300°C. Also steam can be applied in the extractions.

At ambient conditions the relative permittivity, or dielectric constant, of water is high, about 80. Under these conditions water is a polar solvent and it can be applied for the extraction of polar compounds because "like dissolves like". The relative permittivity of water drops when

the temperature is increased with sufficient pressure to maintain the liquid state. At 250°C (pressure 50 bar), for example, the relative permittivity of water is only about 27, which is comparable to the relative permittivity of ethanol at 25°C and 10 bar [20]. Consequently, water at 250°C and above can be applied for the extraction of non-polar analytes. If thermally unstable analytes are to be extracted and high extraction temperatures cannot be applied, it is advisable to use a co-solvent, such as ethanol or urea, to reduce the hydrogen bond density of water so that the extractions can be performed at lower temperatures [21].

It is worth noting that pressurised hot water and supercritical water have found diverse applications in other areas than extraction. Both have been exploited in the destruction of harmful organic compounds [22], and pressurised hot water has been used as an environmentally friendly HPLC eluent [23,24].

5.1. Equipment

No commercial PHWE equipment is available, but the apparatus is easy to construct in the laboratory. PHWE equipment resembles commercial SFE and accelerated solvent extraction (ASE) equipment, which are restricted, however, to temperatures of 200°C or below. Since polymeric PEEK material cannot be applied at temperatures above 200°C, Vespel (graphitised carbon) and stainless steel seals are often used in PHWE equipment. Although PHWE is usually carried out in dynamic mode with flow-through extraction vessels, it can also be carried out in static mode. In static mode a sufficient headspace has to be left in the vessel for safety reasons unless the equipment is designed for both static and dynamic extraction and it is possible to control the pressure during heating (before closing the vessel for static extraction). Static and dynamic modes can also be combined in a single extraction [25]. The integral parts of a dynamic PHWE unit are the following: pump/pumps, extraction vessel, oven for the heating of the extraction vessel, pressure restrictor and sample collection system. One of the pumps is employed for pumping the extractant water and another pump, if needed, is employed for pumping the collection solvent and flushing the tubings. A pressure restrictor is needed to maintain the appropriate pressure in the equipment. This could be a micro-metering valve. The basic construction of the PHWE equipment is presented in Figure 3. Usually it is constructed of stainless steel. Although conventional stainless steel (316L) is suitable for most applications, if temperatures are very high or the water is modified, for example with acids or other corrosive chemicals, it is advisable to find more resistant materials, such as Hastelloy or Inconel. Unlike conventional stainless steel, which is mostly made of iron, Hastelloy and Inconel consist mainly of nickel and chromium. A preheating coil is normally mounted in front of the extraction vessel. The purpose of this preheating coil is to heat the water to the extraction temperature before it enters the extraction vessel. Likewise, after the oven, it is advisable to install a cooling coil to cool the water. The cooling coil can be inserted into a water bath or an ice bath. It is important to rinse the cooling coil with solvent after the extraction in order to collect the deposited analytes quantitatively. It has been found that an average 3% of PAHs in PHWE was left in the cooling coil in front of the trap when the cooling coil was not washed with the solvent after the extraction [26].

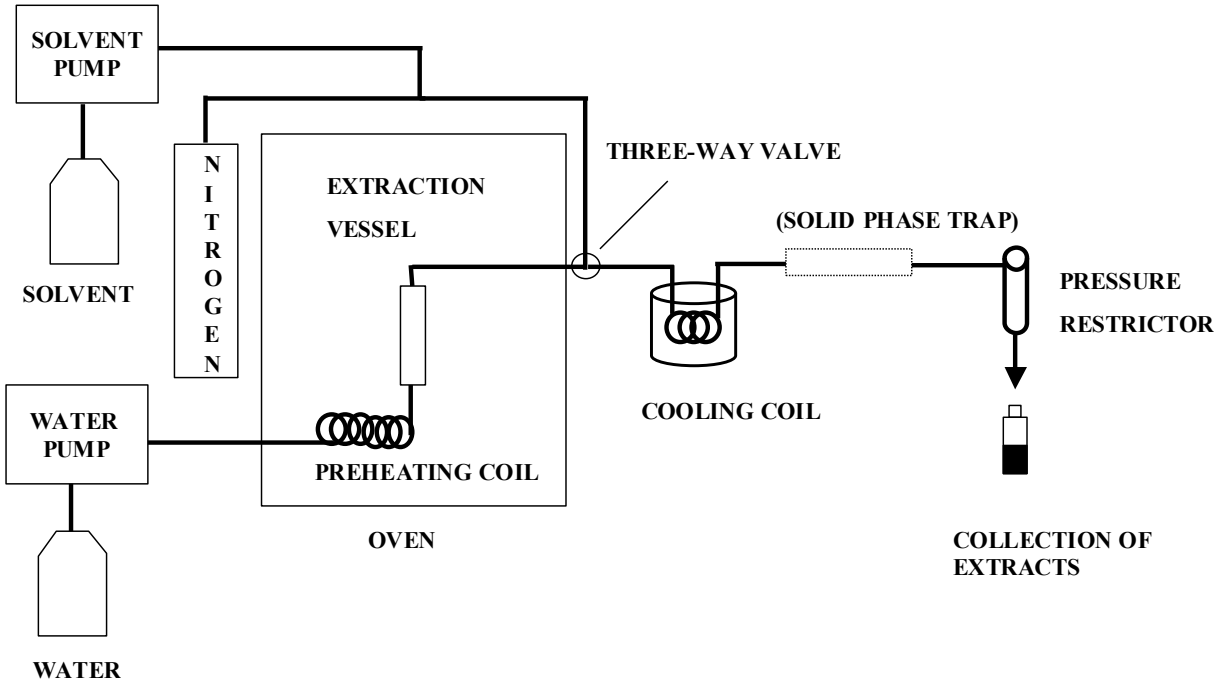


Figure 3. PHWE equipment.

There are several ways to collect the analytes from PHWE. Solid-phase trapping [27] and collection in organic solvent [28] are applied in most cases. Analytes can also be collected by microporous membrane liquid–liquid extraction (MMLLE) [29], hollow fibre microporous membrane liquid–liquid extraction (HF-MMLLE) [30] or anion exchange discs [31]. A study of the suitability of SPE, LLE, flat sheet membranes and hollow fibre membranes for trapping in PHWE showed that the choice of system depends on the application. However, if on-line coupling with chromatography is desired, LLE cannot be used [32]. Solid-phase microextraction (SPME) [33] and stirbar sorptive extraction (SBSE) [34] have also been applied with PHWE. If PHWE is coupled to LC [35], GC [29,30] or LC-GC [36], trapping of the analytes is done on-line with one of the techniques mentioned above (but not LLE). For fast screening purposes with high sample throughput, PHWE has been coupled with enzyme immunoassay [37]. Also, capillary electrophoresis coupled to mass spectrometric detection has been applied in the analysis of PHWE extracts [38]. If destruction of compounds is desired, as in soil remediation, it is possible to couple PHWE on-line with supercritical water oxidation [39,40].

5.2. Parameters affecting the extraction

The most important parameter affecting extractions in PHWE is temperature because a change in the temperature of water changes its relative permittivity and thus its solvent properties. When the temperature of water is increased, the surface tension and viscosity of the water are reduced, while diffusion and thermal desorption of the analytes are increased.

Increase in temperature works to disrupt the solute-matrix interactions and increases the capacity of solvents to solubilise the analytes. These factors, and the improved mass transfer, enhance the recoveries. Relatively low temperatures are sufficient for quantitative extraction of polar compounds, but high temperatures (250-300ºC) are required when hydrophobic organic compounds such as PAHs are extracted. It is not practical, however, to apply very high extraction temperatures (much over 300ºC) due to instrumental problems like corrosion and leakage. The analytes may also degrade or otherwise react at high temperatures. The selectivity in the extraction is often lost at high temperatures, and substantial amounts of matrix compounds and disturbing compounds are extracted along with the target analytes. This may lead to blockages and pressure increase inside the equipment, and the error in the analysis increases. Additional clean-up of the extract may also be needed. Degradation of the analytes and coelution of matrix compounds in analysis is a particular problem when food and plant materials are extracted, and it is often then advisable to use the lowest possible extraction temperature.

Unlike in SFE, pressure does not have a marked effect on recovery in PHWE [41]. As long as the physical state of the water is not changed, the effect of pressure on the recovery is small. In PHWE the pressure needs to be high enough that water exists in liquid state, or if extractions are carried out in steam, that the steam does not condence low enough. In practice, the backpressure of the equipment places a lower limit on the pressure in PHWE. Because the relative permittivity of water increases with pressure, however, a large pressure increase may decrease the recoveries of non-polar compounds. Steam has proved to be more effective than liquid water in the extraction of non-polar organic compounds, for two reasons [42,43]. First, the relative permittivity of steam is lower than that of liquid water at the same temperature, and lower relative permittivity favours the extraction of non-polar compounds. Secondly, steam spreads more uniformly through the sample in the extraction vessel because of its lower viscosity, and it diffuses more effectively than liquid water, and thus undesired channelling leading to lower recoveries is avoided. On the other hand, the capacity of steam to dissolve analytes is lower due to the lower density. Thermal desorption is dominant with steam.

Flow-rate will affect the recovery in PHWE if the extraction is solubility restricted, as it is when compounds have low aqueous solubilities. In that case an increase in the flow-rate increases the recoveries within a certain limit [28].

5.3. Comparison with other extraction methods

Table 2 shows a comparison of PHWE with Soxhlet extraction and three other instrumental techniques. Extraction techniques can be compared in terms of recovery, extraction time, compound class selectivity or selectivity against the sample matrix. Nowthat environmental aspects have assumed importance, solvent consumption and solvent type are also of important consideration, as is the energy needed for the extraction. PHWE is clearly an environmentally friendly extraction technique because it utilises only water as extractant.

Supercritical fluid extraction is also environmentally friendly, at least if unmodified carbon dioxide is used as the extraction fluid.

The extraction time is about the same for all the instrumental extraction techniques included in Table 2, whereas Soxhlet extraction can take as long as two or three days. Soxhlet also requires large amounts of organic solvents, and the most volatile compounds are lost during the extraction. Nowadays automated Soxhlet equipment is available.

The extraction methods based on organic solvent (Soxhlet, PLE and MAE) show poor selectivity for compound classes, and the same is true of SFE. Although selectivity may be achieved through careful selection of solvent and solvent modification, usually the solvent is selected so that quantitative extraction is obtained. In contrast to this, PHWE offers excellent selectivity for a compound class and, with sequential increase in temperature during the extraction, it can be employed for selective extraction of different compound classes, as has been done in the extraction of phenols, benzene, toluene, ethylbenzene and xylene (BTEX), PAHs and alkanes from the same sample [44]. The selectivities of the different extraction methods for the matrix compounds can be seen in the colour of the extracts. Nonselective extraction methods relying on organic solvents (Soxhlet, PLE and MAE) often give dark and turbid extracts because many matrix components are co-extracted. PHWE and SFE extracts are of lighter colour and clearer and contain less matrix interferences, though at temperatures exceeding 300ºC many matrix components may also be extracted in PHWE.

The environmentally friendly extraction methods, SFE and PHWE, offer complementary information. PHWE is more selective than SFE and is well suited for samples containing analytes with a wide range of polarities. SFE is more suitable than PHWE for the most thermolabile and non-polar compounds because of the degradation occurring in the harsh extraction conditions needed in PHWE to achieve quantitative extraction of non-polar analytes. Comparison of PHWE using steam and SFE using a modifier showed similar recoveries for PAHs from sediment [42]. Total recoveries of PAHs from a soil sample by Soxhlet, PLE, SFE and PHWE were almost the same. PHWE gave the highest recoveries for low-molecular-mass PAHs, and PLE the best recoveries for high-molecular-mass PAHs [45].

Hydrodistillation (not included in Table 2) has commonly been used in the extraction of essential oils from plant material. It turns out, however, that PHWE provides a more valuable essential oil with higher contents of oxygenated compounds, and it is quicker and cheaper than hydrodistillation and should therefore be preferred [46].

The benefits and disadvantages of each technique are also listed in Table 2. Among the benefits of PHWE are that no harmful organic solvents are needed and selectivity for the analytes is high. Although PHWE equipment is not commercially available, this is not particularly serious because the equipment can easily be constructed in the laboratory.

Table 2. Comparison of Soxhlet extraction, PHWE, supercritical fluid extraction (SFE), pressurised liquid extraction (PLE) and microwave assisted extraction (MAE) [45,47,48].

	Traditional technique	**Instrumental extraction techniques**			
Extraction technique	**Soxhlet**	**PHWE**	**SFE**	**PLE =ASE**	**MAE**
Typical extraction time	4-48 h	5-30 min	30-90 min	12-20 min	30-60 min
Typical solvent	Acetone-hexane, acetone-dichloromethane, dichloromethane, toluene, methanol	Water	CO_2/CO_2+modifier	Acetone-hexane, acetone-dichloromethane	Acetone-hexane
Typical solvent consumption (ml)	300	- /a few millilitres for elution of analytes	8-50/no solvent needed in on-line SFE-GC	15-40	25-50
Selectivity for compound classes	Non-selective	Selective	Slightly selective	Non-selective	Non-selective
Selectivity for sample matrix	Some selectivity	Selective	Selective	Non-selective	Non-selective
Benefits	Simple well-known procedure, easy to carry out, cheap equipment, also automated	No organic solvent needed, wet samples can be extracted without drying	No or little organic solvent needed, also automated	Fully automated	Generally 14 vessels extracted simultaneously, also automated
Disadvantages	Time-consuming, a lot of manual work, large consumption of organic solvent	No commercial equipment available (ASE, SFE and MAE equipment applicable in some cases), high temperature	Expensive equipment, need for modifiers when CO_2 the extractant	Expensive equipment, blockages, frequent need for additional clean-up	Need for additional clean-up to remove the matrix

5.4. Applications

Since its appearance, pressurised hot water extraction has found many applications in the laboratory. One of the first applications was in 1984, when coal and glucose were extracted both below and above the critical point of water [49]. Samples extracted by PHWE are almost always in solid state, though there are exceptions — oil samples, for example [50]. The applications of PHWE can be roughly divided into two categories, namely the extraction of organic pollutants from soils and sediments and the extraction of flavours, fragrances, pesticide residues and other compounds from plant material [51,52] and food [52]. PHWE is an interesting alternative for the isolation of compounds in plants. Useful compounds, like antioxidants, can then be further incorporated in food because collection is in non-toxic water rather than organic solvent.

PHWE has been applied to the extraction of PAHs (Table 3), PCBs [43,53], pesticides [54,55], herbicides [56,57] and brominated flame retardants [36] from soil and sediment. Essential oils have been extracted from laurel [25], oregano [58], clove [27] and the traditional Chinese medicine *Fructus amomi* [59]. Furthermore, antioxidative compounds have been extracted from sage [60] and roots of *Morinda citrifolia* [61] and iridoid glycosides from *Veronica longifolia* leaves [62]. In the extraction of food, cholesterol has been extracted from solid food (bread, sweets, chips) [63], atrazine from beef kidney [64], and organochlorine pesticides and chlorobenzenes from fruit and vegetables [34]. PHWE has also been applied to the analysis of industrial chemicals such as alquilbenzene sulfonates [65] and a mixture of fluorescent whitening agents and Azo dyes [66]. Recently, interesting matrices including wood [67,68], paper [66], process dust [69] recycled tires [70], squid waste [71], winery by-products [72] and compost [73] have been analysed with unmodified or modified pressurised hot water.

Where polar analytes like phenols are extracted, low temperatures (100ºC or slightly above) can be applied, but for the highly non-polar analytes such as PAHs and alkanes, temperatures of 300°C or more are required. Sometimes pressurised hot water has been modified in order to extract the analytes more effectively at lower temperatures. Low extraction temperatures are often essential in the extraction of plant material and food because the analytes tend to be thermolabile, and co-extraction and degradation of the matrix are serious problems at high temperatures. To avoid excessive temperatures, small amounts of organic solvents have been added to the water, as when berberine, baicalein and glycyrrhizin were extracted from medicinal plants [74]. Nitric acid has been added to water in demetalisation of soils [75] and in extraction of cadmium and lead from plant material [76]. Surfactants, such as sodium dodecyl sulphate (SDS), have been added to facilitate the extraction by increasing the solubility of low polarity analytes [77]. Some additives may impair the environmentally friendly status of PHWE, and extra care is needed in choosing a non-hazardous additive.

Table 3. PHWE applications for the analysis of PAHs.

Matrix	Pretreatment	Temperature (°C)/pressure (bar)	Recovery (%)	Extraction time (min)	Analysis method	Observations	Ref
Environ-mental solids (soil, air particulates)	-	250 / 50	100-187 (soil)/ 57-112 (air particulates)	15	GC-FID/ GC-MS		[28]
Environ-mental solids (soil, sediment, air particulates)	Air-drying, sifting, homogenisation	250 / 40	> 90 soil/ > 75 sediment/ > 69 air particulates	60	GC-FID, GC-MS	Static extraction with styrene-divinylbenzene extraction discs	[78]
Municipal solid waste compost	Mixing, air-drying, milling, sifting	150 / 5 (calculated)	55-106	20	GC-MS/ HPLC	Static extraction applying C-18 resin mixture	[79]
Soil	Sifting, mixing	275 / 100	> 95	35	GC-FID/ GC-MS	Remediation of soil	[80]
Soil	Air-drying, sifting	150 / 50	73.6-110.4	15 (static) + 10 (dynamic)	HPLC with fluorescence detection	Static-dynamic extraction, water modified with sodium dodecyl sulphate (SDS)	[77]
Sand and soil	Not specified	200 / 400	≥ 82	10	HPLC/ GC-FID	Extraction coupled to HPLC	[35]
Urban air particulates and spiked sand	Not specified	250-300 / 50	> 70	15 (dynamic), 120 (static)	SPME-GC	Both static and dynamic extraction applied	[81]
Soil and sediment	Freeze-drying, homogenisation	250 / 50	18.2-163.0 (calculated)	30	Enzyme im-munoassay	PAH screening	[37]
Environ-mental solids	Sifting, mixing	250 or 300 / 100	-	30, 60	GC-FID/ GC-MS	Comparison of Soxhlet extraction, PLE, SFE and PHWE	[45]
Soil	Air-drying	175 / 50	≥ 60 (calculated)	120 or 140	GC-FID/ GC-MS	Comparison of SFE and PHWE	[82]
Sediment and spiked sand	Not specified	300 / 39 (steam)	48.7-104	30	GC-MS	Comparison of PHWE and SFE	[42]
Soil	Sifting, homogenisation, air-drying, grinding	300 / 10 (steam)	-	50	GC-FID/ GC-MS	On-line coupled PHWE-MMLLE-GC	[29]
Soil and sediment	Sifting, homogenisation, air-drying, grinding	300 / 8 (steam)	> 92 for sediment sample (compared with Setoc values)	30	GC-FID/ GC-MS	On-line coupled PHWE-HF-MMLLE-GC	[30]
Soil and sediment	Air-drying, sifting, grinding	300 / 9 (steam)	> 95 (LLE), > 65 (FS-MMLLE), > 50 (HF-MMLLE), > 83 (SPE), recoveries vs. Soxhlet	30	GC-MS, size exclusion chromato-graphy	Comparison of trapping methods for PHWE	[32]

Table 3, continued

Spiked sea sand	-	300 / ~300	> 55	40	GC-MS	PHWE coupled with SCWO	[39]
Sand and soil	Sifting, homogenisation, air-drying, grinding	300 / ~300	42.3-84.7 (spiked sand)	20	GC-MS	PHWE coupled with SCWO	[40]
Soil	Air-drying, homogenisation	300 / -		20	GC-MS	PHWE coupled with SCWO	[83]
Soil	Drying, milling, autoclaving	250 / 400-450 psig	79-99 (spiked soil)	Samples taken hourly for four hours	GC-FID	Static extraction	[84]
Environ-mental solids	Air-drying, grinding *etc.*	200 (static) and 225 (dynamic)/ -	~100	15-45	HPLC with fluorescence detection	Static-dynamic extraction, water modified with sodium dodecyl sulphate (SDS)	[85]
Environ-mental solids	Air-drying, sieving, grinding *etc.*	200 / -	~100	4x15 min static cycles	HPLC with fluorescence detection	Static extraction, water modified with sodium dodecyl sulphate (SDS)	[86]
Spiked sand	-	200 / ~50	-	50	HPLC (UV/VIS)	Extraction and aqueous HPLC coupled on-line	[87]
Environ-mental solids (soil and air particulates)	Sifting	250 / ≤ 40	~60-140	60	SPME-GC-MS	Static extraction	[88]
Soil and sediment	Freeze-drying, homogenisation, sifting	300 or 350 / 200	-	20	GC-MS	Samples cleaned and fractionated in a silica gel column before GC-MS	[89]

As can be seen from Table 3, the matrices in PHWE of PAHs have been, with few exceptions, very similar: sand, soil or sediment. Sample pretreatment typically consists of drying, grinding and sifting. Temperatures giving quantitative extraction (250-300 ºC) have been applied in the extractions, but the extraction behaviour and kinetics for PAHs have also been studied at lower temperatures. The pressure has usually varied between 50 and 100 bar and the extraction time between 15 and 30 minutes. Longer extraction times have been applied in studies of the kinetics of the extraction and when the extractions were performed in a static mode, or when non-exhaustive membrane extraction technique was connected to PHWE. GC-MS has proved to be suitable for the analysis of PAHs in PHWE extracts because the concentrations of PAHs are often low and the identification of PAHs from among many other compounds in the sample may otherwise be difficult. The sensitivity and selectivity provided by LC may not be sufficient for the determination of trace amounts of organic pollutants, but the application of fluorescence detection makes the identification and quantitation easier.

6. LIQUID CHROMATOGRAPHY-GAS CHROMATOGRAPHY

In on-line coupled liquid chromatography–gas chromatography (LC-GC), the analysis technique employed in this work, a liquid chromatograph is coupled to a gas chromatograph using a special interface. The interface depends on the application. LC is applied for sample clean-up, preconcentration and fractionation, and the final separation is carried out in a gas chromatograph with high resolution. Manual sample clean-up and fractionation can usually be totally avoided. The whole analysis is carried out in a closed system, and thus the contamination is minimised, as are the analyte losses by evaporation. The analysis is also reliable and repeatable. The sensitivity is high in LC-GC because the whole sample fraction is introduced to the GC. This is in contrast to the traditional off-line sample clean-up and fractionation techniques like liquid–liquid extraction and solid-phase extraction, where usually only a small fraction of the sample is introduced for analysis, unless the sample is concentrated into a small volume before analysis [90].

Injection volumes in GC are typically only a few microlitres, whereas the volumes transferred from LC to GC may be several hundred microlitres. The larger the internal diameter of the LC column the larger are the transferred fractions. Special techniques are needed when large solvent volumes are introduced to the GC. An uncoated column —also called a retention gap — and a retaining precolumn are often installed to protect the GC separation column and to be able to utilize the solvent effects and obtain sharp peaks. It is also common practice to install a solvent vapour exit in front of the separation column to protect the detector from an excessive amount of solvent and to be able to evaporate the solvent rapidly. The technical aspects of LC-GC have been extensively studied by Konrad Grob and his group [91].

On-line coupled LC-GC has many applications. The technique is most suitable for the analysis of biological samples [92], fuels [93], environmental samples [36], food [94] and other complex samples where analytes are present in trace amounts.

A new trend in LC-GC is comprehensive two-dimensional LC×GC, which means that all the fractions eluting from the LC are analysed by GC [95]. The peak capacity of this kind of system is high and overlapping peaks are seldom a problem. The comprehensive LC×GC system requires careful optimisation. Moreover, the GC detector has to be fast to handle all the data collected and time-of-flight (ToF) mass spectrometry has thus often been applied. Besides being fast, ToF MS offers deconvolution options and a fairly wide mass range.

