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Pressurized Fluid Extraction

A Sustainable Technique with Added Values

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Abstract
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The challenge for the future was defined by the Brundtland Commission (1987) and by the Rio Declaration (1992), in which the fundamental principles for achieving a sustainable development were provided. Sustainable chemistry can be defined as the contribution of chemistry to the implementation of the Rio Declaration. This thesis shows how Pressurized Fluid Extraction (PFE) can be utilized in chemical analysis, and how this correlates to Green Chemistry.

The reliability and efficiency of the PFE technique was investigated for a variety of analytes and matrices. Applications discussed include: the extraction of the antioxidant Irganox 1076 from linear low density polyethylene, mobile forms of phosphorus in lake sediment, chlorinated paraffins from source-separated household waste, general analytical method for pesticide residues in rape seed, total lipid content in cod muscle, and squalene in olive biomass. Improved or comparable extraction yields were achieved with reduced time and solvent consumption. The decrease in use of organic solvents was 50-90%, resulting in minimal volatile organic compounds emissions and less health-work problem. Due to higher extraction temperatures and more efficient extractions, the selection of solvent is not as important as at lower temperatures, which makes it possible to choose less costly, more environmentally and health beneficial solvents. In general, extraction times are reduced to minutes compared to several hours. As a result of the very short extraction times, the amount of co-extracted material is relatively low, resulting in fewer clean-up step and much shorter analysis time. Selective extractions could be obtained by varying the solvent or solvent mixture and/or using adsorbents.

In this thesis, the PFE technique was compared to the twelve principles of Green Chemistry, and it was shown that it follows several of the principles, thus giving a major contribution to sustainable chemistry.

Keywords: ASE, biomass, green chemistry, household waste, lipid, pesticide, PFE, pressurized fluid extraction, sediment

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Till alla mina elever i Ke 3 och Ke 4 under Fyrisskoletiden och till alla studenter på högskoleingenjörsprogrammet Uppsala universitet, som aldrig tröttnade på att fråga och diskutera

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I.   **Accelerated Solvent Extraction of the Antioxidant Irganox 1076 in Linear Low Density Polyethylene (LLDPE)-Granules before and after γ-Irradiation**
        M. Waldebäck, C. Jansson, F. J. Señoráns and K. E. Markides*,
        The Analyst, **1998**, 123, 1205-1207.

II.   **Use of Accelerated Solvent Extraction for Determination of Ecological Important Phosphorus in Lake Sediment**
        M. Waldebäck, E. Rydin and K. Markides*,
        International Journal of Environmental Analytical Chemistry,
        1998, 72, 257-266.

III.   **Pressurized-Fluid Extraction (PFE) of Chlorinated Paraffins from the Biodegradable Fraction of Source-Separated Household Waste**
        M-L. Nilsson, M. Waldebäck*, G. Liljegren, H. Kylin and K.E. Markides,
        Fresenius Journal of Analytical Chemistry, **2001,** 370, 913-918.

IV.   **Pressurised Fluid Extraction (PFE) as an Alternative General Method for Analysis of Pesticide Residues in Rape Seed**
        T. Pihlström, G. Isaac, M. Waldebäck[*], B-G. Österdahl and K. E. Markides,
        The Analyst, **2002,** 127, 554-559

V.   **Total Lipid Extraction of Homogenized and Intact Lean Fish Muscles Using Pressurized Fluid Extraction and Batch Extraction Technique**
        G. Isaac, M. Waldebäck*, U. Eriksson, G. Odham and K. E. Markides,
        Journal of Agricultural and Food Chemistry, **2005,** 53**,** 5506-5512

VI. **Pressurized Fluid Extraction of Squalene from Olive Bio-mass**

M. Waldebäck, F. J. Señoráns, A. Fridström, K.E. Markides, In: *Modern Extraction Techniques for Food and Agricultural Samples*, C. Turner, Ed., American Chemical Society Press, **2005**, in press

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The author's contributions to the articles:

Paper I: Responsible for planning the study, and supervising the practical work. I wrote the paper in co-operation with F. J. Señoráns

Paper II: Initiated the study, planned and performed the experiments, (except performing the traditional fractionation of the sediments), and I wrote the paper

Paper III: Initiated the study, was responsible for planning the study, supervised the practical PFE work, wrote the paper in co-operation with M-L Nilsson, from a first draft by G. Liljegren

Paper IV: Initiated the study, planned the study in co-operation with T. Pihlström, performed the pre-studies, supervised the practical PFE work, wrote the paper in co-operation with T. Pihlström and G. Isaac

Paper V: Initiated, and was responsible for planning the study in co-operation with U. Eriksson, supervised the practical PFE work, G. Isaac wrote the paper in close consulting with the co-authors

Paper VI: Responsible for planning the study, supervised the practical work, wrote the paper in co-operation with F. J. Señoráns

Papers not included in the thesis:

VII.   **Sediment Depth Attenuation of Biogenic Phosphorus Compounds Measured by ^{31}P NMR**

J Ahlgren, L Tranvik, A. Gogoll, M Waldebäck, K E Markides, E Rydin, *Environmental Science and Technology,* **2005**, 39, 867-872

VIII.   **Organic Phosphorus in Lake Sediment: does Humic Bound Phosphorus Constitute a Sink for P?**
K. Reitzel, J. Ahlgren, H. DeBrabandere, M. Waldebäck, A. Gogoll, L. Tranvik and E. Rydin, Submitted to *Limnology and Oceanography,* **2005**

Contents

Abbreviations

ASE	accelerated solvent extraction
BDE-47	2,2',4,4'-tetrabromodiphenyl ether
BDE-100	2,2',4,4',6-pentabromodiphenyl ether
CB 118	2,3',4,4',5-pentachlorobiphenyl
CB 153	2,2'4,4',5,5'-hexachlorobiphenyl
CB 180	2,2',3,4,4',5,5'-heptachlorobiphenyl
CP	chlorinated paraffins
p,p'-DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethene
EPA	Environmental Protection Agency
ESE	enhanced solvent extraction
GPC	gel permeation chromatography
HCB	hexachlorobenzene
α-, β- and γ–HCH	hexachlorocyclohexanes
LCA	life-cycle assessment
LLDPE	linear low density polyethylene
LLE	liquid-liquid extraction
LSE	liquid-solid extraction
MAE	microwave assisted extraction
NFA	National Food Administration
OCP	organo-chlorine pesticides
PAH	polycyclic aromatic hydrocarbons
PHWE	pressurized (hot) water extraction
PFE	pressurized fluid extraction
PLE	pressurized liquid extraction
POPs	persistent organic pollutants
PSE	pressurized solvent extraction
RSD	relative standard deviation
SFE	supercritical fluid extraction
SWE	subcritical water extraction
VOC	volatile organic compounds
XAD	cross-linked(X) ADsorbent

1 Introduction

The United Nations' Brundtland Commission (1987) broadly defined sustainability as meeting the needs of current generations without sacrificing the ability to meet the needs of future generations. At the United Nations' Conference on Environment and Development held in Rio de Janeiro in 1992, fundamental principles for achieving sustainable development were provided. The program of action, the Agenda 21, from 1992 calls for "criteria and methodologies for the assessment of environmental impacts and resource requirements throughout the full life cycle of products and processes" (Agenda 21). In a life-cycle assessment (LCA), the entire life cycle of a product or utility effect - extraction and processing of raw materials, production, distribution and transport, use, consumption and disposal as well as the potential ecological effects - is analyzed. In addition, energy conversions occurring in a life cycle and the resulting burden on the environment are assessed. Sustainable chemistry is understood as the contribution of chemistry to the implementation of the Rio Declaration including its on-going advancements such as the Johannesburg Declaration in 2002 [1].

Chemistry has played, and will play, a major role in the advancement of society, and in making our lives longer, healthier, more comfortable, and more enjoyable. The draw back is unfortunately environmental problems caused by chemicals when present as improper forms, at high concentrations, or by the inherent properties of chemicals for instance, toxic-, endocrine disrupting-, teratogenic-, difficult-to-degrade- or persistent effects. Chemists, chemicals and chemical industry as a whole are commonly blamed for this uncontrolled situation, and regarded to be the cause of many of the environmental issues of today. Therefore, chemicals and chemical industry have been, and are still, regarded as the black sheep by politicians and by people in general, although chemicals generally show vastly more good effects than bad ones. Thus, the challenge for chemists when developing new products, new substances or industrial processes, is to implement LCA in order to provide the society with ecologically and economically efficient processes and products.

Introduced in the early 1990s, Green Chemistry is an approach to address the environmental consequences of products or processes at the design state[2]. Green Chemistry, or environmentally benign chemistry, is defined as the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances. Anastas and Warner also formu-

lated the Twelve Principles of Green Chemistry. These principles provide guidelines for chemists in assessing the environmental impact of their work[3].

1. It is better to **prevent waste** than to treat or clean up after it has been formed.
2. Synthetic methods should be designed to **maximize the incorporation of all materials** used in the process into the final product.
3. Wherever practicable, synthetic methodologies should be designed to use and generate **substances that possess little or no toxicity** to human health and the environment.
4. Chemical products should be designed to **preserve efficacy of function while reducing toxicity.**
5. The use of **auxiliary substances should be made unnecessary** whenever possible and innocuous when used.
6. **Energy requirements** should be recognized for their **environmental and economic impacts and should be minimized**. Synthetic methods should be conducted at ambient temperature and pressure.
7. A raw material **feedstock should be renewable** rather than depleting whenever technically and economically practical.
8. Unnecessary **derivatization should be avoided** whenever possible.
9. **Catalytic reagents (as selective as possible)** are superior to stoichiometric reagents.
10. **Chemical products** should be designed so that at the end of their function **they do not persist in the environment** and break down into innocuous degradation products.
11. Analytical methodologies need to be further developed to allow for **real-time, in-process** monitoring and control prior to the formation of hazardous substances.
12. Substances and the form of a substance used in a chemical process should be chosen so as to **minimize the potential for all chemical accidents**, including releases, explosions, and fires.

Analytical chemistry and chemical analysis must of course also submit to the necessity of green and sustainable chemistry, beyond what is described by principle eleven. The analytical chain can be divided into sampling, sample preparation, separation, detection and data analysis. The focus in chemical analysis has over the last 30 years mostly been on the latter part of the analytical chain, i.e. separation, detection, and data analysis. As the emphasis on method development is moved to the earlier steps in this chain, due to its present ability of efficient automation, it becomes critical that green and sustainable chemistry is considered. Especially in sample preparation, and

extraction processes in particular, more effort has to be made following the twelve principles of green chemistry.

Sample extraction procedures are gaining more and more interest, and today there is a wide variety of extraction techniques to choose from [4]. Traditional liquid-liquid extraction (LLE) and liquid-solid extraction (LSE) procedures that require several hours or even several days to perform are slowly replaced by more attractive alternatives. The most widely used extraction techniques today are still sonication and Soxhlet extraction[5-8]. These classical methods are usually multi-step procedures based on exhaustive extractions from a sample matrix followed by successive clean-up steps prior to analysis. Such sample preparation procedure requires large amounts of sample, sorbents and organic solvents, which are often hazardous and toxic resulting in high costs of both purchase and disposal [9]. These methods also demand lots of manual handling, which oftentimes creates work-health problems.

Organic solvents are emitted as volatile organic compounds (VOC) into the atmosphere. VOC is a significant cause of the formation of photochemical smog, which is a mixture of about 90% ozone (tropospheric ozone), nitric acid, aldehyds, ketones, radicals and oxidants. Regulations aimed at reducing tropospheric ozone have a great impact on any of the regulations on solvent use. Halogenated solvents may also contribute to the depletion of the ozone layer. The use of new of solvents with less negative environmental impact, and even the design of processes that use no organic solvents at all, are therefore subjects of many green initiatives [10,11]. In addition, from a health perspective, it is also important to find alternative, less toxic solvents. Organic solvents generally have anaesthesia properties, of which some are neuro toxic (e.g. hexane), others are carcinogenic (e.g. di- tri- and tetra-chloromethane), and others are suspected teratogenic.

Concerns about using hazardous solvents in the laboratories as well as new restrictions on solvent use, cost of solvents and solvent waste disposal have pushed the development of modern techniques forward. At the same time, both in the industry and in the academia there is a demand for an increasing number of different substances that need to be analyzed. The new solutions have to be cost-effective in terms of increased sample throughput, shortened times of extraction and automation without loosing in accuracy or precision.

Environmentally sustainable but still cost-efficient methods could be obtained either by modifying the techniques already available or by utilizing new principles and/or new techniques. By miniaturization and/or automation of instruments, e.g. micro Soxhlet or automated Soxhlet, like Soxtec™ 2050 (from FOSS Analytical, Denmark), the costs and the solvent consumption can be decreased. The alternative is to develop new techniques, e.g. techniques which make it possible to raise the diffusion coefficient for liquids, which is the most effective way to increase the rate of the extraction

process and decrease the required amount of organic solvent. Diffusion rates in liquids have been shown to increase about 2-20 fold upon increasing the temperature from 25 to 150°C [12]. By using high diffusion fluids the mass transfer rate can be increased and thus the whole extraction process becomes faster. Therefore, during two decades, techniques that employ high diffusion fluids, raised temperature and/or pressure have been developed, including supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and pressurized fluid extraction (PFE) [4].

In this thesis, the PFE technique has been evaluated as an environmentally friendly extraction method for sample preparation in chemical analysis. The PFE technique is a relatively new automated liquid extraction technique that uses only small volumes of conventional solvents at elevated temperatures and pressures.