6.1. Normal-phase and reversed-phase liquid chromatography in LC-GC

There are two fundamentally different modes of LC-GC: normal-phase liquid chromatography–gas chromatography (NPLC-GC) and reversed-phase liquid chromatography–gas chromatography (RPLC-GC). The coupling of NPLC to GC is relatively simple because the non-polar to weakly polar solvents used in NPLC are

compatible with GC. The coupling of RPLC to GC is considerably more complicated because of the high-polarity solvents like water and methanol typical in RPLC. Water destroys the deactivation of conventional retention gaps, and the evaporation of water in GC is difficult due to the high boiling point and to the high surface tension of water, which leads to poor wettability of the capillary wall. Direct and indirect solutions are available to overcome the problems caused by water in RPLC-GC. The direct solutions rely on special retention gaps [96], micro-LC [97], and the use of loop-type or vaporiser interfaces. In indirect solutions the analytes are extracted on-line from water into some organic solvent before transfer of the analyte fraction to GC. The on-line extraction techniques that can be applied include LLE, SPE, MMLLE and open tubular traps (OTT).

6.2. Interfaces and evaporation techniques

LC and GC can be coupled in a variety of ways. The volatility of the analytes determines the choice of the interface, and the interface determines how the solvent is evaporated. The first LC-GC interface was modified from a GC autosampler [98], but sensitivity was poor. Other common LC-GC interfaces are on-column, loop-type and vaporiser interfaces [99]. For volatile analytes, a retention gap technique — conventional retention gap technique or partially concurrent solvent evaporation technique — with on-column interface is appropriate. For analytes with high boiling points, a loop-type interface and concurrent solvent evaporation technique can be applied. The optimisation of transfer conditions is easier with a loop-type than an on-column interface. Various vaporiser interfaces and techniques, like the programmable temperature vaporiser (PTV) are available, and these are most suitable for medium and less volatile analytes. The vaporiser interface is particularly useful for dirty and aqueous samples.

6.3. On-column interface with partially concurrent solvent evaporation

When analytes are volatile and thermolabile, an on-column interface with retention gap technique is suitable. The essential feature of the retention gap techniques is that at least part of the eluent forms a solvent film on the wall of the precolumn during the transfer and traps the volatile analytes. In the conventional retention gap technique, the temperature of the GC oven is well below the boiling point of the eluent and the solvent film on the wall of the pre-column is long. In the partially concurrent solvent evaporation (PCSE) technique, the oven temperature is only slightly below the boiling point of the eluent. Although the solvent film is short, it still allows trapping of the volatile analytes in the solvent film. The optimisation of PCSE is demanding: the factors requiring optimisation are the temperature of the GC oven during transfer, the eluent flow-rate from the LC and the evaporation rate of the solvent. The amount of eluent evaporated during the transfer is commonly 50-90%. When the boiling point of the analytes is lower than 100ºC, the optimisation of the transfer is critical so that the analytes are not lost with the solvent vapours. The closure time of the SVE can be defined by the so-called flame method [100], but that method gives only the average solvent evaporation

rate. In a more exact method, two thermocouples are attached to the precolumn and the movement of the solvent front is monitored directly through measurement of the temperature change of the outer wall of the column [101]. As solvent evaporates the temperature of the capillary wall decreases.

An on-column interface suitable for retention gap techniques is shown in Figure 4. In the on-column interface, the analyte fraction is transferred from LC to GC through a thin fused silica capillary mounted permanently through the septa to the on-column injector of the GC. The transfer technique allows the amount of the LC fraction to be adjusted.

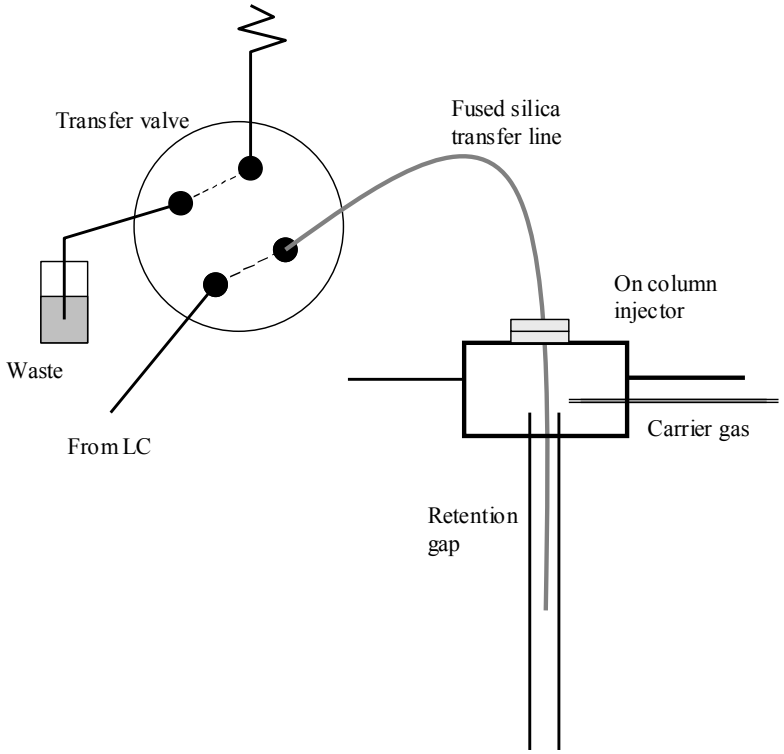


Figure 4. On-column interface and transfer valve for connecting LC and GC.

6.4. Coupling of extraction on-line to chromatography

Many extraction techniques can be coupled on-line to chromatography: for example, SPE, SPME, SFE, LLE, MMLLE, PHWE, dynamic microwave assisted extraction (DMAE) and dialysis [102]. The choice depends, among other things, on the state of the sample. The benefits obtained when the extraction is coupled on-line to chromatography are similar to those obtained when LC is coupled on-line to GC. Contamination and analyte losses are prevented and the sensitivity of the analysis is improved. Automation of the whole system is also possible. Although optimisation of the system is fairly demanding and time-consuming, it is well worth the trouble when analytes are present in small amounts in complex matrices [29,36,103]. A particular benefit when extraction is coupled on-line to LC-GC is that practically no manual sample pretreatment is needed. Thus, the only manual pretreatment needed for soil and sediment samples is drying and homogenisation.

PHWE has been on-line coupled to GC, LC [104] and LC-GC [36]. On-line coupling of PHWE to GC usually requires a cleaning step after PHWE because the extracts tend to be dirty, and non-volatile matrix compounds cannot be introduced to the GC. On-line membrane extraction with flat sheet membrane [29] or hollow fibre membrane [30] offers an efficient cleaning step between PHWE and GC. The separation efficiency and sensitivity of LC alone is often not sufficient for the anlysis of trace compounds and it may be more useful to connect the extraction to GC or LC-GC. The volumes of extracts are often hundreds of microlitres, whereas the volumes normally injected to GC are only 1-2 µl. Special interfaces and solvent evaporation techniques are needed when large-volume extracts are injected to GC (Section 6.2.).

7. DEGRADATION OF ORGANIC COMPOUNDS

Degradation of organic compounds occurs in response to light, elevated temperature and microbial action. Some degradation processes occur in the absence of oxygen and some in the presence of oxygen. In the aqueous environment, the extent of the degradation depends not only on temperature but also on pressure, type of compound, possible catalysts present, and so on. If quantitative extraction is the goal, as in quantitative analysis, then degradation of the analytes is not desired and steps need to be taken to ensure that it does not occur.

Sometimes, degradation of compounds, as well as degradation of matrix compounds, can be used to advantage, as when supercritical water oxidation (SCWO) and pressurised hot water oxidation (PHWO) are utilised in the destruction of harmful organics to carbon dioxide and water [22]. SCWO is carried out in water at temperatures above 374.0ºC and at pressures above 220.6 bar with the help of an oxidant (typically oxygen or hydrogen peroxide), whereas PHWO is restricted to lower temperatures (100-374.0°C) and pressures. In biological methods, the harmful compounds are destroyed by living organisms, whereas in photodegradation either artificial light or sunlight causes the degradation.

Since different degradation techniques may result in similar degradation products, note is taken here not only of degradation at elevated temperature and pressure in aqueous environment, which was studied in this work, but also of photodegradation of PAHs in water and other solvents and of degradation of PAHs in biological systems. Desired degradation is also included because it is relevant to understanding and preventing the undesired degradation in analysis. While the focus is on PAHs, some other compounds are introduced for comparison.

7.1. Degradation in pressurised hot water and supercritical water

A separate oxidant is not always needed to destruct the harmful compounds. Water itself can act as a powerful oxidant. Polychlorinated dibenzodioxins are regarded as extremely stable pollutants that are poorly degraded. However, subcritical water at different pH without an oxidant has been used in the degradation of dioxins in fly ash [105]. Dioxins were fully decomposed in all the experiments at 300°C, but the mechanisms and kinetics of the degradation varied widely with the conditions. Degradation of dioxins has also been observed in study in which dioxins were spiked into various types of soils and the soils were extracted [106]. Although some dioxins were found in the water extracts, most of the dioxins were degraded. Subcritical water without oxidant has also been applied for the pilot-scale destruction of explosives [107]. TNT, RDX and HMX were destroyed up to nearly 100% at 275°C in one hour in a static vessel. No degradation products were seen by GC-MS, which could, of course, also mean that the degradation products were too polar for GC detection.

Insecticides [69] and herbicides [73] are degraded in pressurised hot water at elevated temperatures, which means that the extraction temperature must be carefully optimised. Degradation has also been observed in other solvents than water. When nine phenolic compounds were extracted with methanol, some degradation of catechin and epicatechin was observed at 100°C and 150°C. Phenolic compounds, especially catechins, easily oxidise at high temperatures, but with use of inert atmosphere in the extractions the degradation can be prevented [108]. Degradation of the analytes occurs more easily in static extractions than in dynamic extractions, because in static extractions the analytes are exposed for a longer time to high temperature and to possible catalysts, such as extraction vessel material and matrix compounds (typically 5-30 minutes in dynamic PHWE). The possibility of degradation has to be taken into special account in static PHWE, therefore. Degradation of deuterated internal standard anthracene-d_{10} was observed in static PHWE at 250°C with an extraction time of 60 minutes [88]. Anthracene-d_{10} was oxidised to anthraquinone-d_8. Phenanthrene was found to degrade in static experiments in pressurised hot water [109]. Comparison of the degradations in pure deionised water and in deionised water containing 3% of hydrogen peroxide showed the addition of hydrogen peroxide to increase the degradation noticeably. Degradation products, including phenol, benzoic acid and ketones, were detected. The degradation of compounds present in food and plants often occurs at relatively low temperatures, between 100 and 200°C or at even lower temperatures, and the extraction conditions must be selected carefully if quantitative extraction without degradation is desired.

PHWE can be coupled with SCWO to extract and destroy harmful organic compounds. PAHs in soil have been extracted and destroyed with hydrogen peroxide as oxidant using on-line coupled PHWE-SCWO [39,40]. PCBs have also been destroyed with nearly 100% conversion using SCWO [110]. SCWO can be used in industrial scale, for example in the treatment of wastewater. Catalysts have been applied to decrease the temperature and pressure needed for the oxidation [111]. The choice of materials is important in SCWO equipment due to the harsh conditions and oxidants employed, and special materials (Inconel and Hastelloy) are needed in constructing the equipment.

7.2. Biodegradation and photodegradation

Biological methods, in which microorganisms destroy harmful compounds, are an exciting alternative for the remediation of contaminated soils. The drawbacks of biological methods are that they are slow and the pollutants may be toxic to the microorganisms employed. In addition, the mechanisms involved in the remediation are not always exactly known, and the pollutants are sometimes degraded into even more harmful compounds. The ability of microorganisms to degrade compounds is also matrix-dependent. Low-molecular-mass PAHs are destroyed by bioremediation effectively, while high-molecular-mass PAHs often remain undestroyed. The vaporisation of low-molecular-mass PAHs during bioremediation has been shown to be significant [112]. Vaporisation for up to three-ring PAHs varied between 10 and 90% and thus a great deal of "degradation" is in reality vaporisation to the atmosphere. Only the bioavailable fraction of the pollutants in soil can be degraded by bioremediation, and that fraction is readily estimated by SFE [113].

Degradation of organic compounds also occurs in the soil spontaneously, without intentional use of microorganisms. In a creosote-contaminated soil, hetero-PAHs and metabolites, predominantly with ketonic or quinonic structure, were identified in addition to the typical creosote PAHs [114]. These degradation products may originate from biological or abiotic mechanisms [115]. It has been shown that heteroaromatic compounds may inhibit the biodegradation of creosote PAHs [116]. Ketonic and quinonic degradation products were also detected in a study of the degradation of coal tar PAHs in soils and in soil/compost mixtures [115]. The addition of compost material to PAH-contaminated soil enhanced the biodegradation.

Both artificial light and sunlight work to degrade organic compounds in a process known as photodegradation. If the analyte is dissolved in a solvent, the type of solvent affects the degradation as do the substituents in the molecule. When photooxidation of fluorene was studied with use of artificial light from a xenon lamp, 9-fluorenone was the only degradation product [117]. The photooxidation was more pronounced in non-polar solvents. When water was added to these solvents the degradation decreased further. Just the opposite was observed in another study, where faster degradation of PAHs was associated with more polar solvents because radical cation intermediates were formed [118]. In a study of the effect of substituents, 2-nitrofluorene was found to be more resistant to photo-oxidation than 1-methylfluorene and fluorene, evidently because the electron-withdrawing character of the nitro-group depresses degradation by oxidation [117]. In the degradation of acenaphthylene in aqueous solutions, similar oxygenated intermediates were produced regardless of the oxidation treatment employed (UV radiation, ozone and hydrogen peroxide) and these intermediates further degraded to harmless, low molecular end products [119]. In the photolysis of PAHs in dilute aqueous solutions, oxygen concentration had only a minor effect on the photodegradation [120]. This is in accordance with the finding that oxygen is ineffective in most photolyses, and in aqueous solutions the oxygen in the observed products has been suggested to come from water. The mechanism involved probably includes radical cation intermediates [121]. Photodegradation was decreased when the pH of water was increased. Decay in sunlight is an important mechanism for the removal of PAHs in the

atmosphere, and this process is accelerated when the water content of the aerosol particles is higher [122].

8. SOLUBILITY MEASUREMENTS

Information about the solubility of the analytes is essential in any quantitative analysis. The first step in solubility measurements is the generation of a saturated solution of the target analyte. The amount of the analyte can then be determined by a variety of techniques. In the following discussion of methods for measuring solubilities, the focus is on PAHs and aqueous solubilities.

8.1. General

There are different definitions for solubility. According to the usual definition, the solubility of a substance in a solvent is the concentration of the saturated solution, where undissolved solute and dissolved solute are in dynamic equilibrium [123]. Solubility determines the maximum concentration of analyte in an aqueous phase, and provides vital knowledge of the driving force for mass transfer.

Solubilities are usually reported as mole fraction solubilities (x_2), mol/l solubilities or µg/kg solubilities. While a lot of solubility data exists for ambient conditions, little is available for elevated temperatures. It is important to know the solubilities at higher temperatures, as well, because they help in understanding the mechanisms involved. In the case of PHWE and subcritical water chromatography (SWC), the solubility data is needed for optimisation of the processes. In industry, too, the process waters are often warmer than 25°C and it may be difficult to estimate releases from manufacturing facilities, if solubilities and related parameters are not known for higher temperatures [124]. Solubility and corrosion are related to each other and, as the temperature increases, corrosion also increases. Thus, the high temperature water and steam used in central heating systems and the boilers of power plants will be more corrosive. Corrosion is greatest just below the critical temperature of water, and it decreases as the supercritical conditions are reached. Several other factors such as lipophilicity, adsorption and bioconcentration [125] are related to solubility.

The water solubilities (S_w) of non-polar organics are very low at ambient conditions, but they increase dramatically with temperature. This increase is due to a decrease in the relative permittivity of water as the temperature is increased. Water solubility is a fundamental parameter in assessing the extent and rate of dissolution and the persistence of PAHs in the aquatic environment [126]. But while temperature has a highly significant effect on the solubility, pressure has not. In studies of the solubility of anthracene in water and in other fluids at high temperatures and pressures up to 280°C and 3000 bar, Rössling and Franck found that the solubility of anthracene in water decreases by an order of magnitude over the pressure range of 60-2850 bar at 150°C [127]. The change was only negligible, however, at the pressures normally applied for PHWE.

The enhancement of solubility with temperature is less dramatic for liquids than for solid samples. When the temperature of water is increased from 298 K to 473 K, the solubilities of liquid organics increased by about two orders of magnitude [128,129]. In the case of solid organic compounds, like PAHs and pesticides, a similar increase in temperature results in a solubility enhancement of four or five orders of magnitude. If a compound already is noticeably soluble in water at ambient conditions, the increase in temperature does not enhance the solubility as much as for sparingly soluble compounds. As a rule of thumb, the solubilities of solid hydrophobic organics increase by an order of magnitude for every 50ºC increase in the temperature of water.

8.2. Methods for measuring aqueous solubilities

Solubility measurements are done by first generating a saturated solution of the test analyte and then measuring the amount of that analyte in the saturated solution. Although the basic idea is simple, measurements are not always straightforward because true saturated solutions have to be obtained, not just steady-state solutions. Oversaturation may occur at the beginning of the extraction, and the water flow in a dynamic system may push the analytes mechanically forward, both resulting in overestimation of solubilities. Solubilities may be underestimated if the aqueous phase does not have enough time to be saturated with the test analyte, for example if the water flows too quickly in a dynamic system. The conditions during a solubility measurement have to be measured accurately in order to obtain high quality solubility data. Solubilities can be determined in several ways. In some methods the generation of the saturated solution is done on-line with the analysis, and in some methods the two stages are separated. The methods used for the production of saturated solutions are either static or dynamic in nature. It is important that the analytes do not thermally degrade or react in the course of the measurements. On the basis of experimental data, theoretical equations can be derived to predict the solubilities at other temperatures.

8.2.1. Methods for the generation of saturated solutions

Several methods are available for the generation of saturated solutions. A basic set-up that has long been applied is the shake-flask (batch-stirring) method, in which an excess of solid analyte is added to water, the solution is stirred or shaken at a selected temperature, and finally allowed to settle. The saturated solution can be analysed after it has been decanted or filtered to remove suspended particles [126], or a part of the saturated solution can be withdrawn with a pipette through a glass wool plug [130]. The drawback of the shake-flask method is that it is time consuming, and it may take several days or even weeks to prepare a saturated solution. Furthermore, erroneous results may be obtained for hydrophobic substances of low solubility because colloidal dispersions and slow dissolution rates of solutes may lead to incomplete equilibration. The formation of colloidal dispersions leading to overestimation of solubilities can be avoided with the slow-stirring method [131].

In the generator-column method, developed by May *et al.* [132], saturated aqueous solutions are obtained by pumping water through a thermostated generator column packed with a solid support coated with the compound to be studied. As water is pumped through the generator column it becomes saturated with the test analyte coated on the support. The analysis of the saturated solution is done on-line by coupling to liquid chromatography. The apparatus consists of three columns, a generator column, an extractor column, and an analytical HPLC column, all linked by valves. Through switching valves the generated saturated solution is extracted and concentrated in the extractor column using water as mobile phase. Finally, the trapped analytes are eluted from the extractor column with a mixture of water and organic solvent and analysed in the analytical HPLC column with fluorescence and photodiode array detection. In contrast to the shake-flask method, the generator-column method is well suited for the solubility determinations of sparingly soluble hydrophobic compounds. A further benefit of the generator column method is that contamination and adsorptive and evaporative losses of the analytes can be minimised in a closed system. Sensitivity is also enhanced, when the generator column is on-line coupled with liquid chromatography, a factor of particular importance for poorly soluble compounds. The preparation of the generator column takes some time, of course [133,134].

A rapid dynamic method has been developed for hydrophobic solid analytes [135,136]. This dynamic method was applied in this work for the generation of saturated solutions. The method involves pumping water through a cell that is packed with excess of test solute mixed with sea sand or glass beads. The water becomes saturated with the test solute as it flows at constant flow through the cell. It is important to know the flow-rate (volume) of the water accurately. An appropriate equilibration period is needed before the saturated conditions are reached, and this period can be experimentally determined. After the saturation cell the saturated aqueous phase mixes with the selected organic phase, which can be introduced via a stainless steel mixing T-piece. Hydrophobic analytes are partitioned from the aqueous phase into the organic phase in the cooling coil at ambient conditions, because their solubility in water is much lower at ambient conditions than at elevated temperatures. If an organic solvent is not used to collect the hydrophobic analytes they will precipitate in the cooling coil, blocking it. The equipment is actually similar to that in pressurised hot water extraction, and it is easy to carry out solubility measurements successively at different temperatures. The organic fraction obtained via this dynamic method is usually analysed by GC-MS after it has been separated from the water phase. This dynamic method has also been modified for liquids [128,129]. The liquid analyte and glass beads are introduced to the saturation cell. The density of the liquid analyte determines the flow direction of water through the saturation cell: water flows from top to bottom when the analyte is less dense than water (<1 g/ml) and from bottom to top when the analyte is denser than water (>1 g/ml). Recently, a novel dynamic method with a capillary restrictor was realised for minimising the system dead-volume [137].

A static solubility apparatus is also frequently applied in the production of saturated aqueous solutions. There are many types of static cells, differing slightly in their construction. The solubility cell often contains a stir bar for stirring of the solution. The apparatus may be equipped with sapphire or quartz windows to allow either visual observation [138] or direct

spectrophotometric determination [127] of the contents of the cell. Static solubility cells are of either fixed or variable volume and they often withstand elevated pressures [139].

8.2.2. Methods for the analysis of saturated solutions

The amount of analyte in a saturated solution can be determined by a spectroscopic technique, for example by measuring the fluorescence intensity [140,126] or ultraviolet absorption [130] of the solution. Spectroscopic techniques have often been used in combination with the shake-flask technique. Conventional cells are applied with some spectroscopic techniques, but optical high pressure cells are required if the generation of saturated solutions at high temperature and pressure is integrated with the spectroscopic measurement [127]. Radionuclides can also be applied for the determination of solubilities, and the amount of radiolabeled solute in a saturated solution is then determined [141].

Liquid chromatography is useful for the analysis of saturated solutions if the sensitivity is high enough, as in generator column technique [133,134], or if the concentrations of the analytes are sufficiently high [142]. As mentioned earlier, generator columns are often coupled on-line to liquid chromatography. A static solubility apparatus has also been on-line coupled to liquid chromatography [21] and to gas chromatography [143]. Gas chromatography coupled with mass spectrometric detection may be applied where the sensitivity of liquid chromatography is not sufficient for analysis of the solubilities of sparingly soluble analytes [136]. The benefit of GC-MS is that it is both selective and sensitive, and the identification of unknown analytes, for example the potential degradation products of the analytes, becomes possible. In this work, GC-MS was applied for the analysis of saturated solutions.

Sometimes the solubility of a compound in water can be determined merely by visual observation. The temperature at which a heavy hydrocarbon becomes soluble in water was visually observed in a quartz capillary tube as the point at which two liquid phases disappeared when the temperature was slowly raised [138]. In addition to the solution temperature, visual clarity of the phases and the curvature of the meniscuses between the phases were examined. It is also possible to determine the solubility of a compound in water the other way round, as the appearance of two phases at a constant temperature when solute is added to water and the solubility in water is exceeded [144].