In **Paper I**, the feasibility of the PFE technique was demonstrated by developing a new method that provided fast extraction of the antioxidant Irganox 1076 from ungrounded samples of linear low density polyethylene (LLDPE).

In **Paper II,** PFE was applied to lake sediments in order to efficiently and selectively extract the mobile forms of phosphorus in the sediments, the so called ecologically important phosphorus, which is the forms of phosphorus that can be recycled from the sediment into the water column.

In **Paper III,** the strength of the PFE technique was demonstrated, when chlorinated paraffins (CP) were extracted from a very complex organic matrix, the biodegradable fraction of a source-separated household waste.

In **Paper IV,** a PFE multi-method was developed for the simultaneous determination of pesticide residues in rape seed. The method was validated for 25 different pesticides and metabolites.

In **Paper V,** the reliability and efficiency of the PFE technique was evaluated for the extraction of total lipid content from cod. Surprisingly, it was found that the optimized PFE method gave yields of total lipids from fish muscles exceeding those of conventional batch extractions by a factor of about 10% due to the higher temperature, regardless of the fat content of the fish.

In **Paper VI**, the PFE technique was used to obtain environmentally benign and fast extraction of squalene and α-tocopherol from a low-value biomass in the olive oil production; olive oil pomace. The developed method was used in an analytical context but could also be a starting point for upscaling the "green" process.

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2 The Extraction Process

Theoretical and practical concepts of liquid/liquid and liquid/solid extraction processes in chemical analysis differ to some extent from industrial applications mainly, due to the fact that the target molecules are generally in low or very low concentrations in the matrices. Furthermore, analytical applications usually require mild extraction conditions (i.e. moderate temperature and light- and oxygen-free environment) to prevent degradation of the analytes. In addition, there is often a desire to perform selective extractions.

In order to perform efficient and smart extractions, the understanding of the matrix characteristics and the different steps of the extraction are of great importance. The nature of the sample matrix (water and organic/inorganic content) and its physical characteristics (homogeneity, porosity, particle size) should also be considered. To better understand the extraction process, two models showing the distribution of analytes in different types of sample matrices are illustrated below. Figure 1 is a conceptualization of an aggregate of matrix particles from a source separated household waste, and the possible sites where analytes are expected to be found, in this case contaminated with chlorinated paraffins, see **Paper III**.
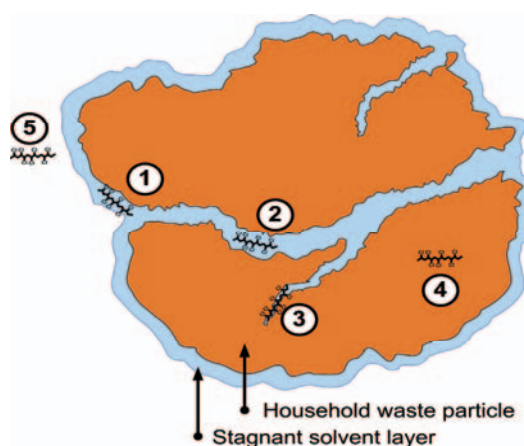

Figure 1. Schematic of a household waste particle and some possible sites where the analyte (CP) might be adsorbed or chemically bonded.

Figure 1 works as a model showing the variety of possible (and likely) positions and status of analytes in many different types of sample matrices, i.e. the analyte can be;

1. Adsorbed at the surface of the matrix
2. Dissolved in the pore solvent and/or adsorbed at the pore surface
3. Dissolved/adsorbed in a micro/nano pore
4. Chemically bonded to the matrix
5. *Dissolved in the bulk solution*

In **Paper I,** the antioxidant Irganox1076 was extracted from the polymer LLDPE. The characteristic of a polymer particle differ to a large extent from the model of household waste, and another model was therefore suggested as shown in Figure 2. The extraction process of compounds from polymer particles generally follows the steps as described below:

1. Diffusion of the solvent into the matrix
2a. Desorption of the analytes from the matrix (including breaking of chemical bonds)
2b. Solvation of the analyte into the extraction solvent
3. Diffusion of the analyte out from the matrix
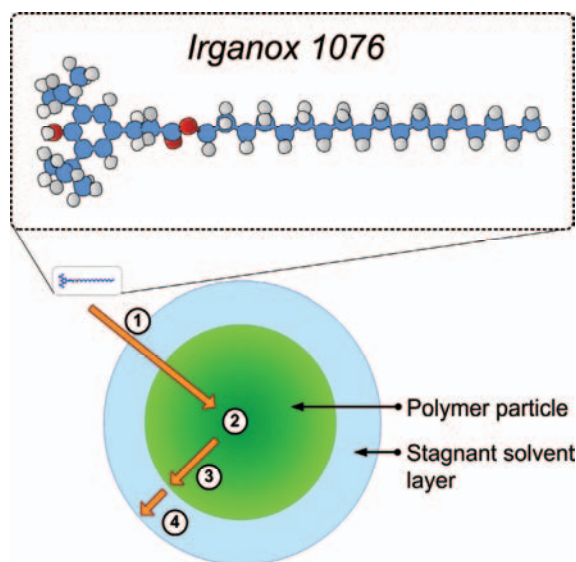4. Diffusion of the analyte through the stagnant solvent layer and into the bulk solvent

Figure 2. Schematic showing the extraction of Irganox 1076 from of an LLDPE polymer particle.

Knowledge about distribution coefficients and distribution ratios are useful tools to provide guidance to the selection of solvent for the extractive separation process. Solubility of the analytes, their diffusivity in the solvent and matrix characteristics are the main factors to consider, when choosing a solvent for a successful extraction process. It is also important to understand the mass transfer mechanism across chemical/physical interfaces in order to design liquid/liquid and liquid/solid extraction processes. Toxicity and sustainability aspects of the solvent should also be considered. Additionally, in most of the analytical scale applications, the concentration of the target molecule is very low, and thus the rate of the extraction is not limited by the analyte concentration in the extraction solvent, but rather determined by the rate of mass transfer out of the matrix.

2.1 Extraction strategy

In order to perform a fast and quantitative extraction, a solvent has to be chosen that has the right chemical properties to dissolve and release the analyte, but should preferably not dissolve other solutes in the sample, i.e. the solvent power should not be higher than needed.

Solubility theory has been discussed and proposed in classic works by J. Hildebrand, who combined the correlation between vaporisation and intermolecular forces, van der Waal forces and hydrogen bonding, to the correlation between vaporisation and solubility behaviour [13,14]. The same intermolecular attractive forces have to be overcome to vaporize a liquid as to dissolve an analyte. The term "solubility parameter" (δ) was described by Hildebrand as the square root of the cohesive energy density (c) giving a numerical value indicating the analyte behaviour in a specific solvent, see equation 1, where ΔH is heat of vaporisation (J/mol), R is the gas constant (J/K mol), T is the temperature (K) and V is the molar volume of the analyte.

$$\delta = \sqrt{c} = \sqrt{\frac{\Delta H - RT}{V}} \tag{1}$$

C. Hansen [15] took Hildebrand's work further and assumed that the total cohesive energy is a linear addition of three components; δ_h (hydrogen bonding ability contribution), δ_d (dispersion coefficient contribution), and δ_p (polarity contribution). They are linked by equation 2, where δ_t is the total solubility parameter [16].

$$\delta_t^2 = \delta_h^2 + \delta_d^2 + \delta_p^2 \tag{2}$$

Fitzpatrick et al. [16] predicted a suitable solvent to extract persistent organic pollutants (POPs) from contaminated soil and certified reference material using the Hildebrand solubility parameter and confirmed the results by experiments. The ideal extraction solvent from the calculations was a mixture of acetonitrile and dichloromethane (1:1 v/v), perhaps the best solvent from the view of solvent power, but not from a health and environmental point of view.

When choosing a solvent it is a good start to have the Hildebrand and Hansen's theories in mind. However, the choice of solvent in a particular situation involves other factors except the solvent power. As described in Figure 2 above the solvent has to penetrate the matrix thoroughly, break the bonds between the matrix and the analytes of interest, help the dissolved analytes to diffuse out from the matrix and finally get dissolved and trapped in the extraction solvent. The process of solute/analyte transfer across an interface between two liquid phases may be rate-controlled by molecular diffusion, by motion of eddies, by irregular surface disturbances or even by chemical reactions in the bulk of a phase or in the interface region. Local velocities in the interface region could be of importance as well as other factors affecting the local conditions, not least the presence of surface active agents. The dependence of matrix geometry was reported by Björklund et al. [17], when mineral oils applied to metal devices were extracted by SFE.

Partitioning processes have a central role of concern in the extraction procedure. These involve partitioning of the analytes between the surface of the matrix and the solvent, as well as chemisorption of the analytes on active surface sites and within the solvent. Different matrices behave somewhat differently, i.e. *polymeric samples* usually build up a layer of stagnant liquid around the polymeric particles, as seen in Figure 2, through which the analyte has to transfer into the extracting solvent. In this case, the partitioning of the analyte between the stagnant liquid and the extraction solvent has to be considered. *Soils* differ strongly in surface physico-chemical properties and grain-size characteristics. *Sediments,* on the other hand, contain water of different kinds of bonding, from free water available for the plants, to water strongly bound to the particles. Therefore a variety of equilibriums take place.

2.2   Extraction by high diffusion fluids

High diffusion fluids are in this thesis defined as fluids created by raising and/or controlling the temperature and the pressure. High diffusion fluids provide different physico-chemical properties compared to those obtained at ambient temperature and pressure. These properties are intermediate to those of liquids and gases, which also make them attractive as extraction

solvents as described below. In Table I, essential parameters for extraction media are compared including; density, viscosity and diffusion coefficients.

Table I. Approximate values of densities, viscosities and diffusion coefficients of gases, supercritical fluids and liquids, modified from Wasen et al. [18].

Fluid	Density (g/cm^3)	Viscosity (g/cm s)	Diffusion coefficient (cm^2/s)
Gas	$(0.6 - 2)10^{-3}$	$(1-3)\ 10^{-5}$	0.1-1.0
Supercritical fluid	0.2-0.9	$(1-3)\ 10^{-4}$	$(0.1-5)\ 10^{-4}$
Liquid	0.6-1.6	$(0.2-3)\ 10^{-3}$	$(0.2-3)\ 10^{-5}$

As shown in Table I, liquids have the strongest solvating power, i.e. the highest density, whereas the solvating power of supercritical fluids approach those of liquid solvents only as their density is increased. In fact, the maximum solubility of an organic compound is frequently higher in liquid solvents than in supercritical fluids [19]. However, mass transfer properties determine the rate at which an extraction can be performed; hence viscosity and diffusivity are especially important parameters. The viscosity values of high diffusion fluids (such as supercritical fluids) are one order of magnitude lower than those of liquid solvents, and the values of diffusion coefficients are one order of magnitude higher than those of liquid solvents as seen in Table I. This explains why these fluids render to higher diffusivities, resulting in significantly faster extractions [20].

An increase in temperature usually tends to promote solubility, as the thermal kinetic energy raises. Increasing the temperature will also facilitate analyte diffusion and/or reduce interactions between analytes and the matrix by disrupting intermolecular forces such as van der Waal's forces, hydrogen bonding and dipole attractions. Higher temperatures also decrease the viscosity of a liquid solvent, thus enabling better penetration of matrix particles. An increased temperature will also decrease the surface tension of the solvent, allowing the solvent to better "wet" the sample matrix [21]. Both lower viscosity and lower surface tension facilitate better contact of the solvent with the analytes and thereby enhance the extraction.

2.3 Techniques using high diffusion fluids

Due to the properties of high diffusion fluids such as liquid-like density, relatively low viscosity, low surface tension and fast diffusivity, which also can be adjusted continuously by small changes in pressure and temperature,

the use of high diffusion fluids are attractive in many fields. Areas of interest are analytical and preparative separations, organic and inorganic synthesis, waste management, cleaning (semiconductors, textiles, microelectronics, etc) and material processing (polymers, nano-materials, nano-structured materials, thin films, coatings, etc) [22].

The focus in this thesis will be in the *domain of analytical chemistry*. Techniques that will be discussed here are; SFE, which was established at the end of 1970's, MAE, which turned up ten years later, and PFE, which was introduced in 1995 as accelerated solvent extraction (ASE®). However, this thesis will not try to compare these different techniques but will rather focus on the PFE technique and its usefulness in environmentally sustainable method development obeying the twelve principles of green chemistry. For those of you who are more interested in reading about SFE, MAE and PFE, there are several excellent reviews articles [23-26] and books [20,27].

2.3.1 Supercritical fluid extraction

Since the PFE technique has been developed from the SFE technique, and most of the theory regarding high diffusion fluid extraction has been described for SFE, this technique will be discussed more deeply. Supercritical fluids have gas-like viscosities, as seen in Table I, which results in fast analyte mass transfer. Hence, supercritical fluids can easily penetrate different types of sample matrices [28]. The solvent power of a fluid is, to a first approximation, proportional to its density [27]. The density of a supercritical fluid is closely related to the pressure (higher pressure gives higher density) and to a lesser extent to the temperature [20]. By varying the extraction pressure, a wide range of densities can be obtained and selective extractions of compounds can be performed, which is a great advantage compared to other extraction techniques. On the other hand, if the pressure is kept constant, the density of a supercritical fluid decreases when the temperature is increased.