9. EXPERIMENTAL

Chemicals, materials, equipment and analytical procedures are described in this section. For more information see papers **I-V**.

9.1. Chemicals and materials

The chemicals and materials used in the experiments (**I-V**) can be found in Table 4. Real sediment samples were applied in the extractions, to obtain a reliable estimation of the extraction efficiency of the method. Analytes are less tightly bound in spiked samples than in the matrix of a real sample, and they are more easily extracted, resulting in different extraction kinetics in the two cases. The stock solutions of PAHs were prepared by weighing the analytes into a measuring flask and adding solvent to the mark. A dilution series of a PAH standard mixture was prepared for the calibration of GC-MS. The oxygen dissolved in the extractant water was eliminated by sonication to avoid corrosion of the equipment and degradation of the analytes.

Table 4. Chemicals and materials used in the experiments (**I-V**).

Compound	Manufacturer or supplier	Comments	Paper
Acenaphthene	Fluka	Model compound (≥99%)	IV, V
Acetone	Lab Scan Analytical Sciences	For cleaning vessels and tubings in PHWE (99.8%)	III, IV
Anthracene	Fluka	Model compound (~99%)	IV, V
1,1'-Binaphthyl	Acros Organics	Internal standard (98%)	III, IV
Chloroform	Rathburn Chemicals Ltd	Collection solvent (HPLC grade)	V
4,4'-Dibromoocta-fluorobiphenyl	Aldrich	Internal standard (99%)	I-IV
Dichloromethane	Lab Scan Analytical Sciences	Solvent for standards and for liquid–liquid extraction (HPLC grade)	IV
Diphenylene oxide	Aldrich	Internal standard	I
Ethyl acetate	J. T. Baker Chemicals B.V./ Lab Scan Analytical Sciences	Solvent (HPLC grade)	I/ II
Fluoranthene	Fluka	Model compound (≥97%)	IV
Fluorene	Schering-Kahlbaum	Model compound	IV
Helium	AGA	Carrier gas in GC (99.996%)	I-V
n-Heptane	Rathburn Chemicals Ltd	Solvent and collection solvent, also used in liquid–liquid extraction (HPLC grade)	II, III, V
Methanol	J. T. Baker Chemicals B.V.	Solvent for standards (HPLC grade)	I
Nitrogen	AGA	Drying of the Tenax trap, thermal desorption experiments (99.5%)	I-IV
PAH standard mixture	AccuStandard Inc.	Z-013-17/ Z-014G-R, contains 17 PAHs, for identification and quantitation of PAHs	I/II,III
n-Pentane	Rathburn Chemicals Ltd	Solvent (HPLC grade) in LC-GC and solvent for standards, distilled in laboratory before use	I
Perylene	EGA-Chemie	Model compound (99+%)	IV
Phenanthrene	Fluka	Model compound (97%)	IV
Pyrene	Fluka	Model compound (~97%)	IV, V
Sea sand	Riedel-de Haën	Acid washed and calcined sea sand, grain size 0.1-0.3 mm	I-V
Sediment EC-1	Environment Canada, National Water Research Institute (Burlington, Ontario)	Certified Reference Material, a Hamilton harbour sediment containing toxic organics [12]	II, III
Sediment Setoc	Supplied by Dr. Hanne Lund (SINTEF, Oslo, Norway)	Channel sludge sediment, sample 4 (98.4) from Setoc, the Netherlands	I
Sodium sulphate	Merck	For drying the extracts and the samples	II-V
Toluene	Lab Scan Analytical Sciences	Collection solvent and solvent for standards (HPLC grade)	I, IV
Water		Distilled and deionised, PHWE solvent and solvent in stability and solubility studies	I-V

9. 2. Equipment

Devices and materials used in the research can be found in Table 5. Both off-line PHWE equipment (**II,III**) and on-line PHWE-LC-GC equipment (**I**) were applied. Stainless steel frits of 5 or 10 µm were employed in the drying column (**II**), extraction vessels and solid-phase trapping column (**II**). Exceptionally, the outlet frit in the solid-phase trap in on-line coupled PHWE-LC-GC had a pore size of 2 µm (**I**). Modified PHWE was employed in the solubility measurements (**V**).

Table 5. Devices and materials used in the research (**I-V**).

Device/material	**Manufacturer and model**	**Comments**	**Paper**
Autosampler	Hewlett-Packard 7636/ Agilent Technologies 7683	On-column injection (2.0 µl)/(1.0 µl)	II-IV/ V
Batch-type reaction vessels	Laboratory-made	Stainless steel, volume 2.5 ml, i.d. 10 mm, also a PEEK vessel having a volume of 2.2 ml was tested in some experiments	IV
Drying column		10 mm × 4.6 mm i.d., packed with sodium sulphate	II
Extraction vessel	Keystone Scientific Inc	Stainless steel, V = 2.2 ml, 100 mm × 5.0 mm i.d.	I, II
Extraction vessel	Laboratory-made	Stainless steel, volume 2.0-3.0 ml, length 1.3-7.7 cm, i.d. 0.7-1.5 cm, o.d. of all vessels was 2.0 cm	II, III
Gas chromatograph	Hewlett-Packard 5890/ Agilent 6890N		II-IV/ V
GC column	Hewlett-Packard/ Agilent Technologies	25 m × 0.2 mm i.d. HP-5 column, phase thickness 0.11 µm/ 30 m × 0.25 mm i.d. HP-5 column, phase thickness 0.25 µm	II, IV/ III-V
GC column in LC-GC	BGB Analytik AG	4 m × 0.53 mm i.d. retaining precolumn + 22 m × 0.25 mm i.d. analytical column (both BGB-5, phase thickness 0.25 µm)	I
GC oven for heating batch-type reaction vessels	Hewlett-Packard 5890		IV
GC oven for PHWE	Hewlett-Packard 5790A/Carlo Erba Fractovap Series 2150		I/ II, III
GC oven for solubility studies	Hewlett-Packard 5790 A (modified)		V
Glass wool	Merck		III-V
Heating module	Pierce ReactiTherm	Sample concentration by N_2-evaporation, no heating	III,IV
High-pressure pump	Jasco PU-980	For delivering water and organic solvents in PHWE and in the solubility measurements	I-III, V
LC-GC	CE Instruments, Fisons Instruments Dualchrom 3000 Series on-line HPLC-HRGC	Contains Phoenix 30CU pump	I

Table 5, continued

Mass spectrometer	Hewlett-Packard 5989 A/ Agilent 5973 N	EI ionisation, 70 eV, used in both SCAN and SIM mode	II-IV / V
Pressure restrictor	Jasco Inc.	Manual micro-metering high-pressure valve	I-III, V
Retention gap	BGB Analytik AG (Agilent Technologies paper V)	DPTMDS-deactivated, 10 m × 0.53 mm i.d./ DPTMDS-deactivated, 3 m × 0.53 mm i.d	I/ II-V
Saturation cell	Laboratoty-made	Stainless steel, volume 3 ml, length 3.7 cm, i.d. 1.0 cm (below the m.p. of the analytes)/ volume 11 ml, length 3.5 cm, i.d. 2.0 cm (above the m.p. of the analytes) [+ cartridge (V = 2.4 ml, length 3.0 cm, i.d. 1.0 cm)]	V
Software for GC-MS	Hewlett-Packard Chemstation	For GC-MS data analysis (incl. Wiley MS library)	II-V
Solid-phase trapping column	Tenax TA adsorbent, Alltech Associates Inc.	7.5 cm × 2.1 mm i.d. (80/100 mesh), worked also as the LC column, inlet frit 10 µm, outlet frit 2µm/ 10 cm × 2.1 mm i.d. (60/80 mesh)	I/ II
Stainless steel capillary		Tubings in PHWE and in solubility apparatus, i.d. 0.5 or 0.75 mm	I-III, V
Thermocouple data logger	Pico TC-08, Pico Technology	Temperature monitoring in the solubility measurements	V
Three-way valve	High Pressure Equipment Co., HIP 30-15 HF4-HT	For directing water, drying gas and solvent in PHWE	I-III
Three-way valve	High Pressure Equipment Co., HIP 15-15AF1 HT#712730	A smaller three-way valve for directing water, drying gas and solvent in PHWE	III

9.2.1. Equipment for pressurised hot water extraction

The equipment used in PHWE is illustrated in Figure 3. All the capillaries were constructed of conventional stainless steel. Internal diameter was 0.5 mm, except for the capillary connecting the extraction vessel to the three-way valve, where it was 0.75 mm to avoid blockage. The length of the preheating coil was 3 m and the length of the cooling coil was 1 m. The cooling coil was immersed in a water bath containing ice. Both laboratory-made (**II,III**) and commercial (**I,II**) extraction vessels were applied. The laboratory-made extraction vessel is illustrated in Figure 5. This flow-through vessel was made of stainless steel 316L. The sealing ring made of copper was inserted between the vessel body and the cover and the whole set-up was tightened with four bolts. The temperature of the oven was monitored during the extraction with a thermocouple installed in the oven. Either solid phase trapping (**I,II**) or solvent trapping (**III**) was employed. The high pressure pumps were operated in constant flow mode, and the pressure was adjusted with a manually adjustable pressure restrictor. The pressure variation was wider in the extractions at high temperatures and pressures because the pressure sometimes began to fluctuate due to co-extracting matrix compounds.

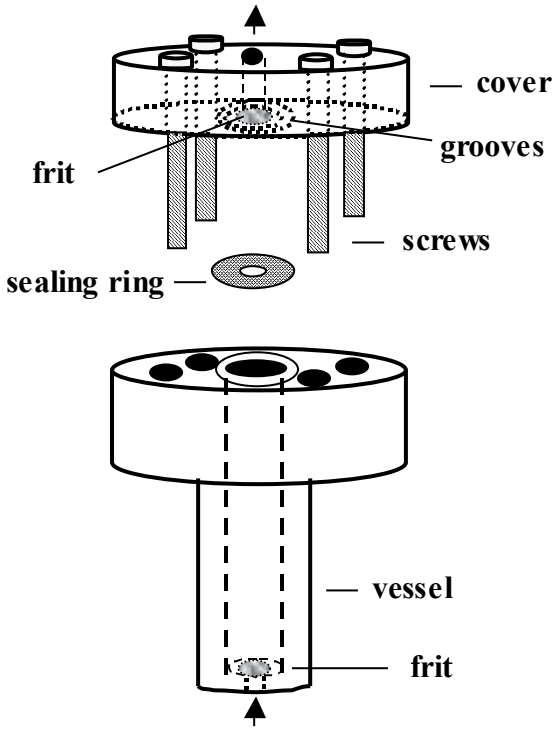


Figure 5. The laboratory-made extraction vessel used in PHWE (Papers II, III).

9.2.2. Equipment for pressurised hot water extraction–liquid chromatography–gas chromatography

The on-line coupled PHWE-LC-GC equipment is presented in Figure 6. The equipment consists of a laboratory-constructed PHWE unit connected to a commercial Dualchrom LC-GC instrument. Commercial extraction vessels were used in the on-line coupling with LC-GC. The solid-phase trap for collecting the analytes in PHWE also served as the LC column, and matrix interferences were removed before GC in this solid phase trap. The pore size of the stainless steel frit was 10 μm at the inlet side of the trap and 2 μm at the outlet side. The frit at the inlet side had a larger pore size to prevent plugging. The on-line coupling of PHWE, LC and GC was realised with an on-column interface. The column system in GC consisted of a retention gap, retaining precolumn and analytical column. The columns were connected by glass pressfit connectors. A solvent vapour exit was installed between the retaining precolumn and analytical column to get rid of the solvent and facilitate the evaporation. A flame ionisation detector could be used for the detection due to efficient clean-up. The PHWE-LC-GC system contained four valves and three pumps.

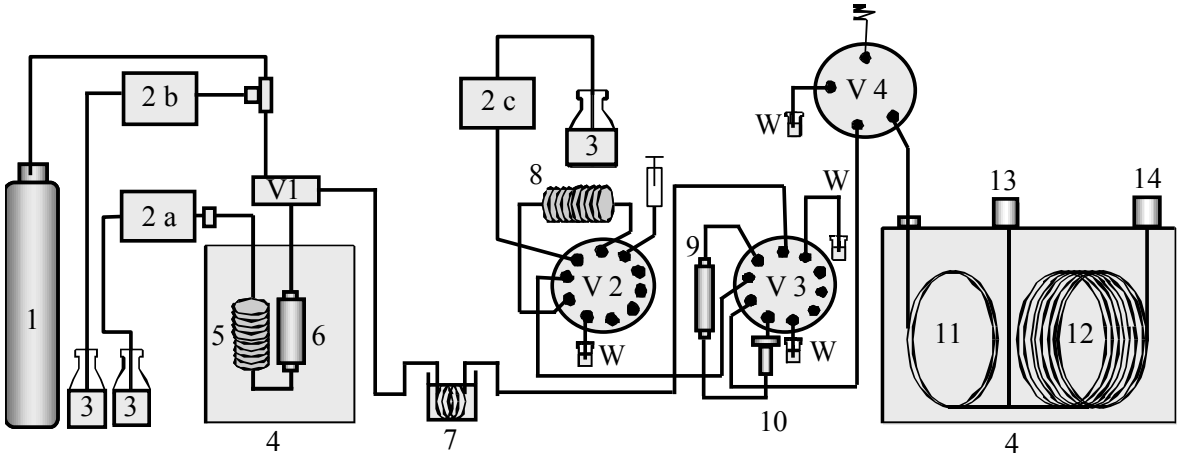


Figure 6. PHWE-LC-GC equipment. 1 Nitrogen, 2 a-c. High-pressure pumps, 3 Eluent, 4 Oven, 5 Preheating coil, 6 Extraction vessel, 7 Cooling coil, 8 Eluent coil, 9 Trapping and LC column, 10 Pressure restrictor, 11 GC precolumns, 12 Analytical GC column, 13 Solvent vapour exit (SVE), 14 Flame ionisation detector (FID), V1 Extraction valve, V2-V4 Multiport valves (Paper I).

9.2.3. Equipment for stability measurements

The stability studies were carried out in two types of closed vessels. The vessels were similar to the laboratory-made extraction vessels except that there was no inlet or outlet. The two types of vessels, differing slightly in their construction for sealing, are shown in Figure 7. A GC oven was used for heating the vessels.

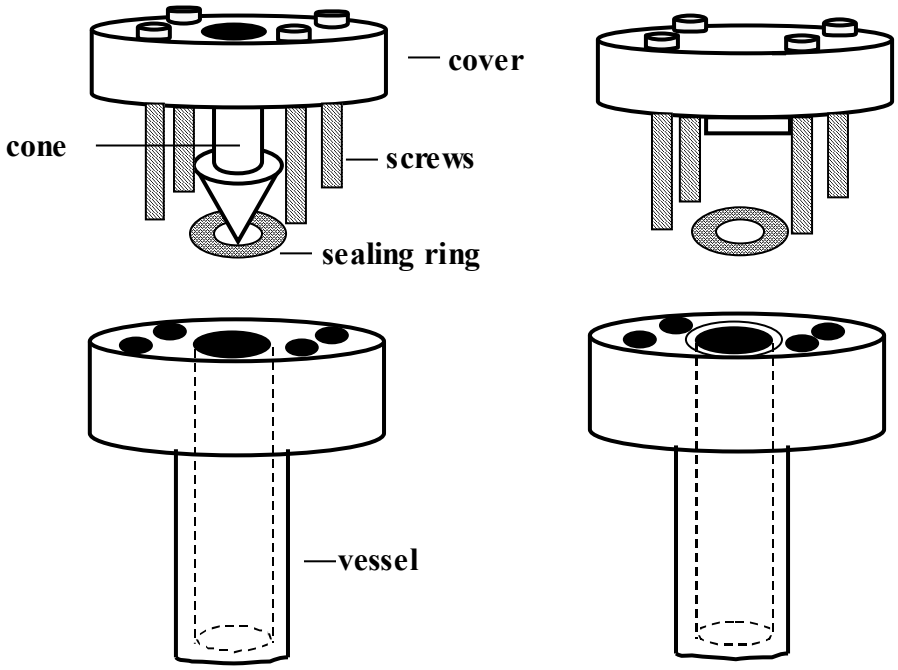


Figure 7. Reaction vessels used in the stability studies (Paper IV).

9.2.4. Equipment for solubility measurements

The equipment employed in the solubility measurements was, with a few exceptions, like that used for pressurised hot water extraction (Figure 3). A different GC oven was used for heating purposes, and a T-piece instead of a three-way valve was utilised to mix the organic collection solvent, usually toluene, with water saturated with PAHs. The T-piece was placed in the oven to prevent the deposition of PAHs in the tubings. On-line liquid–liquid extraction from water to the organic solvent was done in the tubings, and the PAHs in the aqueous phase were transferred to the organic phase as the aqueous phase cooled. This dynamic system for measuring solubilities was developed by D. J. Miller *et al*. [135,136]. Below the melting point of the PAHs, the laboratory-made extraction vessels were used as saturation cells. Above the melting point a different set-up had to be applied, and the flow-through saturation cells were replaced by the cell depicted in Figure 8, where the water seeps through the lid of the vessel. Flow-through vessels could not be used above the melting point of the PAHs because the melted PAHs would otherwise have been swept away from the cell with the water-flow. An inner cartridge was applied in some of the measurements with the idea of ensuring the analyte transfer only by diffusion. A thermocouple was installed in the oven and the temperature of the oven was monitored via computer with the aid of a Pico TC-08 thermocouple data logger. Precise adjustment of the oven temperature (accuracy to the second decimal) was possible by changing the heating power of the oven. The maximum deviation in the temperature was ±0.5ºC, although the usual deviation during the measurement was only ±0.2ºC.

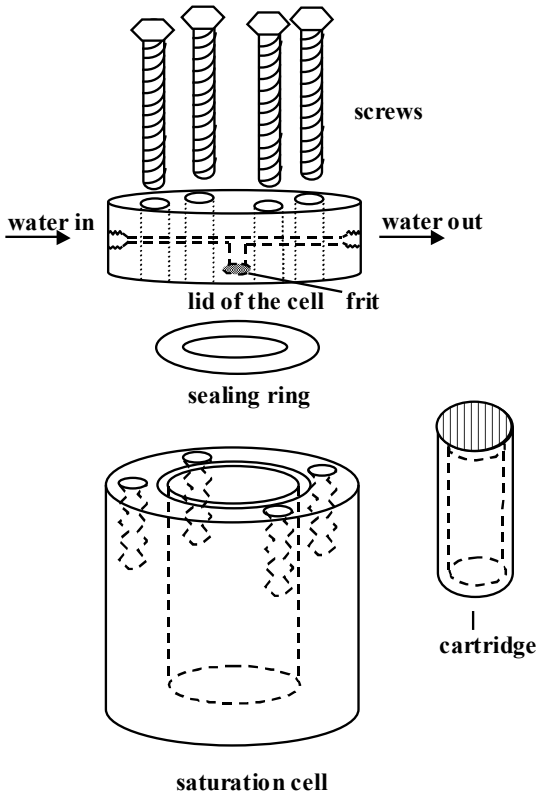


Figure 8. The saturation cell applied in the solubility measurements (Paper V).

9. 3. Procedures

The procedures used in the PHWE and the stability and solubility studies are described in this section. The parameters and conditions applied in the analysis of the extracts by GC and by GC-MS are listed in detail in the corresponding publications (**I-V**).

9.3.1. Procedure for pressurised hot water extraction

1) 0.5 g of sediment was mixed with 0.5 g of sea sand and packed into the extraction vessel. The vessel was then filled with sea sand (**II,III**). Also some other packing styles were tested (**III**). When PHWE was coupled on-line to LC-GC (**I**) 10 mg of sediment was used for extraction. In PHWE-LC-GC (**I**) the ISTD was added to the sediment before the extraction, and in other studies it was added after the extraction (**II,III**).
2) The vessel was closed and connected to capillaries in the oven. The water flow-rate was set to 1 ml/min and pressure was adjusted to the desired value.
3) The oven was heated to the extraction temperature. The heating time varied because it was dependent on the extraction temperature.
4) Extraction was carried out for 30 min under the selected conditions. The extracted analytes were collected into a solid-phase trap (**I,II**) or into 4 ml of heptane (**III**).
5) The solid-phase trap was dried with nitrogen for 25 min (**II**) or 40 min (**I**). Capillaries were flushed with nitrogen for 2 min when the solid-phase trap was not applied (**III**).
6) The trapped analytes were eluted with a mixture of 10% ethyl acetate in *n*-heptane (1 ml/min) directly into a 1.5-ml GC vial (**II**) or with 10% ethyl acetate in pentane (170 µl/min) into the GC (**I**). When the solid-phase trap was not applied the capillaries were rinsed with heptane (1 ml/min) for 3 min directly into the measuring flask (**III**).
7) The solid-phase trap was washed with solvent after the extraction and finally dried with nitrogen (**II)**
8) The PAHs were analysed by GC either on-line (**I**) or off-line (**II,III**). The solid-phase trap was washed and dried simultaneously with the GC analysis (**I**).

9.3.2. Procedure for stability measurements

1) 100 µl of PAH solution (2.0 mg/ml) in dichloromethane was pipetted into a weighed reaction vessel.
2) Dichloromethane was evaporated to dryness in 1 h.
3) 1 ml of degassed and de-ionised water was added and argon atmosphere was blown into the vessel.
4) The vessel was closed, weighed and heated in the oven for a predetermined time.
5) The vessel was cooled.
6) The vessel was weighed to check that it had not been leaking during the heating.

7) The internal standard was added and the contents of the vessel were extracted with 3 × 2 ml of dichloromethane.
8) The combined dichloromethane phases were dried, filtered and concentrated and another ISTD was added.
9) The extract was analysed by GC-MS.

Special attention was needed when working with closed vessels. Sufficient headspace had to be left in the vessel to prevent excessive pressure build-up, which could lead to explosion and personal injury. In this work, 40% of the vessel volume was filled with water. The maximum pressure during the work was 165 bar at 350°C.

9.3.3. Procedure for solubility measurements

1) Below the melting point of the PAHs, 20 wt% of a thoroughly mixed mixture of PAHs in sea sand was packed into the saturation cell; above the melting point of the PAHs neat PAH (0.6 or 1.2 g) was used.
2) Water was pumped through the saturation cell, and after the cell was pressurised and found to be leak-free the oven was heated to the desired temperature. The flow-rate of water was set to 0.1 ml/min and the flow-rate of toluene to 0.4 ml/min.
3) The system was equilibrated at each temperature for 20 (below the melting point) or 30 min (above the melting point).
4) Five (above the melting point) or six (below the melting point) 10 min fractions were collected.
5) The samples collected were diluted if necessary and the internal standard, fluoranthene, was added.
6) The toluene phase was dried with Na_2SO_4 and analysed by GC-MS.

The uncertainty of the temperature in the measurements was ± 0.5°C, and that of the pressure was ± 5 bar.

9. 4. Data analysis by self-organising maps

The self-organising map (SOM) is a well established tool for visualising high-dimensional data [145,146]. The data in SOM can be analysed in one dimension, two dimensions or even multidimensionally. Interesting data and variables are chosen for the data analysis, and after that the data is clustered. Trivial results can be eliminated. Two-dimensional honeycomb-like hit-histograms characterise the data visually and show the correlation between variables. The location of clusters on a chart tells which conditions are valid and what kind of factors are most strongly correlated with the results. Variables can be grouped on the basis of the similarity of the hit-histograms, and variables correlated with each other can be easily observed. The data in SOM can be illustrated visually and clearly in many ways. SOM is more robust to the outliers in the data than, is, principal component analysis (PCA).