If the rate-limiting parameter of the extraction is diffusion of the analytes or desorption of the analytes from active sites of the matrix, increasing temperature will increase the extraction yield [29]. This is because an increased temperature will facilitate solute diffusion and reduce the interaction forces between matrix and the analytes to be extracted.

Theoretical models for different extraction techniques using high diffusion fluids and different samples have been suggested over the years. Even if these models are simplified by assumptions, they can in some cases be extended to predict extraction rates and conditions. One of these models suggested for analytical SFE, has been proposed by Bartle et al [30,31], i.e. the "hot ball" model. This model describes the diffusion of a uniform initial concentration of extractable non-bonded compounds out of homogenous spherical particles into a medium in which the extracted species is infinitely dilute. The described model uses several assumptions, but experimental

results validate the applicability of the model to a wide variety of sample types [30]. In the "hot ball" model, the ratio of mass remaining (m_1) in the particle of radius (r) at time (t) to the initial amount (m_0) is given by equation 3:

$$m_1 / m_0 = (6/\pi^2) \sum_{n=1}^{\infty} (1/n^2) \exp(-n^2\pi^2 Dt / r^2) \qquad (3)$$

where n is an integer and D is the diffusion coefficient of the analyte in the spherical particle. Equation 3 may be simplified by defining a quantity (t_r) which is proportional to time for any given system (and is therefore a reduced or scale time) by the equation 4:

$$t_r = \pi^2 Dt / r^2 \qquad (4)$$

In terms of scaled time equation 3 becomes:

$$m_1 / m_0 = (6/\pi^2)[\exp(-t_r) + \exp(-4t_r) + \exp(-9t_r) + ...] \qquad (5)$$

The solution is therefore a sum of exponential decays, and at long times the later terms will decrease in importance and the first exponential term in square brackets will be dominant. A plot of $\ln(m_1/m_0)$ vs time, or a quantity proportional to time, therefore becomes linear at long times, as seen in equation 6, Chapter 3.1.

Another model, suggested for analytical SFE, has been proposed by Pawliszyn [32]. He assumed in his kinetic model that a matrix particle consists of an organic layer surrounding an impermeable core, and that the analyte is adsorbed onto the core surface. Thus, the extraction process can be modelled by considering several steps based on adsorption, diffusion and partition equilibrium, as illustrated in Figure 1 and Figure 2.

Several fluids have been tested in SFE. Hawthorne et al. [19] listed a number of fluids that have been used together with their Hildebrand solubility parameter (at maximum pressure). By far the most widely used extraction fluid has been carbon dioxide [33] with critical values of 31.1°C and 7.38 MPa, as shown in Figure 3. Other supercritical solvents that have been used are ammonia [34], dinitrogen oxide [19], freons [35] and water [36]. However, dinitrogen oxide is now avoided due to the risk of oxidation and explosion when the matrix organic content is high [37]. The other alternatives are less appropriate because of health (ammonia) and environmental (freons) hazards, or high critical temperatures and pressures (water), as seen in Figure 3.
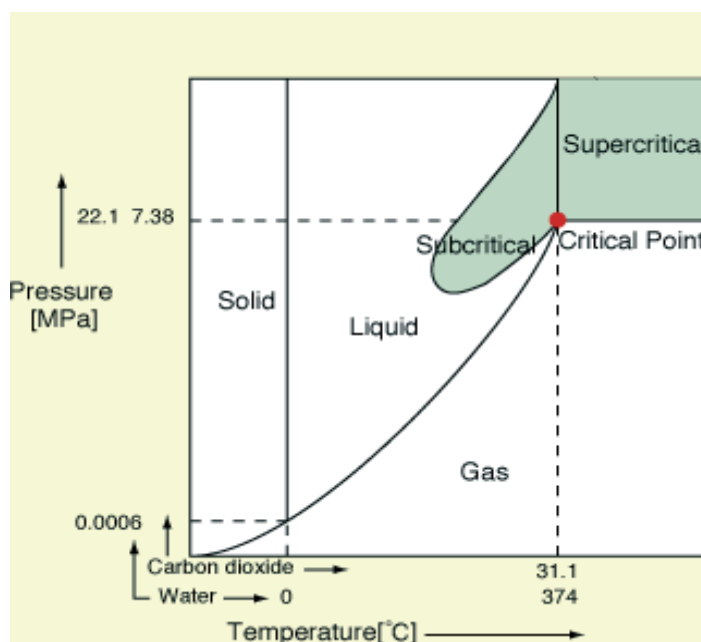
Figure 3. Phase diagram for carbon dioxide and water.

The popularity of carbon dioxide is due to the fact that it is chemically inert, easy to clean, environmentally friendly, "solvent free" and inexpensive. The polarity of supercritical carbon dioxide is however poor, at low pressures corresponding to that of hexane, and at high pressures comparable to that of dichloromethane. Therefore, modifiers have to be added to the supercritical carbon dioxide in order to extract more polar compounds, but still low extraction yields of many polar and/or high molecular weight compounds are commonly obtained [37].

The main advantages of using the SFE technique are that analyte-selective, solvent-free extractions can be performed [38] and thus clean-up step is usually not necessary before analysis. Extraction times are often relatively short and the equipment can be automated and integrated with on-line sample preparation and detection [39]. The main shortcomings are high investment cost, as well as difficulties in extracting polar analytes and high molecular-weight analytes. In addition, method development is needed for every new application.

SFE has been applied to a wide range of samples from numerous applications, and has become popular for complex sample matrices such as environmental solids [40,41], polymer resins [42], food samples [28], drug samples [43] and biological tissues [44]. According to Valcárel et al. [39,45] the major fields are within environmental analysis (40%) followed by food and natural products

analysis (38%). Industrial, biomedical and other applications account for 11, 4 and 6%, respectively.

2.3.2 Microwave assisted extraction

High diffusion fluids can be obtained using microwave energy in combination with high temperature and controlled pressure. The absorption of microwave radiation results in the disruption of weak hydrogen bonds, which improves solvent penetration and enhances analyte solvation [37].

To be able to absorb microwaves, either the solvent or the matrix has to contain dipolar molecules. The effect of the microwaves is strongly dependent on the nature of both solvent and matrix [46]. Solvents generally used cover a wide range of polarities. Usually the chosen solvent possesses a high dielectric constant and strongly absorbs microwave energy. The extracting selectivity and the ability of a medium to interact with microwaves can be modulated by using mixtures of solvents [47]. The solvent will be heated from inside-and-out when placed in a non-microwave-absorbing pressure-resistant vessel to a temperature above the atmospheric boiling point [48] and at a pressure of a few bars (0.68 – 1.36 MPa) [49]. The hot, pressurized solvent increases the efficiency of extraction process as described in Chapter 2.2. In some cases, the matrix itself interacts with the microwaves, while the surrounding solvent, if it has a low dielectric constant, remains cold [50]. This latter situation has some advantages when extracting thermo-sensitive compounds. The major advantages achieved using the MAE technique include the speed of the extraction and the low solvent consumption. One disadvantage is that the samples need to be filtrated before analysis [51], another disadvantage is the long cool-down periods [26]. For more reading on MAE, please consider the references [49,52].

2.3.3 Pressurized fluid extraction

In PFE, high diffusion fluids are generated at elevated temperatures and pressures. This technique is also known as pressurized liquid extraction (PLE), pressurized solvent extraction (PSE®), ASE® or enhanced solvent extraction (ESE). The solvent is kept in a liquid phase even at temperatures much above the atmospheric boiling point as a result of the applied pressure. This technique was originally introduced at the Pittsburgh conference (Pittcon) in 1995. The inventors of ASE also had a standard method, Method 3545A, approved by the U.S. Environmental Protection Agency (EPA), for the extraction of water-insoluble or slightly water-soluble organic compounds from soil, clays, sediments and waste solids [53]. The process and theory of PFE will be discussed in more detail in Chapter 3.

2.3.4 Subcritical water extraction

Water, the most environmental friendly solvent of all, has during the last few years become an increasingly popular extraction solvent when operated under subcritical conditions. This technique is known as subcritical water extraction (SWE), pressurized hot water extraction (PHWE), or similar. Temperatures should by definition be between the boiling point and the critical point of water, i.e. 100 to 374°C, and pressures have to be high enough to keep the water in a liquid state [9], see Figure 3. When the temperature of water is increased, the dielectric constant is significantly decreased [36]. For example, if increasing the temperature from 20 to 250°C at a pressure of 5 MPa, a significant reduction of the dielectric constant from 80 to about 27 is achieved. This later value is similar to that of ethanol at 25°C and 0.1 MPa, which is low enough to dissolve many compounds of intermediate or low polarity [54].

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3 Pressurized fluid extraction in chemical analysis

PFE was originally developed for the extraction of organic compounds from solid and semisolids samples. The main principle of this technique is to use organic liquids as solvents at elevated temperature (40-200ºC) and pressure (3.4-20.4 MPa or 5-15 bars) with standard liquid solvents. Today most of the published applications of PFE have been in the area of environmental analyses [46,55]. Other areas where PFE has been applied are in polymer resin [5,31,56], food [57,58] and natural product [46,59,60] analysis.

3.1 PFE theory

The PFE technique utilizes the same basic principles as traditional liquid solvent extractions (LSE), but the extractions are carried out at higher temperatures and pressures.

As discussed above in Chapter 2.2, an increased temperature during the extraction gives more efficient extraction, which results in both a gain in time and lowered solvent consumption [21]. The principle of PFE is very similar to MAE, where the solvent is heated by microwave energy, although in the PFE technique both higher temperatures and pressures can be obtained.

An increased pressure in the PFE technique is mainly applied to keep the solvents as liquids, even at temperatures above their atmospheric boiling point. Most PFE studies observe no difference in extraction yield when the pressure is varied in the range of 3.4 – 20.4 MPa [61], although Richter et al.[21] reported a trend that a higher pressure rendered a higher extraction yield, when a standard mixture of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated hydrocarbons from a reference material was spiked onto wet silica (different pore size) at 100°C, using methylene chloride/acetone (1:1 v/v) as solvent mixture. Higher pressure gave higher extraction yield, when the pore size of the wet silica was 300 Å, but no difference in recovery was observed with the dry silica. It was suggested that higher pressure could probably facilitate the extraction of analytes trapped inside matrix pores because the pressure would force the solvent into the pores of the matrix that normally would not be contacted by solvents at atmospheric pressure. Pressurized flow would also aid in the solubilization of air bubbles so that the

solvent more easily could penetrate the sample matrix. Kremer et al. [62] also obtained higher yield of acidic herbicides in soil when pressure was increased from 6.9 to 13.8 MPa, using 100°C and dichloromethane as solvent.

There are only a few theoretical models suggested for the PFE technique. For instance Vandenburg et al. [31] applied the "hot boll" model, originally described for SFE, see Chapter 2.3.1, for the extraction of additives from polymeric samples using PFE. By plotting $\ln(m_1/m_0)$, where (m_1) is the mass of analyte remaining in the particle of radius (r) at time (t), and (m_0) is the initial amount of analyte, a linear portion is given in equation 6 [63]. In this case when $\ln(m_1/m_0)$ is plotted against time, the line falls steeply initially and shortly after becomes linear where it follows equation 6 [31]:

$$\ln(m_1 / m_0) = -0.4977 \ - (\pi^2 Dt / r^2) \tag{6}$$

The physical explanation of the shape of the curve is that the additive near the surface is rapidly extracted until a smooth falling concentration gradient is established across the particle. The extraction rate is then completely controlled by the rate at which the additive diffuses to the surface. By plotting the amount of extracted additives versus the extraction time for different solvents at different temperatures, the resulted curves showed a good fit to the "hot ball" model. With a good kinetic model of the extraction process it would be possible to predict experimental parameters, and find out where the extraction is expected to be only diffusion dependent. However, the models mentioned here can at most be considered to give useful hints when developing new extraction methods based on PFE.

There is no theoretical model that includes strong solute-matrix interactions caused by chemical bonding between the solute molecules and active sites on the matrix. In addition, sample matrices are seldom homogenous, thus the penetration of the solvent is difficult to foresee. As a result, accurate descriptions are by no means easy to provide because of experimental difficulties, as well as chemical and mathematical complexity of the total extraction process. Empirical approaches, in combination with multivariate chemometric methods, are expected to be the most widely used in many years to come.

3.2 PFE instrumentation

The commercial PFE instrument used in this thesis is a fully automated sequential extraction system, ASE® 200, Dionex Co. (Sunnyvalle, CA, USA). A schematic of the instrument is shown in Figure 4. Temperatures are ranging from room temperature to 200°C and pressures are ranging between 3.5 and 20 MPa. A filter paper is inserted into a stainless steel extraction cell followed by the sample, if needed mixed with a drying agent. The cell is