Self-organising maps were used in the data analysis of the PHWE results. The dependence of the experimental results on different parameters was studied. From our results we made a matrix in Excel comprising parameters related to conditions and instrumental parameters in PHWE. Solvent and PAH properties were included as well. Table 6 lists all the parameters studied. Because many of the parameters are strongly cross correlated, it is difficult to clarify the effect of a single parameter on the results. Three PAHs — phenanthrene, pyrene and benzo[a]pyrene — were included in the data-analysis.

Table 6. Parameters included in the data analysis (Papers II, III).

Conditions or instrumental parameters	**Solvent properties**	**PAH properties**
Temperature	Relative permittivity	Melting point
Pressure	Viscosity	Boiling point
Vessel volume	Density (gas/liquid)	Molecular mass
Vessel length	Heat of vaporisation	Experimental Henry's law constant (H_{exp})
Vessel i.d.	Internal energy	Solubility (log S)
Trap type	Solubility parameter	Octanol–water partition coefficient (log K_{ow})
Solvent	Fugacity	Number of rings
Commercial or laboratory-made vessel		Number of carbons
Flow direction		Number of hydrogens
Sample packing		Enthalpy of formation (ΔH_f)
		Volume
		Surface area
		Electron affinity (EA)
		Measured solubility

10. RESULTS AND DISCUSSION

Study was made of the suitability of PHWE coupled on-line to LC-GC for the determination of PAHs in sediment samples (**I**). After the suitability had been established, the effect of thermal desorption was compared with the solvating properties of water in PHWE (**II**). The significance of the extraction vessel geometry and flow and packing conditions for the recoveries was investigated (**III**). Finally, the stabilities (**IV**) and aqueous solubilities (**V**) of selected PAHs at the temperatures normally applied in PHWE were determined.

10.1. On-line coupled pressurised hot water extraction–liquid chromatography–gas chromatography

On-line coupled PHWE-LC-GC was applied to the extraction and analysis of polycyclic aromatic hydrocarbons in sediment samples. The extraction and chromatographic analysis were optimised separately before the coupling, and the final optimisation was carried out with the extraction coupled on-line to LC-GC. Sediment samples were then quantitatively analysed.

10.1.1. Analytical steps and optimisation of conditions

The PHWE-LC-GC analysis can be divided into five steps, namely PHWE extraction, drying of the solid-phase trap, LC (trap) clean-up, transfer of the selected fraction to GC and GC analysis. The change from one analytical step to the next is made simply by switching the valves in the equipment. Each analytical step is characterised by specific valve positions (**I**). Spiked sea sand and sediment samples were studied. With the on-line sample clean-up, the only sample preparation required for the sediment samples was drying and homogenisation.

The PHWE conditions were adopted from a study carried out earlier in our laboratory, but a Tenax material of smaller particle size (80/100 mesh) was used to be able to obtain some separation of the analytes in the solid-phase trap [42]. The smaller particles created a larger back pressure than in the trap used earlier (Tenax 60/80 mesh), however, making the extraction with steam difficult, and, for practical reasons, liquid water was chosen for the extraction.

As noted above, the trapping column was used for the clean-up and initial fractionation of the extract. The sediment sample contained a large amount of hydrocarbons that needed to be separated from the PAHs before the GC analysis to prevent overloading of the GC column and enable the analysis and FID detection of PAHs. The initial idea was to transfer the hydrocarbon and PAH fractions to the GC one after the other, but this proved to be impossible because of the large difference in the concentrations of the two fractions. Thus only the fraction containing PAHs was transferred and analysed in GC. The hydrocarbon

fraction was eluted to waste with pure pentane, after which a stronger eluent (90% pentane-10% ethyl acetate) stored in a loop was used to elute the PAHs as a 780 µl fraction to GC.

The three parameters to be optimised in the partially concurrent evaporation technique that we applied for solvent evaporation in GC were the transfer rate from LC, the length of the flooded zone, and the rate of solvent evaporation. The temperature during the transfer was 32°C. The eluent flow-rate in LC was set to 170 µl/min and the evaporation rate in GC was adjusted to 160 µl/min to keep the solvent evaporation time as short as possible. Thus, a high portion of the eluent — approximately 94% — evaporated during the transfer. Estimations under these conditions indicated that the length of the flooded zone was shorter than the length of the retention gap (10 m). It was not possible to transfer the PAHs to GC with pure pentane alone, because the size of the fraction would have been far too large for GC. Consequently 10% of ethyl acetate was added to pentane. That was also the maximum amount of ethyl acetate that could be added because, with a larger amount, the solvent trapping was disturbed, and there were problems with FID as well. The fraction size could be kept to a reasonable 780 µl with this 10% ethyl acetate in pentane, and at the same time sufficient separation between the hydrocarbon and the PAH fractions was obtained in the Tenax TA column.

10.1.2. Quantitative analysis of sediment samples

The transfer efficiency from the Tenax trap to the GC was over 90%, and the total recoveries of the method were better than 69% for all PAHs, the average being 103%.

The developed method had good linearity (0.984-1.000) over the concentration range of 0.01-2 µg/g (**I**) except for phenanthrene and anthracene for which a baseline separation was not always obtained because they eluted near each other. The repeatability (RSD%) of the retention times varied between 0.03 and 0.63% and the repeatability (RSD%) of the peak areas between 3 and 28% (**I**). The limits of quantification ranged between 0.001 and 0.01 µg/g with PHWE-LC-GC, as compared with the substantially higher value of 0.1 µg/g achieved in a Nordtest study with the off-line SFE-GC-MS [147]. The sensitivity of our on-line coupled system was thus higher. In another interlaboratory comparison study (Setoc), the limits of quantification obtained by 50 participating laboratories in 15 European countries ranged from 0.005 µg/g to 4.3 µg/g [148].

A sediment sample, also used as sample 4 in the just mentioned Setoc study, was analysed by the PHWE-LC-GC technique under optimised conditions, and the results were compared both with the results obtained earlier in our laboratory using off-line GC-MS and with the values obtained in the Setoc study (Figure 9). A detailed description of the analytical conditions can be found in Paper I. In the Setoc study, a multistep sample pre-treatment was carried out including Soxhlet extraction and open-column chromatography. In almost every case the recoveries obtained by PHWE-LC-GC were highest, followed by those of SFE off-line GC-MS. The recoveries obtained in the Setoc study were the lowest. The most striking difference in the recoveries was for naphthalene, the most volatile analyte. The recovery obtained with PHWE-LC-GC was 35 times that obtained in the Setoc study. The probable reason for the

larger value for naphthalene with PHWE-LC-GC is that, in our closed system, analyte losses are kept to a minimum, and volatile analyte like naphthalene is particularly prone to loss. Probably, in the Setoc interlaboratory comparison study, most of the naphthalene was evaporated to the atmosphere during the sample concentration. The RSDs with our closed system were substantially smaller than those in the SFE-GC-MS study or the Setoc study, further confirming the performance and reliability of our closed on-line coupled system. The average RSD was 13% with PHWE-LC-GC, 22% with off-line SFE-GC-MS [147] and 24% in the Setoc study [148].

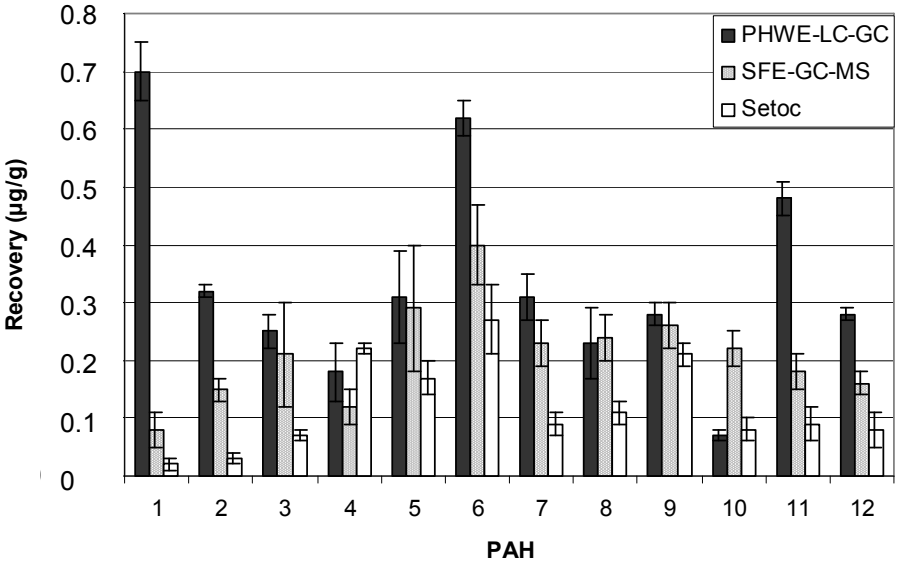


Figure 9. Comparison of recoveries and RSDs obtained by PHWE-LC-GC and by other techniques. Peak identification: 1 naphthalene, 2 fluorene, 3 phenanthrene, 4 anthracene, 5 pyrene, 6 fluoranthene, 7 benzo[a]anthracene, 8 chrysene, 9 benzo[b]fluoranthene, 10 benzo[a]pyrene, 11 indeno[1,2,3-c,d]pyrene and 12 benzo[g,h,i]perylene. PHWE at 300ºC with liquid water (pressure 118 bar), LC clean-up with pentane at 170µl/min, elution of a 780 µl fraction to GC with a mixture of pentane/ethyl acetate (9:1), GC analysis (oven programmed from 32ºC (15 min) to 150ºC (2 min) at 8 ºC/min, from 150ºC to 200ºC at 4ºC/min, and finally to 300ºC (5 min) at 11ºC/min) using PCSE and FID (Paper I).

Due to the high sensitivity of our method — 400–800 times better than in the corresponding off-line methods — impurities present in the transferred fraction are concentrated together with the PAHs in GC. The amounts of the hydrocarbons were several times larger than those of PAHs, and because a "tail" of the hydrocarbon fraction co-elutes with the PAHs, some hydrocarbons appeared in the GC chromatograms. These can be seen as a hump in the chromatograms (**I**). Since the sensitivity of the on-line coupled system is extremely high, only a small amount of sample is needed for the extraction and analysis. A 10-mg sample was sufficient, whereas a 500-mg-sample was used in the off-line method. With small sample size, however, the homogenisation and weighing of the sample need to be done extremely carefully. The developed PHWE-LC-GC method is best suited to the determination of analytes present in very low concentrations in samples with relatively small amounts of matrix components.

10.2. Pressurised hot water extraction

Parameters related to PHWE were separately optimised and their effects on recoveries were examined. The instrument for PHWE had been constructed and the solid-phase trapping conditions optimised in an earlier study [149]. A laboratory-made extraction vessel was tested for the extraction (**II,III**); liquid water and steam were compared (**II**) and the effect of thermal desorption on the extraction was investigated (**II**). In addition, the effect of the geometry of the extraction vessel was studied (**III**) and the performance of solid-phase trapping versus solvent trapping was evaluated (**II,III**). Real sediment samples were used in the extractions.

10.2.1. Choice of experimental conditions

Since earlier studies in our laboratory had shown that a temperature of 300°C is needed for the PHWE of PAHs from naphthalene to benzo[g,h,i]perylene, this temperature was applied in most of the extractions. A few other temperatures were tested as well. Based on the same study an extraction time of 30 minutes was selected [42]. The best location for the solid-phase trap has been found to be in front of the pressure restrictor [53]. Water flow-rate was set to 1 ml/min, because a decrease in recoveries is likely at lower flow-rate [28].

Tenax TA was used in the solid-phase trapping of the analytes. Tenax TA (80/100 mesh) material was tested early in the study (**II**), but it easily became blocked and the drying of the trap with nitrogen was not successful. In addition, the Tenax TA (80/100 mesh) material retained the analytes too effectively. These kinds of problems were not encountered in PHWE-LC-GC (**I**) where the sample size was much smaller. Tenax TA (80/100 mesh) was replaced by Tenax TA (60/80 mesh) in further off-line PHWE studies. The possibility of breakthrough was checked from time to time by extracting the water collected during the extraction with organic solvent and analysing that extract by GC-MS. The trap was always conditioned with solvent before the first extraction in the morning by pumping solvent through it and drying it with nitrogen. Stainless steel frits (5 µm or 10 µm) were used in the extraction vessel and in the solid phase trap. Earlier in PHWE-LC-GC, a 2-µm frit was applied at the outlet of the trap (**I**). The drying of the trap with the 2 µm frit took longer and drying of the trap after the extraction was increased to 40 minutes.

In PHWE-LC-GC (**I**), internal standard was added before extraction of the sediment, and in off-line PHWE studies (**II**,**III**) it was added after the extraction. In pressurised liquid extraction (PLE), it has been found that if the internal standard (ISTD) is added after sample insertion to the extraction vessel, its elution is not representative for the extraction process because it is more a chromatographic elution than an extraction [150]. It is advisable therefore either to mix the ISTD thoroughly with the matrix prior to extraction or to add it immediately after extraction.

The colour of the water collected during the extraction was brownish and a sulphurous smell originating from the matrix components was observed. The pressure typically increased at the

beginning of the extraction, when the first foaming drops were extracted. The colour of the PHWE extract collected in the GC vial varied from light to deep yellow depending on the extraction temperature. Sometimes water drops were seen in the collected fractions in GC vials, and an on-line Na_2SO_4 drying column was accordingly installed (**II**). Another approach is to dry the collected fractions in a pipette packed with Na_2SO_4 (**III**) [151]. Even small amounts of water can seriously disturb the GC analysis.

A smaller on/off three-way valve (Table 5) for directing water, solvent and drying gas was tested to reduce the dead volume of the extraction system. The dimensions of this new valve were 5 cm x 3 cm x 1 cm. The results obtained with this smaller valve and with the valve normally employed are presented in Figure 10. The recoveries obtained with the smaller valve are, in general, lower than those obtained with the valve normally employed, on average 90% of the recoveries with the conventional valve. In addition, the smaller valve warmed up to 170°C during the extraction at 300ºC and often started to leak (a hissing sound could be heard) at the end of the extraction so that it could not be used for practical reasons. The normal valve is larger and of greater thermal mass, and as it resists heating more efficiently it is more suitable for high temperature use. The best alternative for the valve configuration would be a valve with small dead volume but with a sufficiently high thermal mass to resist heating.

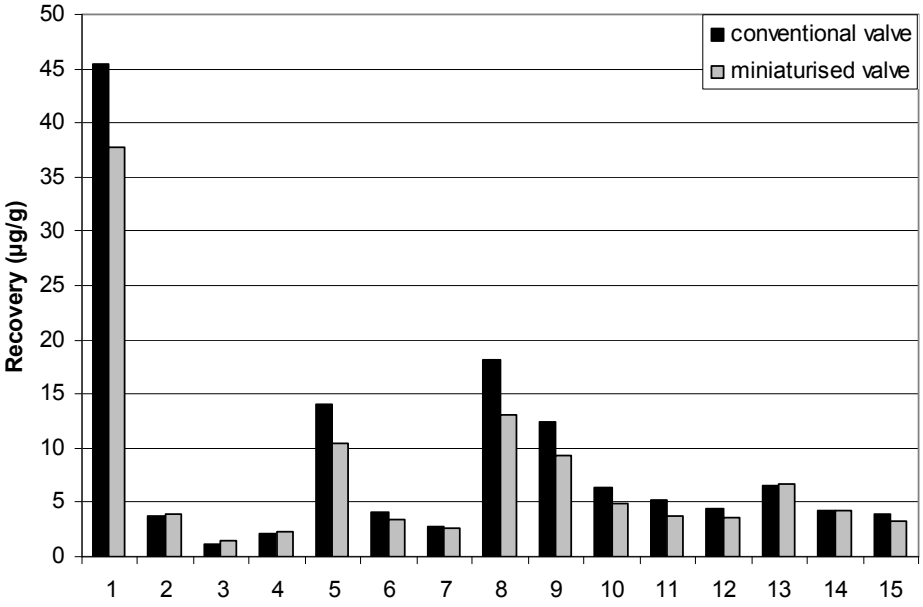


Figure 10. Comparison of conventional and miniaturised valves. Peak identification: 1 naphthalene, 2 acenaphthylene, 3 acenaphthene, 4 fluorene, 5 phenanthrene, 6 anthracene, 7 carbazole, 8 fluoranthene, 9 pyrene, 10 benzo[a]anthracene+chrysene, 11 benzo[b+k]fluoranathene, 12 benzo[a]pyrene, 13 indeno[1,2,3-c,d]pyrene, 14 dibenzo[a,h]anthracene and 15 benzo[g,h,i]perylene. Temperature was 300ºC and pressure 50 bar. *Unpublished results

10.2.2. Comparison of laboratory-made and commercial extraction vessels

Commercial extraction vessels from Keystone Scientific were used at the beginning of our research (**I,II**). However, there were some problems related to the sealing of the vessels. In the commercial vessels, two hard metal surfaces are tightened against each other by turning, and without a separate seal. Tightening of the vessels is thus a demanding task and much force is required. Damage to the vessel is always a risk. The void in the vessel is filled with sea sand, and if just a small grain of sand is accidentally left on the sealing surface the vessel will leak. With these problems in view, new, more robust vessels were constructed in our workshop (Figure 5).

The results obtained with a laboratory-made extraction vessel were compared with those obtained with a commercial extraction vessel (Keystone Scientific) at 300°C using steam at 50 bar (Figure 11). With the RSDs taken into account, the recoveries obtained with the two vessels are similar (**II**): the recoveries obtained with the laboratory-made vessel were on average 98% of those obtained with the commercial vessel. When the two vessels were compared in terms of repeatability, however, the repeatability was slightly better with the laboratory-made vessel: the average RSD value with the laboratory-made vessel was 15%, and that for the commercial vessel 19%. To conclude, the performance of the laboratory-made extraction vessel is comparable to that of the commercial vessel. Laboratory-made vessels were used in our further research (**II,III**). Occasionally, the vespel ferrules applied in tightening the laboratory-made vessel deformed and split and had to be changed. This was probably caused by the imperfect joint geometry in some of the vessels.

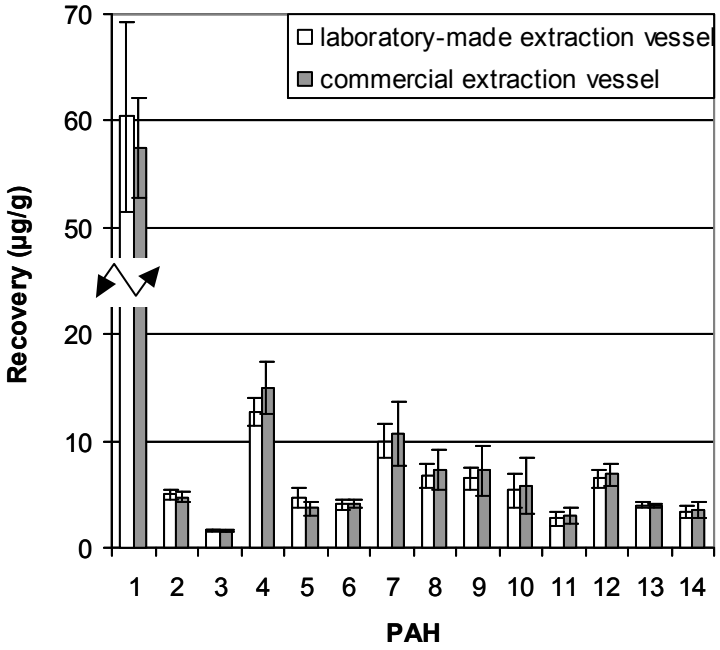


Figure 11. Performance of laboratory-made and commercial vessels in extractions at 300ºC (n=6) with steam (50 bar). Peak identification: 1 naphthalene, 2 acenaphthylene, 3 acenaphthene, 4 phenanthrene, 5 anthracene, 6 carbazole, 7 fluoranthene, 8 pyrene, 9 benzo[a]anthracene+chrysene, 10 benzo[b+k]fluoranthene, 11 benzo[a]pyrene, 12 indeno[1,2,3-c,d]pyrene, 13 dibenzo[a,h]anthracene and 14 benzo[g,h,i]perylene (Paper II).

10.2.3. Liquid water versus steam

In general, extractions with steam give better recoveries for non-polar compounds because the relative permittivity of steam is lower than that of liquid water. Likewise, the repeatability of steam extractions tends to be better because steam spreads more evenly through the sample. When total recoveries and average RSDs obtained with steam and liquid water under different conditions were compared (Figure 12), no clear trend was evident: sometimes the recoveries were better with steam and sometimes with liquid water, and the same was true for the relative standard deviations. When the average RSDs are taken into account the results obtained with steam and liquid water can be considered similar. However, examination of the results for individual compounds (**II,III**) shows the extractions of naphthalene with liquid water to be clearly more repeatable, than those with steam. In addition, recoveries for naphthalene with liquid water were usually higher. The reason for these results may be the more efficient trapping of volatile naphthalene in extractions with liquid water because it is unlikely that liquid water would be clearly more efficient than steam in extracting naphthalene. Differences larger than the standard deviations were occasionally observed for other individual PAHs. Such differences were exceptions, however; the results with steam and liquid water were usually on the same level.

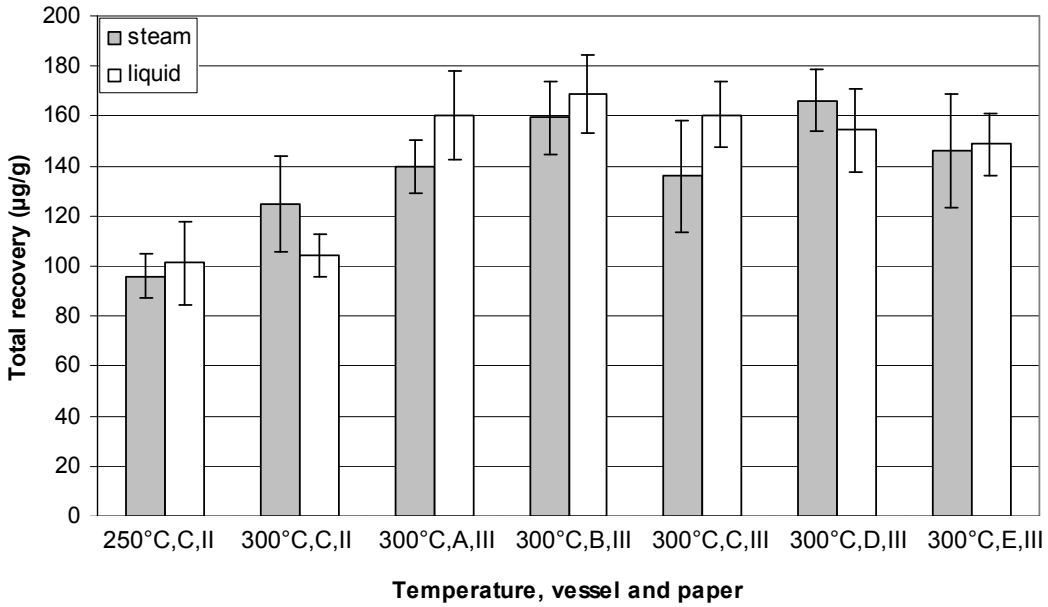


Figure 12. Comparison of steam (pressure 30 bar at 250ºC, 50 bar at 300ºC) and liquid water (pressure 250 bar): total recoveries and average relative standard deviations. PAHs included were naphthalene, acenaphthylene, acenaphthene, phenanthrene, carbazole, fluoranthene, pyrene, benzo[a]anthracene+chrysene, benzo[b+k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene. Details of the extraction vessels (A-E) can be found in Table 7.