loaded on a carrousel, automatically placed in an oven, and filled with ex-traction solvent. A static valve in combination with a pressure relief valve control the pressure in the cell during the static extraction by adding more solvent to the cell or by opening the static valve, whichever is needed to maintain the desired pressure. The first part of the extraction is a pre-heating step to reach thermal equilibrium. During this heating, thermal expansion of the solvent occurs and causes an increase in pressure within the cell. When the set values are achieved static extraction is performed during a selected time, typically 5-10 min. After the static time, part of the solvent in the ex-traction cell is replaced with fresh solvent. The so called flush volume can vary from 5 to 150% of the extraction volume. If required, one to five static cycles can be performed. The flush volume is divided by the number of static cycles. Finally, pressurized nitrogen will purge the remaining solvent from the cell and the lines to a vial that contains the extract. Sequential ex-tractions can easily be performed by repeating the procedure with a new solvent.
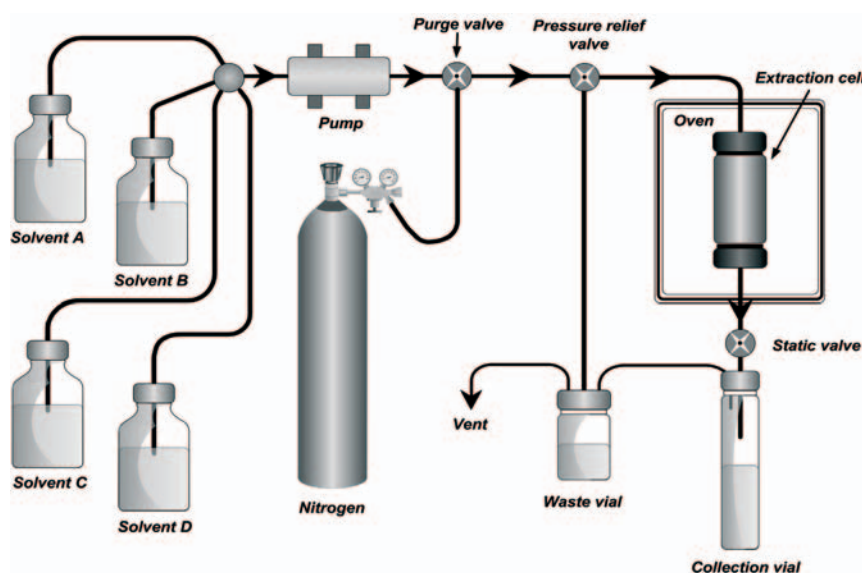

Figure 4. Schematic diagram of a PFE system, Dionex ASE® 200.

There are only a few different types of commercial instruments available on the market, but lots of home-made instrumentation is described in the litera-ture [64]. Some of them perform both dynamic and static extractions as well as temperatures above 200°C [5,31,38,65].

3.3 Possibilities and limitations of PFE

Is PFE the solution to the need for more cost-effective and environmentally friendly extraction methods without loosing in sensitivity, accuracy and precision? Before answering this question it is important to consider the controllable variables of the extraction. To develop an extraction method using the PFE technique, Richter et al.[21] suggested to start with the same solvent as used in the corresponding traditional extraction method. This approach was used in **Paper IV**, where the objective was to investigate the reliability and efficiency of the PFE technique for extraction of pesticide residues from rape seed with high lipid content. The same solvent mixture as the one currently used in the standard method at the National Food Administration (NFA) in Uppsala, Sweden, was used throughout the study. Totally 25 pesticides and metabolites with different chemical properties were incorporated in the method development; see Table I**, Paper IV**. The reliability of the method was tested and compared to the standard LLE method that is currently used in the monitoring program at NFA. A comparison of the extraction scheme is shown in Figure 5. In general, LLE gave more precise results in terms of lower RSD values, as seen in Table 2 and 3 in **Paper IV**. However, both techniques recover the pesticides studied within acceptable values, with overall recoveries of 96% for LLE and 91% using PFE.

By varying other parameters such as solvent/solvent mixtures; extraction temperature; extraction time; number of flushes; sample size; and adsorbents, new advantages compared to other techniques can than be achieved.
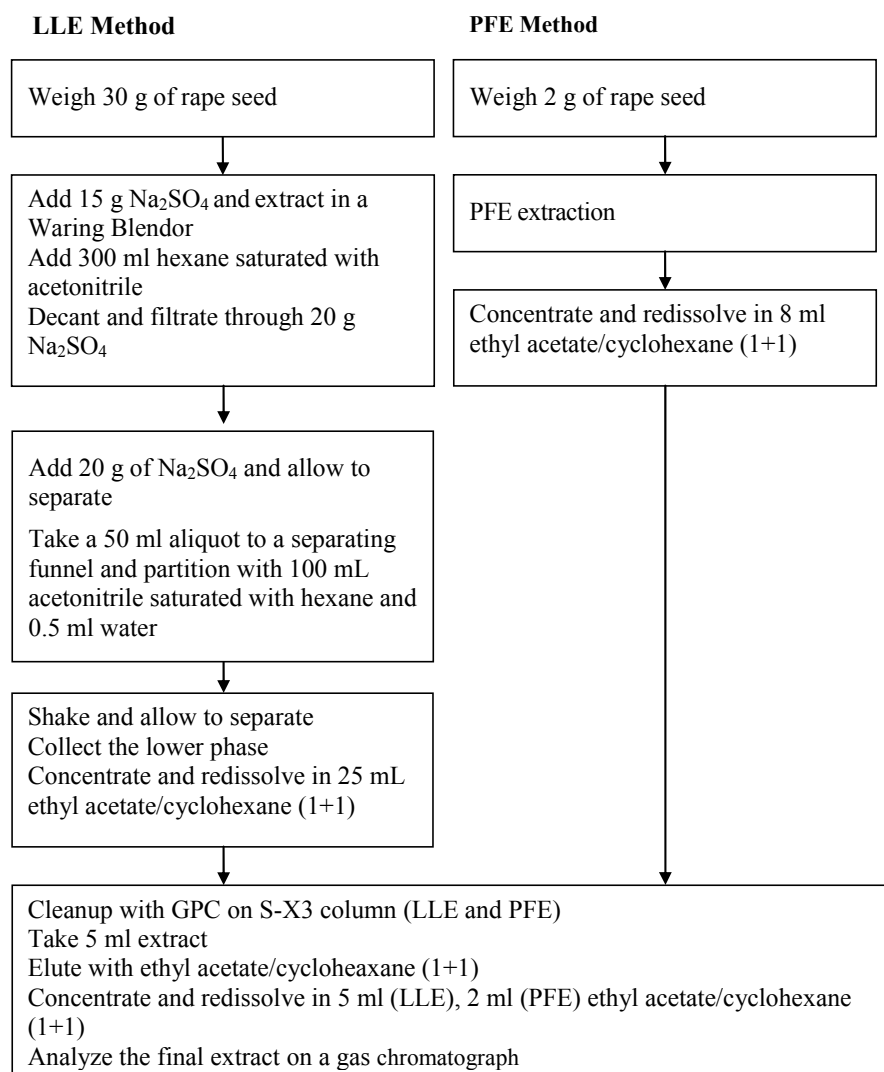
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LLE Method

PFE Method

Weigh 30 g of rape seed

Weigh 2 g of rape seed

Add 15 g Na$_2$SO$_4$ and extract in a Waring Blendor Add 300 ml hexane saturated with acetonitrile Decant and filtrate through 20 g Na$_2$SO$_4$

PFE extraction

Concentrate and redissolve in 8 ml ethyl acetate/cyclohexane (1+1)

Add 20 g of Na$_2$SO$_4$ and allow to separate Take a 50 ml aliquot to a separating funnel and partition with 100 mL acetonitrile saturated with hexane and 0.5 ml water

Shake and allow to separate Collect the lower phase Concentrate and redissolve in 25 mL ethyl acetate/cyclohexane (1+1)

Cleanup with GPC on S-X3 column (LLE and PFE) Take 5 ml extract Elute with ethyl acetate/cycloheaxane (1+1) Concentrate and redissolve in 5 ml (LLE), 2 ml (PFE) ethyl acetate/cyclohexane (1+1) Analyze the final extract on a gas chromatograph

Figure 5. Comparison of the extraction scheme of the NFA´s multi residue method LLE and the developed PFE method.