10.2.4. Role of thermal desorption

Thermal desorption experiments were carried out at 200ºC, 250ºC, 300ºC and 350ºC (**II**) to determine the role of heat in the desorption of compounds in PHWE. Thermal desorption is a method in which thermal energy is used to vaporise adsorbed or absorbed compounds. Volatile and semi-volatile compounds present in a sample or in some sorbents, like Tenax, are vaporised and then introduced to an analytical instrument by thermal desorption [152]. We applied thermal desorption to the analytes present in a sediment sample. The situation is similar to that in direct thermal extraction (DTE) [153,154]. In DTE, the sample is introduced to the empty glass tube that forms the extraction unit. Heating releases the analytes, which are transferred with carrier gas flow into the injector of the GC-MS, which is cooled with liquid nitrogen. The analytes are trapped and focused and then released by heating of the injector. In our study, the sediment sample acted as a sorbent and the PAHs were desorbed from it in the PHWE vessel with hot nitrogen gas. The pressure of nitrogen was 13 bar. The nitrogen bottle was installed in place of the water pump, and the extraction was carried out with nitrogen instead of water. The pressure restrictor was kept fully open. No drying step was needed for the solid-phase trap. Slightly reduced trapping of the volatile PAHs is to be expected with nitrogen as extractant because the solid-phase trap is totally dry during the extraction. The trapping performance of the Tenax material is usually better if it is wetted with some solvent before the extraction as is the case in PHWE. This problem would have been avoided if LLE instead of SPE had been applied.

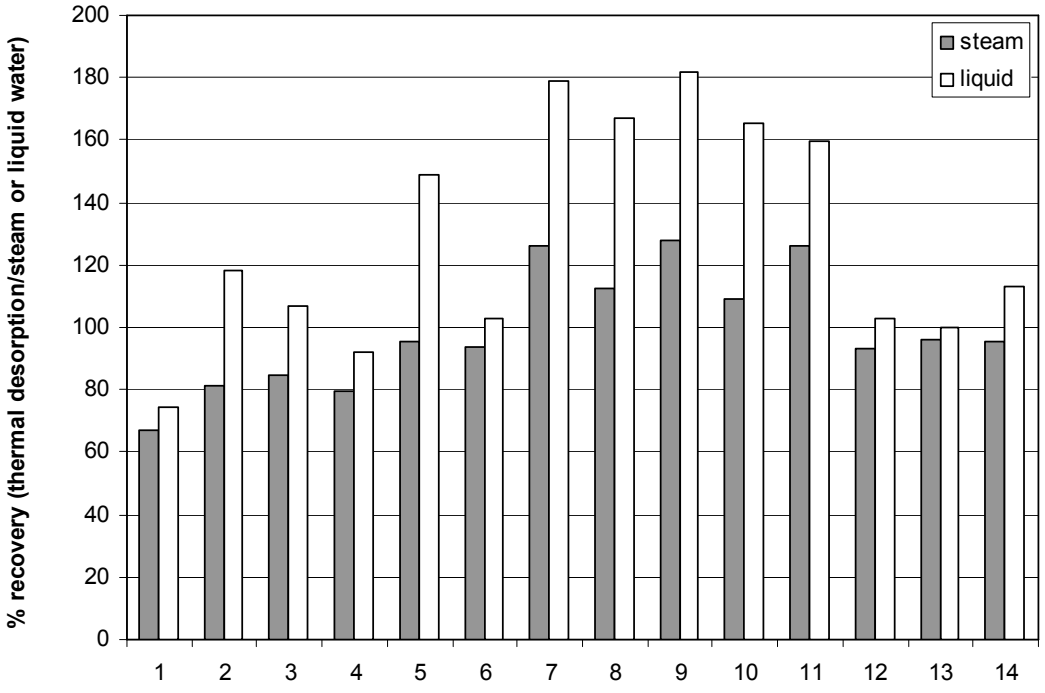


Figure 13. Comparison of thermal desorption (pressure 13 bar) with PHWE using steam (pressure 50 bar) and liquid water (pressure 250 bar) at 300ºC. Peak identification: 1 naphthalene, 2 acenaphthylene, 3 acenaphthene, 4 phenanthrene, 5 anthracene, 6 carbazole, 7 fluoranthene, 8 pyrene, 9 benzo[a]anthracene+chrysene, 10 benzo[b+k]fluoranthene, 11 benzo[a]pyrene, 12 indeno[1,2,3-c,d]pyrene, 13 dibenzo[a,h]anthracene and 14 benzo[g,h,i]perylene (Paper II).

Pyrene was the heaviest PAH that could be thermally desorbed at 200ºC. Similarly, benzo[b+k]fluorenes were the heaviest PAH that were thermally desorbed at 250ºC. The situation was quite different at 300ºC, where thermal desorption was nearly as efficient as PHWE, and for medium volatile PAHs it was even more efficient. Figure 13 compares the recoveries obtained with thermal desorption at 300ºC with those obtained by PHWE with steam and liquid water at the same temperature. For medium volatile PAHs, nitrogen is more effective than steam and much more effective than liquid water. Still, the solvating effects of water are needed when PAHs with high molecular mass are extracted. The finding that the recoveries obtained with nitrogen for low molecular mass PAHs were less than those of PAHs of medium molecular mass is probably related to the inefficient trapping of low molecular mass PAHs in a Tenax trap. When nitrogen flows through a trap not wetted by a solvent, a portion of the most volatile PAHs may remain untrapped and the recoveries of those PAHs will be too low. Inefficient cooling of the nitrogen entering the trap may also have reduced the recoveries of the most volatile PAHs.

Thermal desorption with nitrogen could be carried out at 350°C. With steam and liquid water at 350°C, however, the pressure fluctuated widely and there were leakages in the equipment. It is not practical, therefore, to carry out extractions with water at such a high temperature, at least with a sample size of 0.5 g. At very high extraction temperatures the selectivity in the extractions is lost, and more matrix compounds are extracted, and the analysis of the extracts is more demanding. The cleaning of the extraction system between the extractions also becomes more difficult because the frits and tubings are sometimes seriously blocked. In the test extractions with water at 350°C, it was necessary to wash the extraction vessels with 1M HCl to prevent plugging of the frits.

The instrumental problems encountered with steam and liquid water were not as severe with nitrogen. Perhaps nitrogen did not extract the matrix compounds as efficiently as water, and the equipment was not blocked. The recoveries obtained with nitrogen at 350°C and 300°C were similar. Although the recoveries decreased slightly for some of the PAHs and increased for others, there was no clear trend. The decrease in recoveries for some of the PAHs when the temperature was increased from 300°C to 350°C may be related to thermal decomposition of PAHs or to undetected leaks in the extraction system when it was used near its upper temperature limit.

In a study of phenanthrene, the relative error was larger in the extractions with nitrogen than in the extractions with water, and smallest in the extractions with steam (Figure 14). No clear trend in the relative error was seen for pyrene and benzo[a]pyrene. The relative error is the value obtained when the experimental value is subtracted from the true value and the result is divided by the true value. A positive value indicates that the experimental value is smaller than the true value, and a negative value that it is larger.

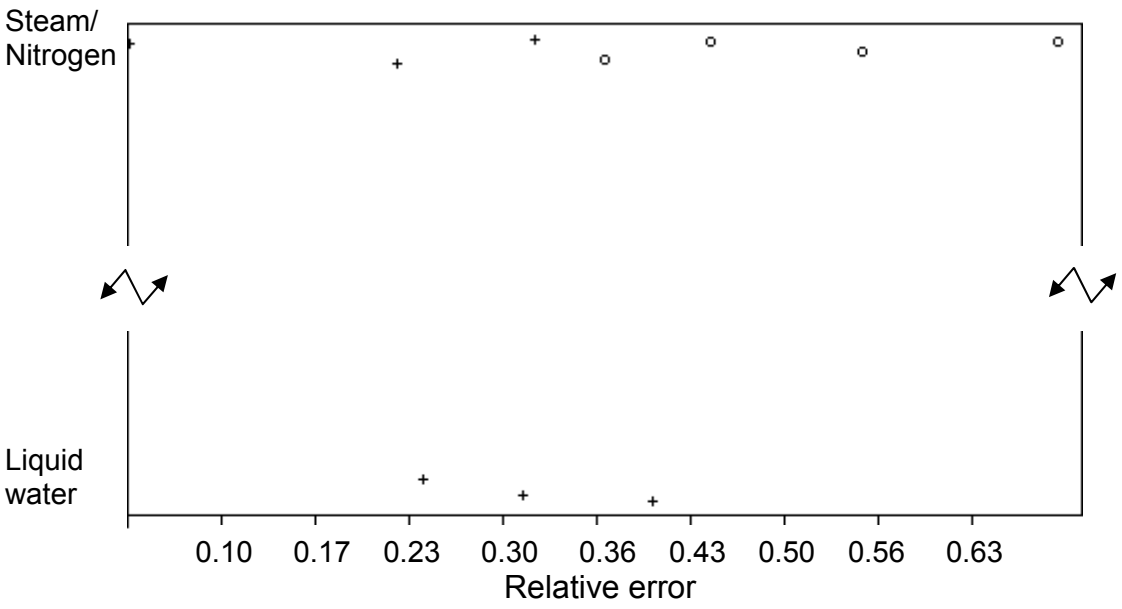


Figure 14. Comparison of the relative error in extractions of phenanthrene with nitrogen (o) and pressurised hot water (+). The + -signs in the lower part of the figure represent liquid water and those in the upper part of the figure steam (Paper II).

Although the use of PHWE at 350ºC failed in this study, PHWE has been performed at 325ºC and 350ºC earlier in our laboratory. The temperature of 325ºC was found optimal for the extraction of brominated flame retardants from 500 mg sediment in off-line coupling [155]. Later, PHWE-LC-GC was applied for the extraction of the same compounds at 325ºC, but the sample size needed for the on-line coupling was only 100 mg [36]. The sample matrix did not block the capillaries and frits when the amount of sample was only 100 mg. In the extraction of polychlorinated dibenzofurans and naphthalenes from industrial soil, a temperature as high as 350°C was used [151], but substantial co-extraction of matrix components occurred, and sometimes the equipment became plugged.

10.2.5. Effect of extraction vessel geometry

The effect of extraction vessel geometry on recoveries was studied with the vessels desribed in Table 7. The outer diameter of all the vessels was 2.0 cm. Thus long vessels with small i.d. were heavier and had larger thermal mass than short vessels with large i.d. There might, therefore, have been some differences in the warming rates of the vessels. In any case, the differences are not large, and all the vessels had enough time (extraction time 30 minutes) to heat up to the extraction temperature. The total weights of the vessels (vessel + lid + sealing ring +screws) ranged from 239 g to 384 g. The preheating of water also ensured that the temperature of the extractant water was the same for every vessel during the extraction.

Table 7. Dimensions of the extraction vessels (Paper III).

Vessel/dimensions	Length (cm)	i.d. (cm)	Volume (ml)
A	7.7	0.7	3.0
B	5.4	0.7	2.1
C	3.7	1.0	2.9
D	2.5	1.0	2.0
E	1.3	1.5	2.3

The effect of extraction vessel geometry was studied with both steam and liquid water. With steam, the best total recoveries for certified PAHs were obtained with vessel D and the worst with vessel C (**III**). Comparison of the recoveries for the individual PAHs showed the same general trend (Figure 15 a). With liquid water the best total recoveries were obtained with vessel B, and the worst with vessel D (which gave the best total recoveries with steam) (**III**). The failure of vessel D to give high recoveries with liquid water can be explained by channelling. Unlike steam, which spreads uniformly in the extraction vessel, liquid water finds paths along which it preferably flows. This channelling phenomenon is more pronounced in broad extraction vessels and it may lead to lower recoveries because part of the sample may remain unextracted. Channelling was also observed for the broadest vessel E. The same general trend in the recoveries that was observed for total recoveries was also observed for individual PAHs with liquid water (Figure 15 b).

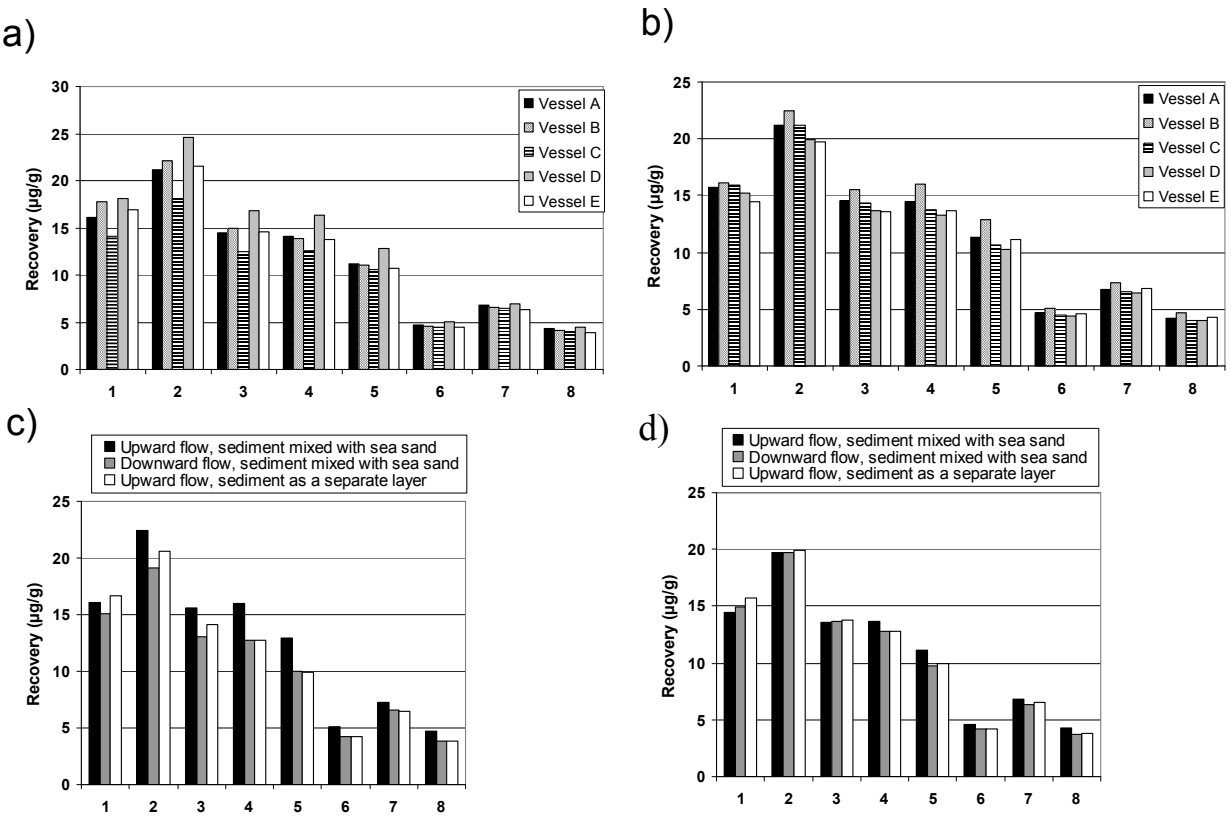


Figure 15. Recoveries for PAHs in certified EC-1 sediment with a) vessels A-E and steam (50 bar), b) vessels A-E and liquid water (250 bar), c) vessel B and liquid water (250 bar) with different set-ups and d) vessel E and liquid water (250 bar) with different set-ups. Peak identification: 1 phenanthrene, 2 fluoranthene, 3 pyrene, 4 benzo[a]anthracene+chrysene, 5 benzo[b+k]fluoranthene, 6 benzo[a]pyrene, 7 indeno[1,2,3-c,d]pyrene, 8 benzo[g,h,i]perylene (Paper III).

The length and internal diameter of the vessel as well as the vessel volume affect the recoveries. However, the differences in the recoveries with the five vessels were quite small with both steam and liquid water (**III**). Surprisingly however, the differences in the recoveries were larger with steam than with liquid water. The volume of the vessel somehow

affects the recoveries. Comparison of the total recoveries with steam and liquid water showed that the recoveries obtained with the 2-ml vessels (B, D and E) were, on average, slightly better than those of the 3-ml vessels (A,C). This is quite logical because, in the course of the extraction, the small vessel is flushed with more fresh water than the large vessel. If the vessel is fully packed, as in this study, however, the difference is not large. It is always advantageous to pack extraction vessels fully to avoid dead volume in the system.

The RSDs in the extractions were, on average, slightly larger with liquid water (9.4%) than with steam (9.2%) when the PAHs with certified reference values were included. This is in accordance with earlier findings that extractions with steam are more repeatable [42,43]. The RSDs for naphthalene, the most volatile analyte, were nevertheless very high with steam, probably because of the inefficient trapping of naphthalene in steam extractions. The recoveries for naphthalene were also lower with steam than with liquid water.

Vessel C was the vessel that was used in early work (**II)**. When steam was applied, the recoveries were lower and RSDs higher with vessel C than with vessels A, B, D and E. However, with liquid water and vessel C the RSDs were low and recoveries average. A correlation between relative error and vessel volume was observed only for phenanthrene (not for pyrene and benzo[a]pyrene) and only when steam was applied as extractant. The relative error was smallest with small-volume vessels as illustrated in Figure 16.

It can be concluded that geometry has only minor effect on the recoveries: relatively large changes in the vessel geometry do not cause dramatic changes in the recoveries. This is a good finding as it indicates that PHWE extraction is a robust method.

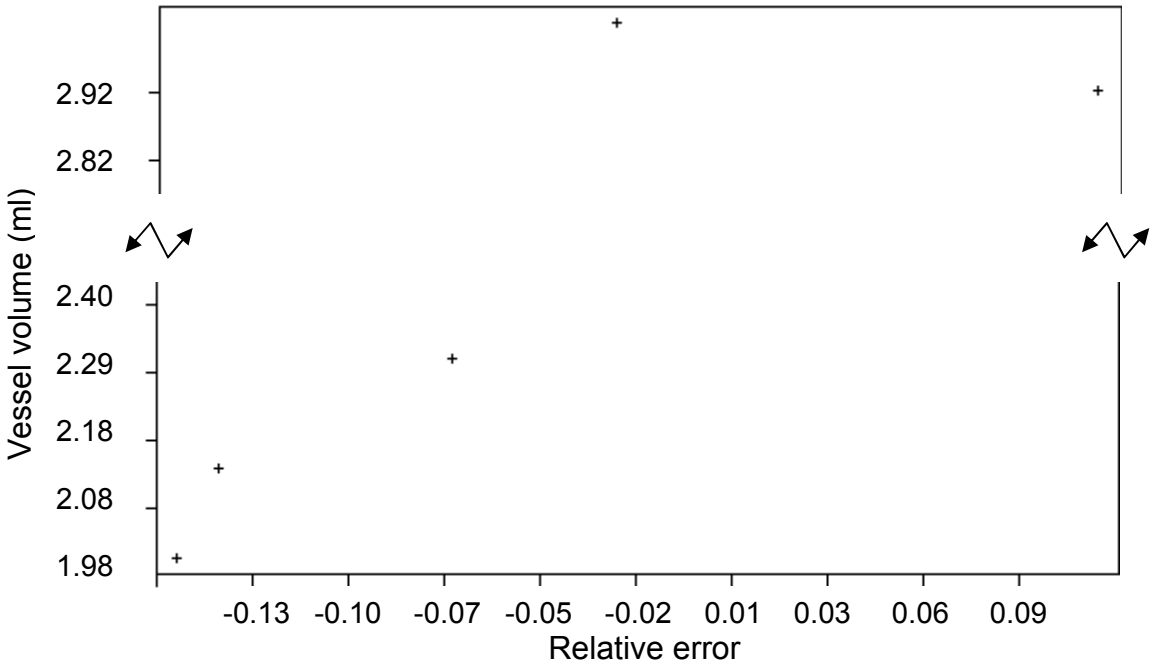


Figure 16. Relative error as a function of vessel volume for phenanthrene at 300ºC using steam (50 bar) (Paper III).

10.2.6. Effect of water flow direction and sediment packing

In the first extractions of this work, the direction of water flow in the vessel was upwards and the sediment was mixed with sea sand (1:1). The effects of flow direction and sediment packing on recoveries were studied with liquid water and vessels B and E. Vessel B was selected because it gave the highest recoveries with liquid water, and vessel E because it gave low recoveries and differed most from vessel B geometrically. The results for vessel B (**III**) and vessel E (**III**) followed the same trend: the best total recoveries were obtained when the water flowed upwards in the vessel and the sediment was mixed with sea sand. The worst recoveries were obtained with water flowing downwards in the vessel. With vessel B the recoveries were especially low for the most volatile PAHs — phenanthrene, fluoranthene and pyrene — when the water flow was downwards. Again, the differences in the recoveries were not large. The reason why the preferred water flow direction is upwards is probably that the air present in the equipment at the beginning of the extraction is then removed more effectively. Air in the equipment may disturb the flow and cause the degradation of analytes at high temperature. Recoveries were better when the sediment was mixed with sea sand before it was packed into the vessel than when it was packed into the vessel as a separate layer. Exceptionally, with both vessels B and E, recoveries of the volatile compounds were usually best when the sediment was packed as a separate layer. The recoveries for the individual PAHs with different flow directions and packing styles are shown in Figure 15 c) and d). As can be seen, the recoveries with the various set-ups were closely similar with the short and broad vessel E and slightly larger with the longer and narrower vessel B. More pressure might be needed to push water through the narrow vessel B, and the sediment can pack more tightly, more easily blocking the vessel than with the wide vessel. This would explain the larger differences with vessel B. There are also more contacts with the walls of the vessel in the narrow vessel, which more probably would lead to degradation than with the broader vessel E.

With liquid water the upward flow direction produced slightly smaller relative errors than downward flow (Figure 17). As well, mixing of the sediment with sea sand produced smaller relative errors (Figure 18). In general, both upward and downward flows have been applied in PHWE extractions, while downward flow is preferred in SFE.

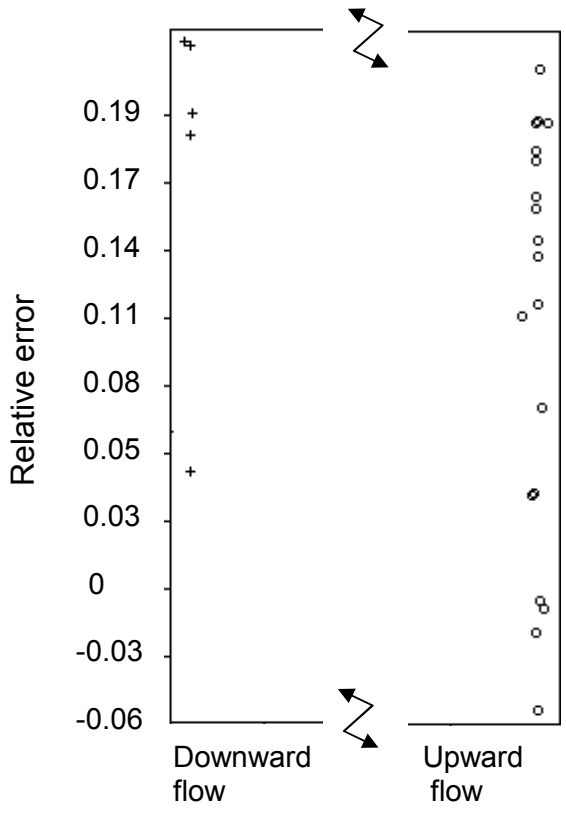


Figure 17. Comparison of relative error with upward flow (o signs) and downward flow (+ signs) direction of liquid water. PAHs are phenanthrene, pyrene and benzo[a]pyrene. Temperature was 300ºC and pressure 250 bar (Paper III).

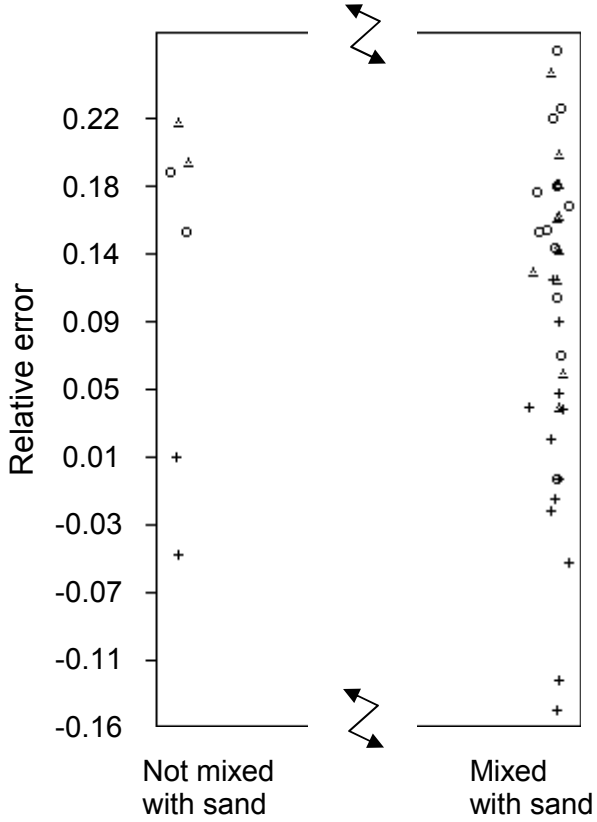


Figure 18. Comparison of relative error when the sediment was mixed and not mixed with sea sand. PAHs are phenanthrene (+), pyrene (o) and benzo[a]pyrene (△). Temperature was 300ºC and pressure 250 bar (Paper III).

To determine if the recoveries for individual PAHs with different vessels and set-ups differed significantly a statistical test, one-way ANOVA, was applied for all PAHs including those without certified values (**III**). Results of one-way ANOVA at the level of significance $\alpha > 0.05$ are collected in Table 8. Only those PAHs with statistically significant differences are listed. No dramatic effect on the results was noticed for the dimensions of the vessel, the sediment packing or the orientation of the vessel. The largest statistical difference in the results was obtained with vessel B when different packing styles and water flow directions were applied with liquid water (**III**). Compounds showing statistically significant differences in the results were four-, five- and six-ring PAHs. No statistically different results were obtained for two- and three-ring PAHs. Thus, the variation in the experimental conditions seems to have greatest effect on those large molecular mass PAHs with low volatility that are not readily extracted due to their limited aqueous solubility. Statistically significant differences in the results of more polar carbazole were found as well. Carbazole is not a true PAH-compound because, in addition to hydrogen and carbon, it contains nitrogen.

Table 8. The results of one-way ANOVA. Only PAHs with statistically significant differences are listed (Paper III).

Vessels A-E, steam, water flow direction upwards, sediment mixed with sea sand	Vessels A-E, liquid water, water flow direction upwards, sediment mixed with sea sand	Vessel B, liquid water, water flow direction up- or downwards, sediment a separate layer or mixed with sea sand	Vessel E, liquid water, water flow direction up- or downwards, sediment a separate layer or mixed with sea sand
• Fluoranthene • Pyrene	• Benzo[a]pyrene • Indeno[1,2,3-c,d]pyrene	• Carbazole • Benzo[a]anthracene + chrysene • Benzo[b+k]fluoranthene • Benzo[a]pyrene • Benzo[g,h,i]perylene	• Carbazole • Benzo[g,h,i]perylene

The effect of the geometry of the extraction vessel was studied earlier in our laboratory with a "large" vessel (V=2.8 ml, i.d.=1.0 cm) and a "small" vessel (V=1.0 ml, i.d.=0.5 cm) [156]. The large vessel gave better extraction efficiencies in a shorter time, probably because it took a longer time for the small vessel to heat up. The inner surface of the small extraction vessel was somewhat rough due to the manufacturing method, and this probably increased the adsorption of analytes onto the walls of the vessel.

In the present work, the small vessel volume, 2 ml rather than 3 ml, was found to be beneficial. Smaller volumes than 2 ml were not examined. In terms of i.d., the smallest vessels studied were A and B, both of which had an internal diameter of 0.7 cm. No noticeable decrease in the recoveries with small volume, small i.d. vessels was observed. Our sample was 25 times larger (0.5 g versus 20 mg) than that used in the earlier research with the small vessel (V=1.0 ml, i.d.=0.5 cm) [156], and this, too, may have affected the extraction.

The differences between the vessels and set-ups would have been more significant if the extractions had been carried out at lower temperatures (200 or 250°C) so that extraction was incomplete, or if the extraction vessels had been only partially filled. For practical reasons, we did the extractions at the optimised temperature we applied earlier, that is, at 300°C.

In a study of the effect of extraction vessel geometry in SFE, better recoveries of PCB standards were obtained with a broad extraction vessel (increased i.d./length), with octadecylsilane sorbent, but the vessel geometry had no effect on the recoveries when Florisil sorbent was applied [157]. When real samples were extracted using SFE, the extraction vessel geometry at constant internal volume had no effect on the recoveries [158].

10.2.7. Comparison of solid-phase and solvent trapping

The advantages and disadvantages of the solid-phase trapping and solvent trapping applied in this work are collected in Table 9. Although trapping in Tenax is a sophisticated and also environmentally friendly technique for collecting analytes, some precautions are required in the application. After several extractions were carried out, the colour of the Tenax TA at the inlet side of the trap changed as some matrix compounds were irreversibly adsorbed on it and the material became sticky. Fresh Tenax is a white powder. The backpressure of the equipment tends to increase as the Tenax material deteriorates, and it becomes more difficult to dry the trap with nitrogen. LLE requires considerable manual work, but it is simple. The results obtained with SPE (**II**) and LLE (**III**), in terms of both recoveries and RSDs, recommend the use of LLE. Trapping of the analytes in SPE has to be efficient enough that breakthrough does not occur. At the same time, the elution of the trapped analytes should be easy, requiring only a small amount of solvent. Although usually all the PAHs were eluted into one GC vial (V~ 1.7 ml), we always collected two vials from each extraction and we also analysed these two vials because occasionally a small portion of PAHs was eluted in the second vial. The need for two analyses could have been circumvented by using larger vials.

The recoveries of some PAHs were not good with solid phase trapping. The same problem was not experienced with solvent trapping, where the recoveries were consistently good. It is likely that the extraction time applied in PHWE was sufficient and the extraction was quantitative, because the conditions applied in the extractions were otherwise the same. The lower recoveries with SPE were due to inefficient and variable trapping and elution in the Tenax trap.

The relative errors in the data analysis were also significantly larger in SPE than in LLE (Figure 19, **II**, **III**). This result was obtained for all analytes studied in the data analysis, namely pyrene, phenanthrene and benzo[a]pyrene. The difference was probably due to the variations in retention behaviour in SPE.

Solid phase trapping worked better when PHWE was on-line coupled to LC-GC (**I**), but the sample and sample amount were not the same as in the off-line PHWE (**II**).

Table 9. Comparison of solid phase and solvent trapping in PHWE.

	Advantages	Disadvantages
SPE with Tenax TA	• Selective • Little manual work required • Connection to chromatographic techniques possible (**I**) • Fast (convenient)	• Blocking of the trap => problems in the drying of the trap with nitrogen • Functioning/performance of Tenax changes with time =>slight changes in retention behaviour • Careful optimisation required
LLE	• Minimal optimisation • High recoveries • Reliable	• Dirty extracts • Lot of manual work • Higher solvent amount • Formation of emulsions

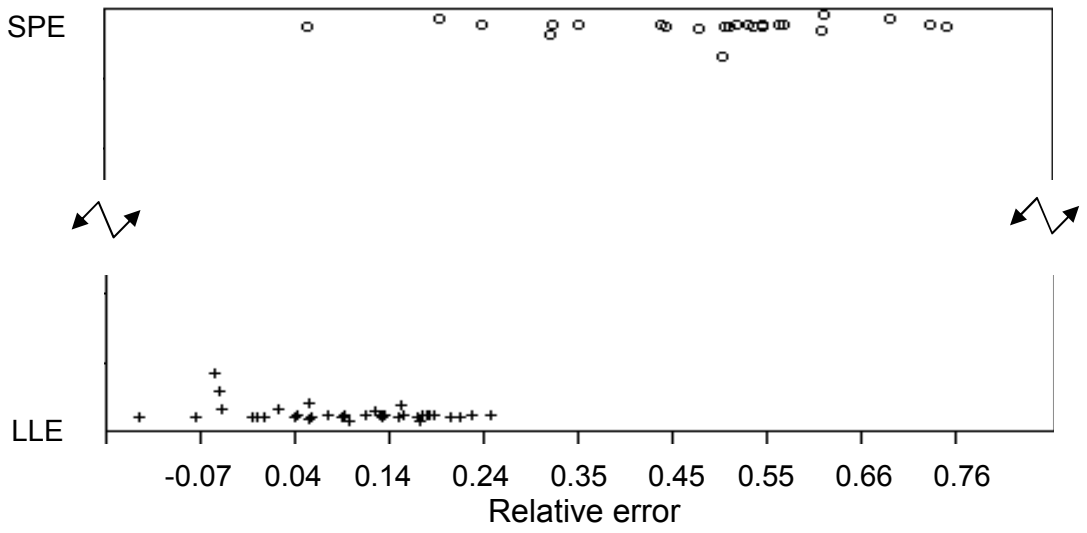


Figure 19. Comparison of relative errors in LLE (+) and SPE (o). The PAHs included in the study were phenanthrene, pyrene and benzo[a]-pyrene. A small amount of variation was added to the plotted data to assist the analysis of the data (Papers II, III).

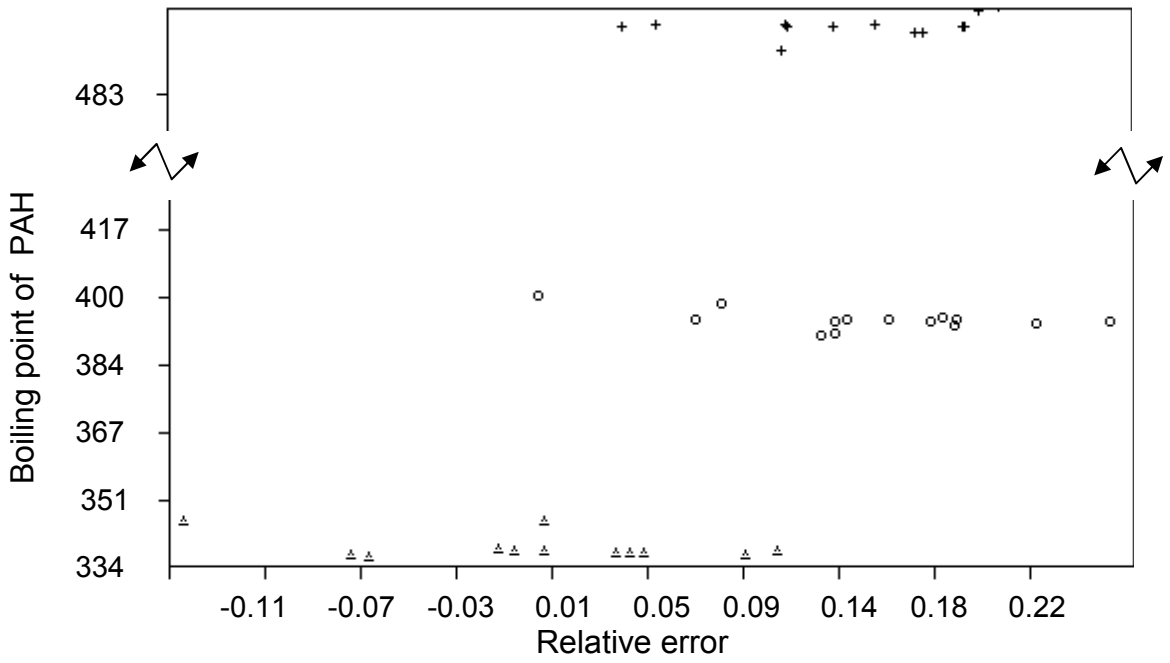


Figure 20. Dependence of relative error on the boiling point of PAH using LLE. Temperature was 300ºC and pressure 50 bar or 250 bar. PAHs included are phenanthrene (△), pyrene (o) and benzo[a]pyrene (+). A small amount of variation was added to the plotted data to assist the analysis of the data (Paper III).

Study was made of the effect of PAH properties on the relative error in LLE. The relative error was smallest with the PAH having the lowest boiling point, namely phenanthrene (Figure 20). This is logical because the PAHs with lowest boiling points are easiest to extract in PHWE. The relative error is larger with the heavier PAHs, which are more difficult to extract.

10.3. Stability of polycyclic aromatic hydrocarbons

The stability of polycyclic aromatic hydrocarbons was studied as a function of time and temperature after the experimental set-up had been optimised. Degradation and reaction products were identified. Degradation products have smaller molecular masses than their parent compounds, whereas reaction products may have either smaller or larger molecular masses. The term degradation/reaction product is applied to cover all kinds of reaction products, but degradation was the dominant reaction.

10.3.1. Selection of experimental set-up; background and observations

Background for the stability studies is discussed as well as observations made during the measurements. Seal material and the vessel atmosphere were investigated in selecting the experimental set-up for the stability studies. In addition, PEEK was tested as a vessel material to check if the catalysing action of metals is needed in degradation.

PAHs were introduced into the reaction vessel in dichloromethane. Dichloromethane was evaporated to dryness before addition of water and heating of the vessel (9.3.2.) because

otherwise it would have led to the formation of hydrochloric acid and caused extensive corrosion at high temperatures [159]. The presence of dichloromethane would also have affected the results.

Copper, Teflon modified with fibreglass, and graphite were tested as seal materials for the reaction vessels. Neither Teflon nor graphite withstood the high temperature (250-300ºC) and pressure without deformation. Aluminium seals were tested earlier, but they oxidise rapidly and leakage occurred. Copper withstands the high temperatures well, and copper seals were chosen for the stability studies. Since copper deforms slightly when the vessel is tightened, a firm seal is obtained.

When the reaction vessels in the stability studies were opened after heating, some gas escaped. The same observation was made previously when pressurised hot water in a static vessel was used for the destruction of explosives [107]. When organic compounds are fully oxidised, the only reaction products should be carbon dioxide and water, so the gas escaping from the vessel may well have been carbon dioxide. However, oxidation is not the only reaction in the vessel during heating; pyrolysis and hydrolysis are also taking place. In conditions free from oxygen in aqueous environment, pyrolysis and the decomposition of analytes is favoured at lower temperatures, whereas hydrolysis is favoured at temperatures above the critical point of water. In a study of the hydrolysis of cellulose to sugars at 320-400ºC with reaction times varying from 0.05 to 10.0 s, decomposition products of glucose were the main products in the low temperature region from 320 to 350°C [160]. But hydrolysis products predominated at 400ºC. Our stability studies were always carried out below the critical point of water and the highest temperature applied was 350ºC. If PAHs behave like cellulose, then pyrolysis and decomposition products would be favoured over hydrolysis products in the temperature region studied. In pyrolysis, organic molecules are fragmented into smaller molecules and free radicals are formed. At high temperature the self-ionisation of water is enhanced, and oxonium and hydroxide ions are formed. If the reaction mechanism in pressurised hot water resembles that in photolysis, radical cations would take part in the reaction and the oxygen in the end products would originate from water, not from residual oxygen left in the vessel after the argon purge. Free electrons are needed for the production of these radical cations.

There were two possible sources of oxygen in our studies: residual oxygen that was left in the vessel despite the purge with argon before closing the vessel and oxygen originating from water molecules during the heating. While no conclusions can be drawn about the origin of the oxygen in the reaction products of PAHs, it is probable that the vessel walls catalyse the reactions taking place, which result in the degradation/reaction of PAHs and the introduction of oxygen to the reaction products.

Polymeric PEEK was tested as an alternative to stainless steel as material for the reaction vessel. PEEK cannot be used much above 200°C because it starts to deform [161]. Although the melting point of PEEK is about 350°C, deformation starts well below that, as is expected of a polymeric material. PEEK was used in some experiments to test if degradation occurs in vessels made of other materials than metal.

Argon and air atmosphere were tested as atmosphere for the vessels in the stability studies. Degradation was expected to be slower in argon atmosphere, but for some compounds it was, in fact, faster in argon. The reason for this might be that other factors than the atmosphere in the vessel affect the degradation. The condition of the vessel might be one. On average, however, the argon atmosphere slowed the degradation, and we used argon atmosphere in the stability studies. As an example, at 350°C with a heating time of 4 h, an average 88% of PAHs degraded in argon and 93% in air. Argon was the inert gas chosen because it is heavier than air and argon atmosphere is easily generated in the vessel.

It should be added that the effect of atmosphere on the degradation is not totally clear. Divergent results have been obtained. In one study, the removal of oxygen had only a negligible effect on the degradation of some PAHs, whereas a purge with inert gas slowed down or inhibited the photodegradation of others [120,121]. These photodegradation experiments and our stability studies cannot be directly compared, however. Photodegradation experiments are conventionally carried out at ambient temperature or slightly above, whereas our studies were carried out at high temperature, where the role of molecular oxygen in degradation may be quite different. At least, oxygen is more soluble in pressurised hot water of low polarity than in polar water at ambient temperature. In addition, the mechanisms involved in photodegradation most likely differ from the degradation mechanisms in our stability studies, where light was not involved. The degradation products of different reaction mechanisms may, of course, be similar.

10.3.2. Effect of time and temperature on stability

Stability of the PAHs was investigated as a function of both time and temperature. The degradation of PAHs at 300°C as a function of time is shown in Figure 21. The experiments were carried out at 300°C because extractions are often performed at 300°C. As can be seen, for most of the compounds the degradation is rapid at 300°C. The degradation was substantial for most of the PAHs even with 10 minutes heating, the shortest time applied. The degradation varied widely among the PAHs with this 10 min heating time. In fact, the analytes were exposed to high temperature for a longer time than the actual heating time because the vessels were also exposed to heat during the warming up and cooling down of the vessels.

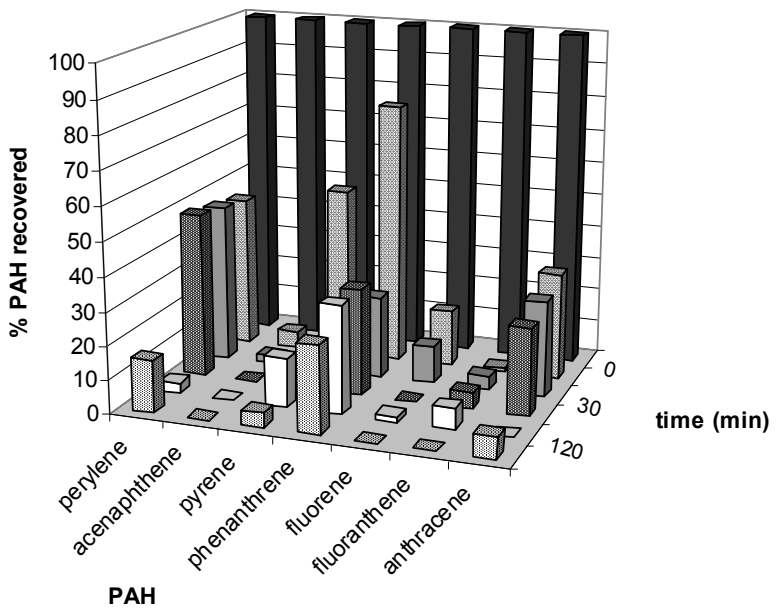


Figure 21. Degradation of PAHs at 300ºC as a function of time (Paper IV).

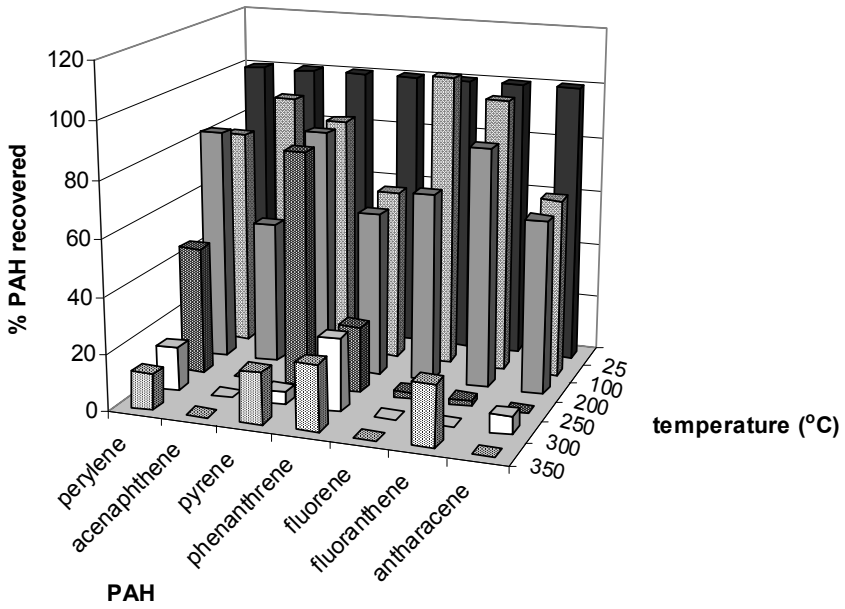


Figure 22. Degradation of PAHs in 240 min as a function of temperature (Paper IV).

The effect of temperature on the degradation was studied with a heating time of 240 min (Figure 22). At 100°C the degradation of most of the PAHs was less than 20%, and at 200°C 51-86% of the parent PAHs remained undegraded. At 100ºC many of the PAHs are only slightly soluble in water, and this may affect their tendency to degrade. Taking into account the amount of PAHs in the vessel, at 200ºC most of the PAHs are already totally dissolved in aqueous phase and this most likely enhances the degradation. At 250°C most of the PAHs were degraded. At 300°C and 350°C the degradation was on the same level and almost complete. The anomaly observed for pyrene and fluoranthene, namely that the degradation decreased when the temperature was increased from 300 to 350°C, was probably due to the new vessels used in the experiments at 350°C. Degradation is probably slightly faster in oxidised vessels used for some time than in new unoxidised vessels because the layer of metal oxide on the wall of the old vessel is a more effective catalyst than the surface of the new vessel with only a very thin metal oxide layer. Metal ions and small metal particles may also detach from the surface of the old vessel and enter the solution, and they probably catalyse the degradation more effectively than the metal and metal oxide surfaces. Finally, PAHs can be assumed to adsorb more abundantly on the porous oxide layer of the old vessel.

Study of the degradation of PAHs as a function of time and temperature showed that PAHs with small molecular mass degraded faster than those with large molecular mass. Thus, perylene and pyrene showed more resistance against degradation than acenaphthene and fluorene. Since PAHs with small molecular mass also have low melting points and boiling points and low amount of aromatic rings, it is not entirely clear which of these properties is responsible for the increased tendency to degrade.

It may be that not all the PAHs considered as degraded were in fact degraded but instead were adsorbed on the wall of the reaction vessel. This is nevertheless unlikely because three rinses with 2 ml of dichloromethane were applied to recover the PAHs and their degradation products after the heating in each stability study. PAHs are readily soluble in dichloromethane at ambient conditions.

Tests were carried out with reaction vessels made of stainless steel and reaction vessels made of PEEK to test for the reversible adsorption of PAHs on the vessel wall. When a used and washed reaction vessel was rinsed three times with 2 ml of dichloromethane, without the addition of water or PAHs and without a heating cycle, no PAHs or their degradation products were detected in GC-MS. No extra peaks were seen in runs with stainless steel vessels, but 1,1’-sulphonylbisbenzene, probably originating in PEEK material, was detected in runs with PEEK vessels. It needs to be noted, however, that these tests were carried out not at high but at ambient temperature where any PAHs reversibly adsorbed on the walls of the vessel would not be easily detached. It can be concluded that if PAHs are left on the walls of the reaction vessel after a stability study, they are not easily detached.

10.3.3. Effect of vessel on stability

In addition to temperature and heating time, the effect of three other parameters on the degradation was studied, using anthracene as model PAH. These parameters were the addition of sea sand to the vessel, vessel material and roughness of the vessel inner wall. The results of these studies are discussed in detail in paper **IV**. The addition of sea sand to the vessel had no effect on the degradation. Degradation was observed, in the form of a 9,10-anthracenedione peak, when the stainless steel vessel was replaced by a PEEK vessel indicating that the catalysing action of a metal surface is not essential for the degradation.

Studies done with a polished vessel and a conventional vessel with slightly rough inner surface showed the degradation to be more intensive in the polished vessel. With the polished vessel, only 0% fluorene, 0.5% anthracene and 0.9% phenanthrene remained undegraded after heating at 300ºC for 240 min, whereas the values for the conventional vessel were 0% fluorene, 6% anthracene and 26% phenanthrene. We expected more degradation in the conventional vessel with slightly rough inner surface because a rough surface, of larger surface area, could be expected to catalyse the degradation more effectively than a polished surface. Evidently, some other properties of the polished vessel than the surface area led to the more extensive degradation. It may be possible that the analytes are spread more evenly on the smooth surface, enabling better contact with the catalysing species. In the rough vessel the analytes are perhaps clustered on the uneven surface, so that the catalysis is less efficient and less degradation occurs. The polished vessel used in this comparison was newly prepared and thus had only a thin oxide layer on its inner surface; the conventional vessel, which had been in use for a longer time, had a thicker oxide layer. The results obtained with a polished vessel coated with tantalum oxide were similar to those obtained with the conventional oxidised stainless steel vessel. This is reasonable, because the tantalum oxide coating should correspond to a fully oxidised stainless steel surface.

On the basis of these stability studies, we would expect most of the PAHs to degrade during the 30 min pressurised hot water extraction at 300°C, because we observed that some PAHs were almost completely degraded within just 10 min at 300°C. The situation in the extraction is better, however, because of the several differences between the stability studies and extractions, as can be seen in Table 10. To confirm that PAHs are not degraded in our PHWE extractions, SIM and TIC chromatograms of several PHWE extracts were studied, and none of the degradation/reaction products found in our stability studies (see section 10.3.4) were detected.

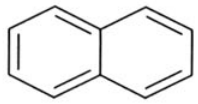
Table 10. Comparison of conditions in the stability study and in PHWE (Papers II-IV).

	Stability study (Paper IV)	**PHWE (Papers II and III)**
Mode	Static	Dynamic
Other compounds	No; only PAHs and water	Yes; sea sand, other analytes and matrix compounds (humic substances, sediment, hydrocarbons, PCBs *etc*.)
Amount of total PAHs	0.2 mg PAH	0.055 mg certified PAHs + several other PAHs
Contact of analytes with walls of the vessel (=> catalyse degradation)	Substantial	Minor
Adsorption onto the walls of the vessel	May be substantial	Minor
Distribution of PAHs	Local	More uniform

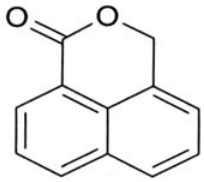
Substantial contact of the PAHs with the walls of the vessel led to more extensive degradation in the stability studies than in PHWE. Also, the adsorption of PAHs on the walls of the vessel was more extensive in the stability studies than in PHWE, leading to overestimation of the degradation. The dynamic nature of PHWE (papers **II** and **III**) decreases the possibility of degradation because most of the analytes are extracted at the beginning of the extraction and transported away from the hot oven, so the time for the degradation is short. The heating and partitioning of PAHs is also dissimilar in the stability studies and PHWE. In PHWE, water flows through the vessel from the beginning of the extraction, before the heating is started. In the stability study, in turn, the PAHs are fixed to the walls of the reaction vessel at the start of the heating. As the temperature increases, they slowly dissolve in the aqueous phase. The typical temperature at which the total 0.2 mg of a PAH is dissolved in the aqueous phase exceeds 150ºC. The presence of other analytes and matrix compounds in the extraction vessel in PHWE decreases the possibility of degradation further, because the analytes have fewer contacts with the walls of the vessel.

10.3.4. Degradation/reaction products

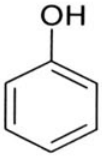
The chemical structures of degradation/reaction products of acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene and perylene detected in the stability studies are depicted in Figure 23. Included are degradation and reaction products for which the library match of the mass spectrum was over 80%, although for most of the compounds the match was over 90%. The formation of 1H,3H-naphtho[1,8-*cd*]pyran-1-one indicates that the solvent molecules have taken part in the degradation [118].



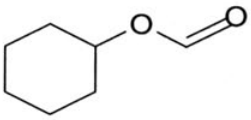
naphthalene



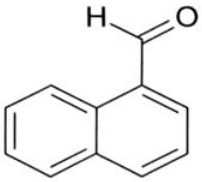
1H,3H,naphtho[1,8-c,d]pyran-1-one



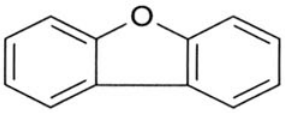
phenol



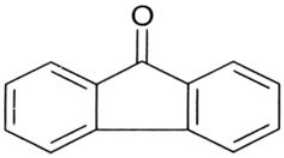
1(3H)-isobenzofuranone



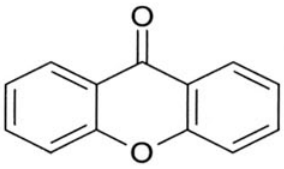
1-naphthalene-carboxaldehyde



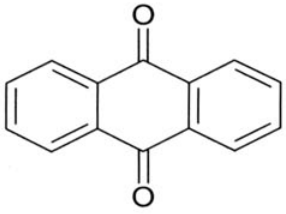
dibenzofuran



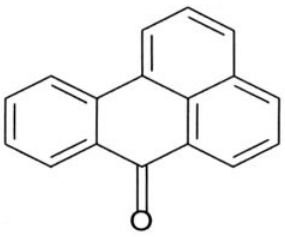
9H-fluoren-9-one



9H-xanthen-9-one



9,10-anthracenedione



7H-benzo[d,e]anthracen-7-one

Figure 23. Chemical structures of the degradation and reaction products of acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene and perylene (Paper IV).

It is not possible to detect all the degradation/reaction products by GC-MS; highly polar and high molecular mass compounds as well as small molecular mass degaradation products such as carbon dioxide and water remain undetected.

We carried out reversed phase LC analysis on the contents of the reaction vessel to check for the presence of polar degradation/reaction products. The analyte selected for this study was anthracene and the reaction vessel was heated at 300°C for 240 min. The contents of the vessel were analysed for phenol, benzaldehyde and salicylic acid because these products have previously been found in the degradation of anthracene by photooxidation [162]. When the contents of the vessel were analysed by LC, phenol was found but not benzaldehyde or salicylic acid. The presence of phenol was confirmed by spiking and by analysis of a standard solution of phenol by LC. It is likely that other polar degradation/reaction products in addition to phenol were formed as well, but the subject was not studied further.

The degradation/reaction products in Figure 23 are mainly oxidation products of PAHs. The most common reaction products are ketones. The origin of the oxygen is not clear. Because the air in the vessel was replaced by argon before the heating of the vessel, however, the most probable source of oxygen is the water in the vessel. The mechanism for the reaction could be hydrolysis or radical cation mechanism. Some oxygen may have been left in the water despite the sonication, or the atmosphere in the vessel may have contained some air in addition to argon. The effect of molecular oxygen on the photodegradation of PAHs in dilute aqueous solutions has been shown to be minor, however, and the oxygen in the degradation products probably comes from water via radical cation intermediates [120].

Although the degradation/reaction products were chemically similar compounds, the products differed for the individual PAHs (**IV**). Thus, a variety of degradation/reaction products was observed for acenaphthene, while only one degradation/reaction product each was detected for fluoranthene and perylene, and no degradation/reaction products were observed for pyrene. Similar products to those observed here have been found in studies where PAHs were photodegraded [119] and creosote-contaminated soils [114] and soil/compost mixtures [115]. It seems, therefore, that similar products are obtained despite the differing degradation methods and matrices. Often, these intermediate oxidation products are then degraded to small acids, and, if degradation proceeds to the end and the mechanism is oxidation, they degrade further to the harmless end products of carbon dioxide and water. Note, however, that oxidation is not the only reaction taking place.

The amounts of selected degradation/reaction products as a function of temperature were studied (**IV**). Except for 1H,3H-naphtho[1,8-c,d]pyran-1-one, the amounts increased when the temperature was increased to 300°C and then levelled off. The exceptional compound was probably a hydrolysis product of acenaphthene and clearly a reaction intermediate. After reaching maximum concentration at 200°C, it probably converted to other more stable reaction products when the temperature was increased. The degradation of the parent PAH was not balanced by a corresponding increase in the amount of degradation/reaction products detected. This is because highly polar and high molecular mass compounds, and also volatile and low molecular mass compounds, are not detected in GC. Another reason for the lack of mass balance is that some PAHs may have adsorbed irreversibly onto the vessel during heating.

10.3.5. Effect of concentration of polycyclic aromatic hydrocarbons on degradation/reaction

The effect of the amount of PAH on the degradation/reaction was studied with pyrene and fluoranthene. The usual concentration of the PAH solution added to the reaction vessel was 2 mg/ml, and 100 µl of the solution was added. We tested the effect of amount of PAH on the degradation by adding five times less PAH (100 µl of 0.4 mg/ml) and five times as much PAH (500 µl of 2 mg/ml solution) to the vessel and keeping the vessel at 300ºC for 240 min. When five times less PAH was added, no PAHs were recovered after the heating. Concentrating of the extract did not help, indicating that all the PAHs may have been degraded. When five times higher amounts of PAH than usual were added, much higher percentages of PAHs were recovered. As shown in Figure 24, when more PAH than usual is inserted to the vessel, a smaller percentage of PAH is degraded. When the amount of PAH is small, in turn, the amount of metal and metal oxide catalysing the degradation/reaction is probably sufficient to degrade all the PAH present. The point at which all the PAHs are degraded may reflect the capacity of the active places on the vessel walls for degradation. Also, importantly, the relative amount of the adsorption is greater when the amount of PAHs is small and a relatively larger amount of PAHs degrades on the wall of the vessel before PAHs have time to be dissolved. As the amount of sample increases, the PAHs and other compounds protect each other from degradation/reaction.

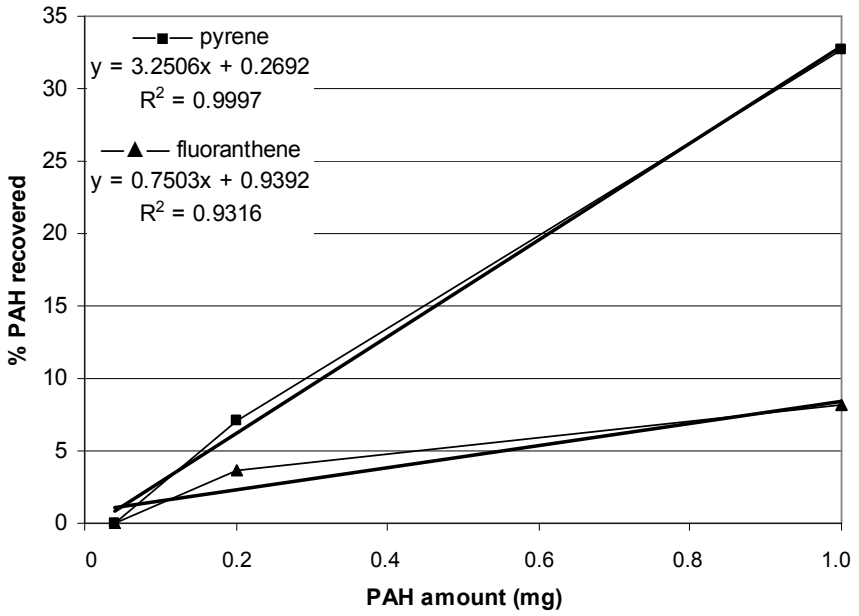


Figure 24. Effect of amount of PAH on degradation/reaction at 300°C with a heating time of 240 min (Paper IV).

10.4. Aqueous solubility of polycyclic aromatic hydrocarbons at elevated temperatures

Better understanding of the behaviour of PAHs in pressurised hot water extraction was sought by measuring the solubilities of selected PAHs at high temperatures. When the solubility of PAH is low, the kinetics of the extraction becomes slower and the extraction is solubility restricted. When the PAH concentrations are high, the solubility is more likely to be the limiting factor in the extraction.

10.4.1. Testing of equipment below the melting point of polycyclic aromatic hydrocarbons

Preliminary solubility tests with pyrene and anthracene were carried out with the equipment for solubility measurements to see that it was working. The tests were done below the melting point of PAHs because literature values measured with similar equipment were available for comparison.

The conditions tested and the conditions chosen for the measurements are collected in Table 11. As can be seen, the repeatability was clearly best when toluene was used as the collection solvent. The poor repeatability with heptane may have been caused by the lower solubility of PAHs in heptane than in chloroform or toluene [163]. The poor repeatability with chloroform, on the other hand, may have been due to its high volatility (b.p. 61°C). Although it did not give the highest solubilities a conventional vessel having a length of 3.7 cm, i.d. of 1.0 cm and volume of 3 ml was chosen over a short and broad vessel because in pressurised hot water extrations with liquid water the conventional vessel gave higher recoveries with smaller average RSD than the short and broad vessel (**III**). With the broad vessel also the possibility of unwanted channelling is larger. The amount of PAH spiked into the sea sand was 20 wt% so that sufficient PAH was available for the saturation.

Table 11. Conditions tested and those chosen with pyrene at 50ºC at 50 bar and the solubilities (x_2) obtained (Paper V). * n means the number of separate solubility measurements. Six fractions were collected and analysed in one measurement (n=1).

	Collection solvent (RSD %)	**Geometry of the vessel**	**Wt% of the PAH in sea sand**
Tested	•Heptane (81) •Chloroform (109) •Toluene (23)	•Short and broad (length 1.3 cm, i.d. 1.5 cm, volume 2 ml): $x_2 = (6.79 \pm 0.61) \times 10^{-7}$, n=1* •Conventional (length 3.7 cm, i.d. 1.0 cm, volume 3 ml): $x_2 = (6.37 \pm 0.25) \times 10^{-7}$, n=4* •Long and narrow (length 7.7 cm, i.d. 0.7 cm, volume 3 ml): $x_2 = (5.40 \pm 0.041) \times 10^{-7}$, n=1*	•10 wt%: $x_2 = (7.18 \pm 0.61) \times 10^{-7}$, n=1* •20 wt%: $x_2 = (6.37 \pm 0.25) \times 10^{-7}$, n=4* •30 wt%: $x_2 = (6.46 \pm 0.80) \times 10^{-7}$, n=1*
Chosen	Toluene	Conventional (length 3.7 cm, i.d. 1.0 cm, volume 3 ml)	20 wt%

The solubilities obtained were compared with literature values obtained with a similar technique [136]. As the agreement was good (**V**), the focus was then on the solubility measurements above the melting point of the PAHs.

10.4.2. Optimal set-up for the saturation cell above melting point

A flow-through saturation cell was applied in measurements below the melting point of the analytes. The set-up could not be used above the melting point because the liquid analytes would have been swept mechanically from the cell with the water flow. In fact, it is possible to use a flow-through saturation cell in solubility measurements of liquid solutes, but then the densities of the solutes have to be known and taken into account at each temperature. The water flow is adjusted from top to bottom when the density of the solute is less than that of water, and from bottom to top when the solute is heavier than water [129]. For measurements above the melting point, we constructed a special saturation cell with inner cartridge, where the PAHs diffuse from the cell into the bypassing water flow (Figure 8). Different set-ups were tested with this new cell (**V**). The kinetics of the measurements (Figure 25), and the solubilities obtained and the RSDs in one measurement and between measurements (Table 12), were compared to determine which of the set-ups is best. Pyrene was used as test analyte. Some set-ups were too hindered to allow efficient diffusion of the PAHs into the water flow and the saturation of water. The solubility measurements with a frit and with a cartridge gave too low solubilities and high RSD values and, accordingly, the use of cartridge and frit in the saturation cell was abandoned. In the preliminary tests, sea sand was also packed into the saturation cell, but this prevented proper diffusion of pyrene into the water flow, and the use of sea sand was rejected too. In the end, a set-up without sea sand, without frit and without cartridge was chosen. Doubling the amount of pyrene from 0.6 g to 1.2 g improved the repeatability both within a measurement and between measurements and thus 1.2 g was chosen (**V**). The same effect as was obtained by doubling the amount of pyrene probably would have been obtained by decreasing the volume of the saturation cell, but since it was easier to increase the amount of solute than construct a new cell, the amount of solute was doubled. The volume of the cell was relatively large (11 ml) because there had to be room for the inner cartridge tested in some of the measurements.

Table 12. Solubilities and RSDs of the solubility measurements for pyrene at 300ºC and 100 bar using different set-ups (Paper V).

	a) Frit, no cartridge, 0.6 g pyrene (n=4)	b) No frit, no cartridge, 0.6 g pyrene (n=4)	c) No frit, cartridge, 0.6 g pyrene (n=2)	d) No frit, cartridge, saturation cell upside down, 0.6 g pyrene (n=2)	e) No frit, no cartridge, 1.2 g pyrene (n=4)
Solubility (x_2)	5.65×10^{-5}	1.21×10^{-3}	4.86×10^{-4}	1.07×10^{-5}	1.41×10^{-3}
Solubility (mg/ml)	0.635	13.8	5.46	0.120	15.8
RSD (%) of different solubility measurements	29	36	123	4	12
Average RSD (%) of single measurement in which five fractions (10 min) were collected	56	31	48	64	8

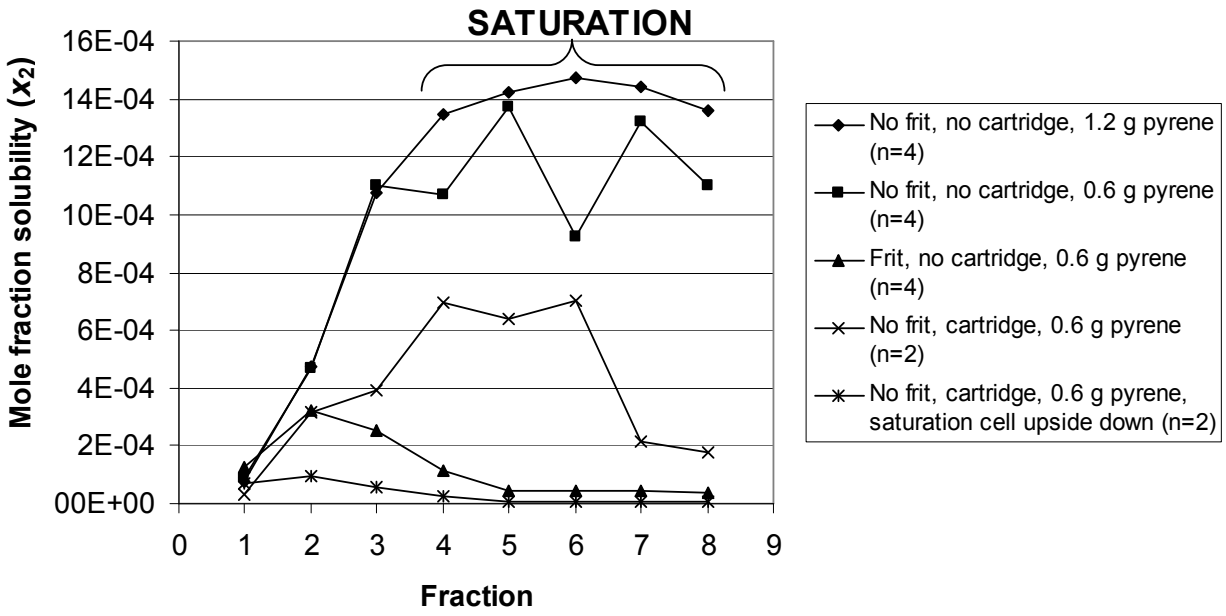


Figure 25. Kinetics of the solubility measurements at 300°C and 100 bar using different set-ups and pyrene. Saturation indicates the region where the samples where collected in the solubility measurements. Each fraction was collected for 10 minutes (Paper V).

Figure 26 shows the kinetics of the solubility measurements under optimised conditions below and above the melting points of pyrene, anthracene and acenaphthene at temperatures from 50°C to 300ºC (for acenaphthene only above the melting point). Although there should be no clear upward or downward trends in the solubilities of the successive fractions, a slight upward trend can be seen in the solubilities at the higher temperatures. The upward trend is most pronounced for acenaphthene at 250°C.

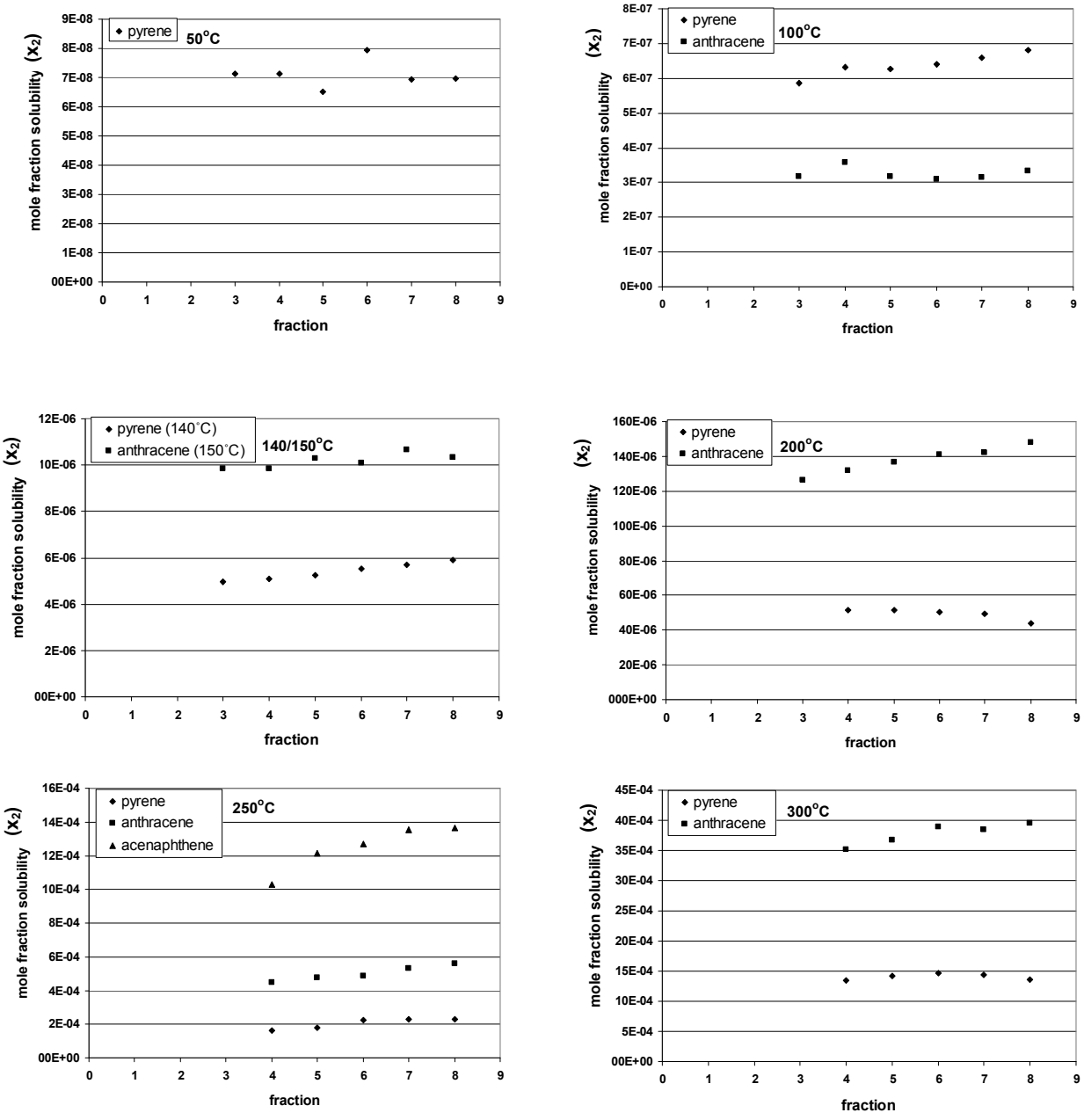


Figure 26. Kinetics of the solubility measurements at 50-300ºC with pyrene, anthracene and acenaphthene. Pressure in the solubility measurements was 50 bar at 50ºC to 250ºC and 100 bar at 300ºC. Only those fractions that were taken into account in calculating the results are marked in the Figure (Paper V).

10.4.3. Measured solubilities

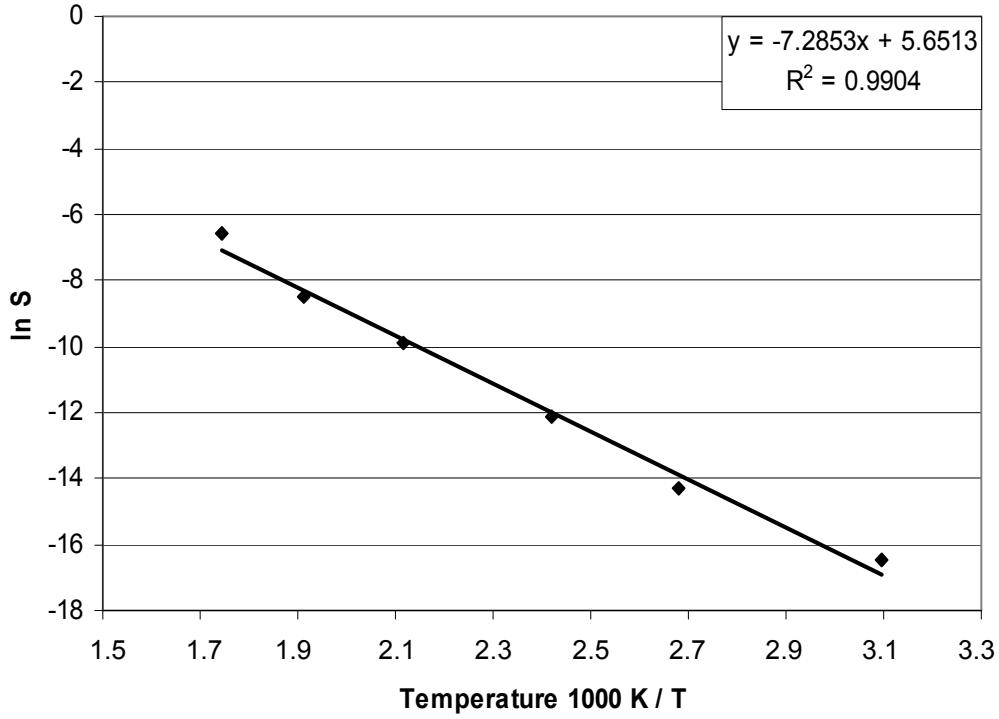
Our main target of measuring the solubilities above the melting point of the analytes was realised only after the equipment had been shown to work reliably, a saturation cell had been constructed and a proper set-up had been found of the PAHs.

The solubilities for pyrene and anthracene, below and above the melting point, are shown in Figure 27. The natural logarithm of the solubility is plotted as a function of 1000 K/T. An excellent linear correlation (R^2 value over 0.99) was found for both analytes. The solubilities at intermediate temperatures can be interpolated from the equations shown in Figure 27. Although to our knowledge, there are no literature values for comparison for the solubility measurements above the melting point of PAHs, our measurements can be considered reliable because repeatable results with a linear correlation as a function of temperature were obtained over the whole temperature range used in the measurements. It is, of course, difficult to obtain a true estimate on the accuracy because of the lacking literature values.

Solubility measurements were also carried out at 200ºC for perylene and chrysene, but those did not succeed because of blockage in the equipment. Probably the solubility of perylene and chrysene in toluene used as the collection solvent was not sufficient and the PAHs precipitated in the tubings of the equipment. Other collection solvents than toluene were not tested. The choice of the collection solvent is not easy since it has to be insoluble in water while dissolving the PAHs easily. The collection solvent cannot be a low boiling solvent because it would then partially evaporate upon mixing with the hot water in the oven, and the liquid–liquid extraction would be disturbed. This criterion excludes, for example, the use of dichloromethane and chloroform, which boil at 40°C and 61°C, respectively.

We also tried to measure the solubility of carbazole at 250 and 300ºC, but carbazole was probably degraded during the measurements since only a small peak was detected in GC-MS after the solubility measurements at 250ºC and 300°C although the aqueous solubility of carbazole should already be significant at much lower temperatures. The structure of carbazole differs from that of the PAHs because carbazole contains a nitrogen heteroatom. Nitrogen heteroatom probably makes it more sensitive to degradation, and it was therefore detected in only small amounts in the collection solvent. No degradation/reaction products were detected in the chromatograms, however. No degradation was observed when the solubility of carbazole was measured earlier at 200°C [136].

a)



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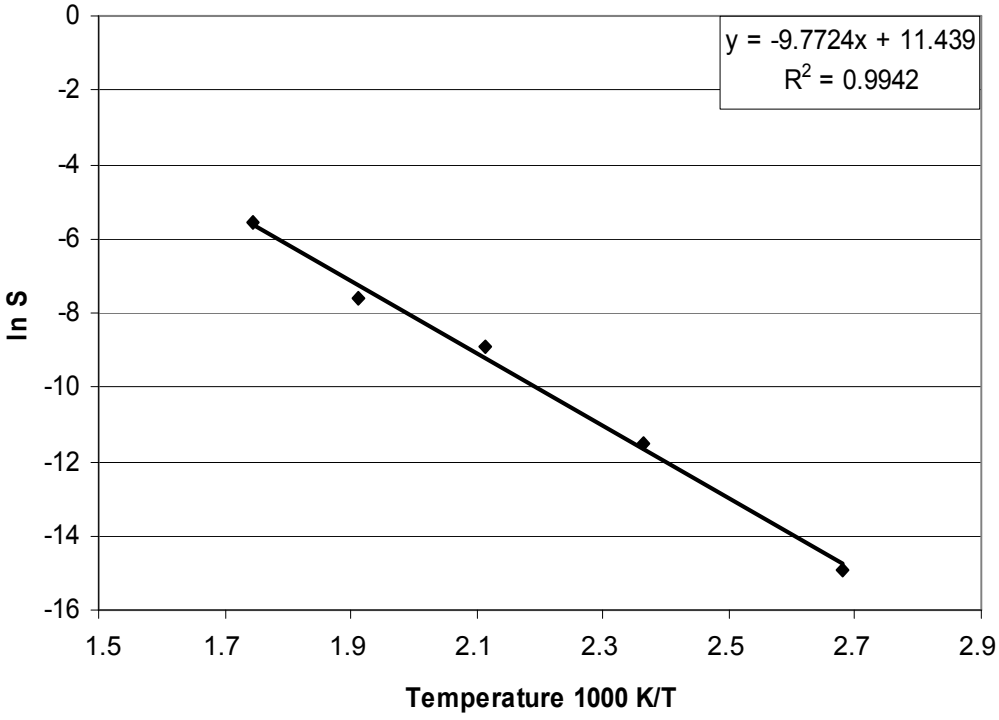

Figure 27. Solubilities of a) pyrene and b) anthracene as a function of temperature (Paper V).

The possible degradation of anthracene, pyrene and acenaphthene was studied during the high temperature solubility measurements but no degradation or reaction products were observed. The amount of PAH in the saturation cell was so much higher than in our stability studies (0.6 g or 1.2 g versus 2.0 $\times 10^{-4}$ g => over 1000× higher) that degradation was assumed to be negligible.

One problem encountered during the analysis of the fractions collected in the solubility measurements was that the retention gap in GC-MS had to be changed fairly often, probably because of small amounts of water in the collected fractions despite the drying of the fractions with sodium sulphate.

The solubilities measured for pyrene, anthracene and acenaphthene at different temperatures are collected in Table 13. The values are compared with literature values where possible. A more detailed list of the solubilities can be found in Paper **V**. For pyrene, our values and the values obtained by *Miller et al.* [136] are not similar, whereas for anthracene the values obtained at 100°C and 150ºC compare very well. Our solubility values for anthracene compare favourably with the value of *Rössling et al.* [127] at 100ºC and the value of Karásek *et al.* [137] at 200ºC.

The increase in the solubilities of anthracene and pyrene with temperature can be seen in Table 13. The rule of thumb, that the aqueous solubilities increase by an order of magnitude for every 50ºC increase in the temperature, applies fairly well in most cases. The solubility of anthracene increases more sharply than the solubility of pyrene when the temperature is increased from 100°C to 200ºC. Once the temperature exceeds the melting points of the analytes, the increase in the solubilities as a function of temperature is no longer so pronounced. This is a common phenomenon, and the solubilities of solids increase more dramatically than those of liquids when the temperature is increased [128]. Similarly, the solubilities of compounds with very low aqueous solubility at ambient conditions increase more sharply with temperature than do those of compounds with substantial aqueous solubility at ambient conditions. Pyrene has a slightly higher aqueous solubility than anthracene at ambient conditions (0.135 mg/l versus 0.073 mg/l) and this could partly explain why the solubility of anthracene increases more sharply with the temperature [126].

The certified reference value for pyrene in EC-1 sediment is 16.7 µg/g and that for anthracene 1.2 µg/g. These are much lower values than the solubilities of those compounds in water at 200-300°C. Thus the aqueous solubility of anthracene and pyrene is not a limiting factor when these compounds are extracted with PHWE above 200ºC. The situation would be quite different if they were extracted at 50-100°C, where the solubility would be a limiting factor (Table 13).

Table 13. Solubilities measured for pyrene, anthracene and acenaphthene and comparison with literature values. Pressure in our solubility measurements was 50 bar at 50ºC to 250ºC and 100 bar at 300ºC (Paper V).

PAH	Tem-pera-ture (°C)	Measured solubility (x_2)[a]	Measured solubility (µg/g)	Increa-se in solu-bility[b]	Literature value 1[c] (x_2)	Literature value 2[d] (x_2)	Literature value 3[e] (x_2)
Pyrene m.p. 156ºC	50	$(6.87\pm1.59)\times10^{-8}$	0.772		$(3.8\pm0.1)\times10^{-8}$	-	
	100	$(6.37\pm0.25)\times10^{-7}$	7.15	9.3x	$(9.0\pm0.5)\times10^{-7}$	-	
	140	$(5.40\pm1.57)\times10^{-6}$	60.6	8.5x	-	-	
	200	$(4.92\pm2.27)\times10^{-5}$	553	9.1x	-	-	
	250	$(2.05\pm0.23)\times10^{-4}$	2300	4.2x	-	-	
	300	$(1.41\pm0.17)\times10^{-3}$	15800	6.9x	-	-	
Anthracene m.p. 214-216°C	100	$(3.25\pm0.34)\times10^{-7}$	3.21		$(3.2\pm0.5)\times10^{-7}$	3.06×10^{-7}	$(4.57\pm0.32)\times10^{-7}$
	150	$(1.02\pm0.13)\times10^{-5}$	101	31.4x	$(9.2\pm0.6)\times10^{-6}$	5.75×10^{-6}	
	200	$(1.38\pm0.19)\times10^{-4}$	1360	13.5x	$(2.1\pm0.25)\times10^{-4}$	2.74×10^{-5}	$(1.30\pm0.029)\times10^{-4}$
	250	$(4.97\pm0.89)\times10^{-4}$	4920	3.6x	-	1.84×10^{-4}	
	300	$(3.78\pm0.13)\times10^{-3}$	37500	7.6x	-	-	
Ace-naphthene m.p. 92-95°C	250	$(1.25\pm0.097)\times10^{-3}$	10700		-	-	

[a] Each solubility value is a mean of four separate measurements. Five (above the melting point of PAHs) or six (below the melting point of PAHs) fractions were collected for each measurement.
[b] The increase in solubility is calculated relative to the previous temperature at which the solubility was measured for a given compound.
[c] Values from Miller *et al.* [136]. Values are based on one solubility measurement in which 10 fractions were collected.
[d] Values from Rössling *et al.* [127]. The literature value for anthracene at 250ºC is measured above the melting point.
[e] Values from Karásek *et al.* [137].

10.5. Observed correlations

Self-organising maps and non-linear data analysis provided a useful tool for studying the data obtained in this work and observing the trends in the data sets. The effect of different parameters on the recoveries and relative errors in PHWE and the dependence of different variables on each other were studied. As an example, the smaller relative errors in LLE than in SPE were easily recognised, as well as the larger relative errors in thermal desorption with nitrogen than in extractions with water. The variables were found in clusters (Figure 28). Those variables that are close to each other and in the same cluster are correlated with each other. Each variable is visualised in a one-component plane. The planes that are similar to each other have a strong positive correlation: viscosity and relative permittivity, for example. Planes with negative colouring have a strong negative correlation: internal energy and density, for example.

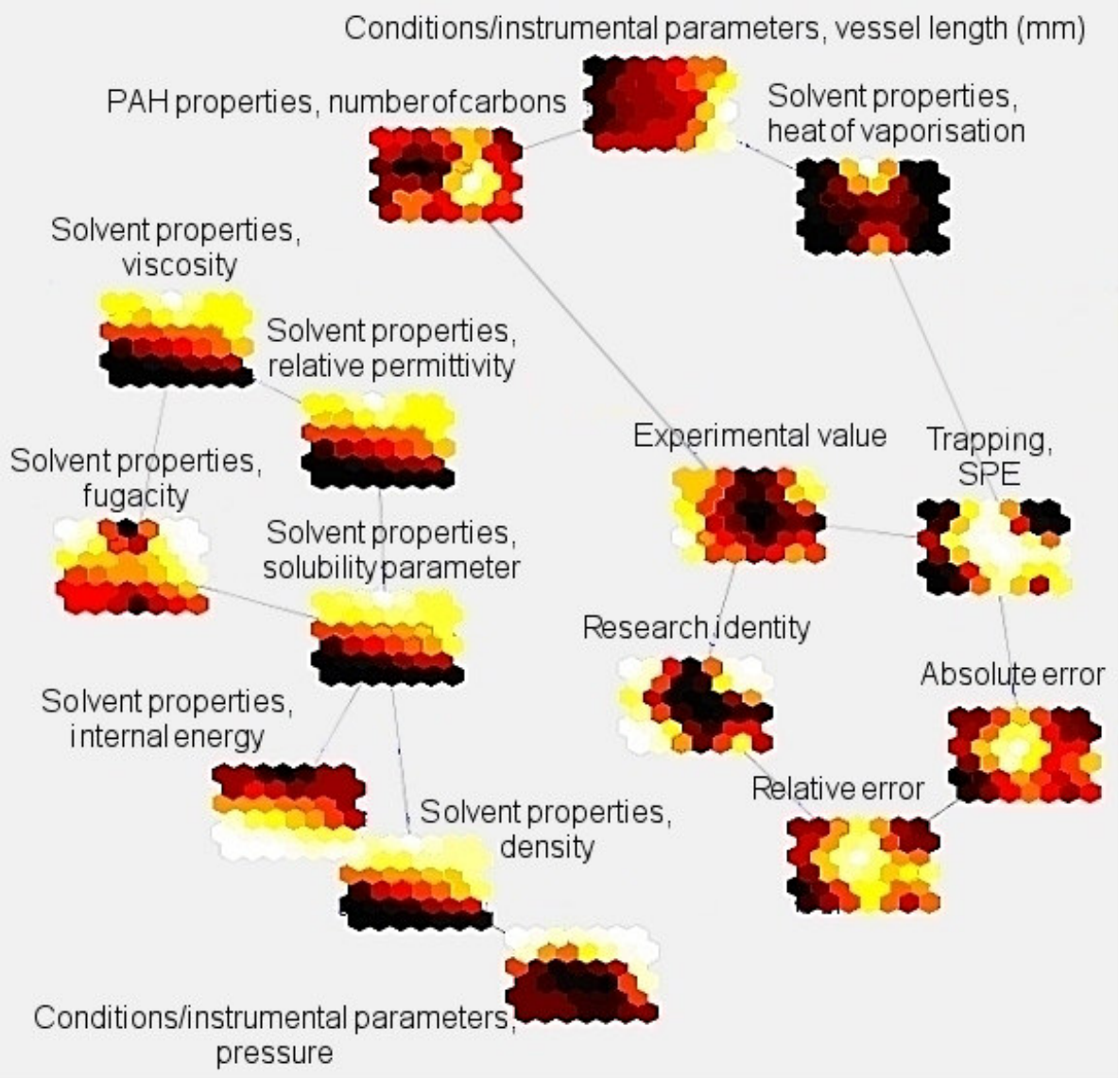

Figure 28. Correlation of properties studied in this work (Papers II, III).

A summary of the most significant parameters that were studied is presented in Table 14. Preferred alternatives are presented as well as the effect of the parameters on PHWE. Correlations were found between some parameters. For example, melting point, boiling point, molecular mass and solubility of PAHs are correlated, as are relative permittivity, viscosity, density and solubility parameter of the solvent. These values are not independent of each other and they increase or decrease in value at the same time.

Table 14. Summary of the parameters studied in this work and their effect on PHWE (Papers I-V).

PARAMETER		**PREFERRED ALTERNATIVE**	**EFFECT ON PHWE**
Solubility		-	Large
Stability		-	Large (at low concentrations)
Thermal desorption		-	Large
Sediment packing	Mixed with sea sand	Mixed with sea sand	Small
	As a separate layer		
Water flow direction in the vessel	Upward	Upward	Small
	Downward		
Vessel type	Laboratory-made	Commercial (slightly better recoveries)/ laboratory-made (lower RSDs)	Small
	Commercial		
Trapping	Solid-phase trapping	Solvent trapping	Large (problems should not arise with SPE)
	Solvent trapping		
Temperature		-	Large
Pressure	Steam	Sometimes steam, sometimes liquid water	Usually small
	Liquid water		
Geometry of the vessel	Length	Vessels with large i.d. should not be used, 2 ml vessels preferred over 3 ml vessels	Usually small, some trends were observed
	Volume		
	i.d.		
Solvent	Water	Water	Quite large
	Nitrogen		
Solvent properties*		-	Large
PAH properties**	Boiling point of PAH	PAHs with low boiling points are easier to extract and their relative errors are smaller	Large

*Solvent properties are often correlated with each other, and a change in the value of one parameter changes the values of other parameters
** PAH properties are also often correlated.

11. CONCLUSIONS

On-line coupled PHWE-LC-GC was successfully applied to the analysis of PAHs in sediment samples. Only 10 mg of sample was needed, and the whole analysis was performed reliably, without manual sample preparation, in a closed system. The sensitivity of the method was up to 800 times better than in traditional systems, and if MS detection instead of FID had been used the sensitivity would have been increased further. The recoveries obtained with PHWE-LC-GC were comparable to those obtained with other techniques, and the repeatability and LOQs were better in PHWE-LC-GC.

A laboratory-made extraction vessel was found to perform equally as well as a commercial extraction vessel. The laboratory-made vessel was more robust, however, and less force was required in the tightening because the soft sealing ring tightened more easily against the sealing surfaces than did the two hard metal surfaces without a separate seal in the commercial vessel.

Surprisingly, the effect of thermal desorption was found to be even greater than the solvating effect of hot water in PHWE. At 200 and 250ºC, thermal desorption was responsible for about 50% of the recoveries in PHWE, and at 300ºC it was clearly the main mechanism. The solvating effect of hot water was more important in the extraction of high molecular mass PAHs.

Comparison of solid-phase and solvent trappings as trapping methods in PHWE showed the latter, although more labour intensive, to be more robust and to give higher recoveries. Solvent trapping can thus be recommended over solid-phase trapping.

Usually steam gives higher recoveries and smaller RSDs for hydrophobic compounds than does liquid water. No clear trend was observed in our study, however. Sometimes the recoveries were larger with steam and sometimes with liquid water, and the same was true for the RSDs in the extractions.

The geometry of the extraction vessel had no great effect on the recoveries, nor did the style of sediment packing in the vessel nor the direction of water flow. The results were compared by one-way ANOVA, and significant differences were observed only for some PAHs. It is an advantage that the vessel geometry does not have dramatic effects on the recoveries, because otherwise the vessel geometry would need to be carefully optimised before the beginning of extractions. Some trends were observed. Short and broad vessels should be avoided with liquid water because of the channelling observed. In addition, a small vessel volume seems to be beneficial (2 ml better than 3 ml). It should be kept in mind that if lower temperatures had been used or the vessels had been only partially filled, the differences would have been larger. We wanted, however, to study the extraction under optimised conditions.

The stabilities of selected PAHs were studied as a function of time (10-240 min) and temperature (100-350°C). Extensive degradation was found at 300ºC even with the shortest heating time. Most of the degradation and reaction products were ketones and quinones. The

results of the stability studies cannot be directly applied to estimate the possible degradation of analytes in pressurised hot water extraction because of some fundamental differences between the two. These differences include, the static nature of the stability studies and the dynamic nature in conventional PHWE. In addition, the absence of matrix and other analytes in the stability studies increases the adsorption of the studied PAH into the walls of the vessel, and this intense contact may be the cause of the substantial degradation observed. Nevertheless, the stability studies indicate that the possibility of degradation always should be considered when extractions are performed at high temperatures and especially in static PHWE. Although PAHs are considered to be more or less resistant to degradation, the stability studies clearly indicated a risk for degradation at high temperatures. Among other things, the analyte type and concentration, the matrix and the material of the vessel affect the degradation.

The solubilities of anthracene and pyrene were measured below their melting points, and the results were compared with the solubilities obtained earlier with a similar method. The measured solubilities and the literature values agreed very well. A novel saturation cell that allowed the diffusion of the PAHs through the lid of the cell into the water flow was constructed and applied in solubility measurements above the melting point of the PAHs. To our knowledge, solubilities of PAHs have not earlier been measured at temperatures up to 300°C. An excellent correlation was obtained between the solubilities of anthracene and pyrene and temperature. The high temperature solubility data is extremely important in optimising PHWE, and also important in industry when high temperature process waters are handled. The existing solubility data has mostly been measured under ambient conditions.

In future, more attention needs to be paid to the material used in the construction of vessels as this may affect the results. A more thorough study of the effect of vessel material on degradation is needed. Metals also oxidise at various rates, and oxidation rate may affect the degradation. In future, the degradation of PAHs could be examined under conditions resembling more closely the conditions of PHWE. To be able to understand the degradation and the basic concepts related to it, it was important, however, first to examine the degradation, as done here, using a simple experimental set-up with one analyte at a time.

The non-linear data analysis and self-organising maps were of importance in determining which of the parameters are relevant for the extraction and under what conditions the best results can be obtained. Data analysis was carried out after all the experimental work was done. In future, such data analysis would be useful in the early stages of the research so that experiments could be directed in the most fruitful and relevant directions.

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