3.3.1 The solvent effect – the main advantage

In general, any liquid solvent or mixtures of solvents, including water and buffered aqueous mixtures, can be utilized in PFE, except those that are corrosive to the hardware of the instrument like strong acids and bases. It is also possible to perform sequential extractions with different solvents or

solvent mixtures. The use of a controlled atmosphere, e.g. nitrogen, between the changes of solvent is also a great advantage compared to conventional extractions, where oxygen in the air might react and oxidize the analyte or the surrounding compounds in the sample.

The recommendation to start with a conventional LLE solvent when developing a new method in PFE can be found to be somewhat difficult if the matrix is a polymer. Solvents that are good swelling agents for polymer matrices in Soxhlet extraction, may dissolve the polymers at the higher temperature of PFE, and thereby clogg the tubing of the instrument. This problem was experienced in one of the studies described in **Paper I,** and was also reported by Vandenburg et al. [56]. The advantage of being able to mix different solvents is also demonstrated in **Paper I.** Here an additive, the antioxidant Irganox 1076, was extracted from a granulized polymer of LLDPE. The solvent mixture was designed so that one of the solvents used in the mixture, ethyl acetate, worked as a solvent for the target molecules and the other solvent, hexane, worked as a swelling agent of the matrix. Compared to traditional Soxhlet [66], where several sequential extractions had to be performed using different solvents, even including hazardous solvents, the PFE method developed in **Paper I** used only around 30 mL of solvent mixture for each sample compared to 50-100 mL using Soxhlet. In this case swelling of the matrix worked well, but there is always a risk that swelling could lead to a smaller pore size, and slow down the extraction.

The PFE technique was initially developed for rapid and high yield extractions of solid and semi-solid samples using organic solvents [67]. One of the first papers published using aqueous solutions and water as solvents in PFE is described in **Paper II**. Here, ecologically important phosphorous in lake sediment was extracted, i.e. the labile phosphorous compounds that will recycle from the sediment into the water column (the lake). The phosphorus that in this way is released from sediments, the so called internal loading of phosphorus, is a key factor in the eutrofication issue [68,69]. In this project it was also interesting to note that by being able to freely choose solvent/solvent mixture together with an inert atmosphere, the nitrogen purge between exchanges of solvents, it was possible to simulate specific conditions or keep a specific environment, like a controlled model for an anaerobic environment in a lake sediment.

In **Paper III** the objective was to develop a faster, easier, more environmentally friendly and health beneficial method to extract CP from an extremely complex matrix, a biodegradable fraction of a source-separated household waste. This was compared to a Soxtec™ method currently used at the Department of Environmental Assessment at Ultuna, Uppsala, Sweden. The solvent commonly used was dichloromethane (dielectric constant 10.6) which is more compatible with watery samples than cyclohexane and hexane and thus gives better recoveries as seen in Table 1, **Paper III**. However, being a more polar solvent, dichloromethane naturally co-extracts more in-

terfering compounds as illustrated in the chromatogram in Figure 2, **Paper III**. This leads to the necessity of a number of time- and solvent-consuming clean-up steps, with the risk of loosing analytes during the evaporation steps. Different solvents and solvent mixtures were tested in this study, as well as varying temperatures, the use of adsorbents and homogenization of the sample. Due to the higher temperature used during the extraction and the shorter extraction time needed, cyclohexane (dielectric constant 2) rather than dichloromethane proved to be the best solvent, see Table 2, and 3 and Figure 2, **Paper III**. This was a surprising result since cyclohexane did not look promising in the pre-study. By choosing cyclohexane, the more toxic solvents hexane and acetone could be avoided, and a more health-beneficial method was developed. The overall solvent volume was also decreased by more than 50% compared to the method used at Ultuna, and dichloromethane was totally excluded. The yield obtained with PFE was equivalent or even higher to the Soxtec™, as seen in Table 3 in **Paper III**.

When extracting samples of high water content, for example sediments, biodegradable household waste, vegetables and cod muscle, using organic solvents, there is always a risk that the non-polar organic solvent will not be able to penetrate water-sealed pores that contain analytes, and thus result in a lower extraction yield and not repeatable data [70]. In **Paper V**, Table 1**,** different solvent mixtures were used to extract total lipids from wet cod muscle. When the lipid contents of a lean and a fat fish were examined using the Blight & Dyer [71] and the traditional Jensen method [72], it was found that the traditional Jensen method gave the same recoveries as the Blight & Dyer method in the case of lipids from fat fish, but from lean fish unacceptably low levels of lipids were obtained. Since the concentration of POPs should be reported on both fresh and lipid weight basis [73], it is of great importance to extract the total lipid content, and also crucial that the used extraction method leads to correct results for the pollutants as well as for the lipids. As lipids include both lipophilic triglycerids and more polar phospholipids, there is a need to use different solvents with a wide range of polarity, in order to quantitatively extract the total amount of lipid. In fatty fish, the phospholipid fraction is a lower proportion of the total lipids compared to in lean fish [73]. Thus, recovery of phospholipids has high influence on the total lipid content from lean fish compared to fatty fish. Moreover, phospholipids such as phophoinositides are most likely bound to other cellular biopolymers by ionic bonds and these are not easily disrupted by simple solvation with organic solvents [74]. When the cod muscle was homogenized, there was no discrepancy in the yield, but when whole muscle was extracted, there was a significant difference in yield when isopropanol/hexan (65/35, v/v) was used, compared to isopropanol/diethylether (25/10, v/v), see Table 6 **Paper V**. The solvent mixture isopropanol/hexan (65/35, v/v) was probably only able to solubilize relatively small amount of the indigenous water resulting in two separate liquid phases and thereby giving a lower extraction yield.

Isopropanol/diethylether (25/10, v/v) on the other hand easily dissolved indigenous water and most likely remained in one phase during the extraction process. Hence, this solvent mixture gave significantly higher total lipid yield than isopropanol/hexan for intact cod muscle, 0.51 weight% compared to 0.36 weight%, respectively. Yields of POPs in cod muscle in the PFE extract and in the extract from the traditional Jensen [72] used at the Department of Applied Environmental Research at Stockholm University, are compared in Figure 6.
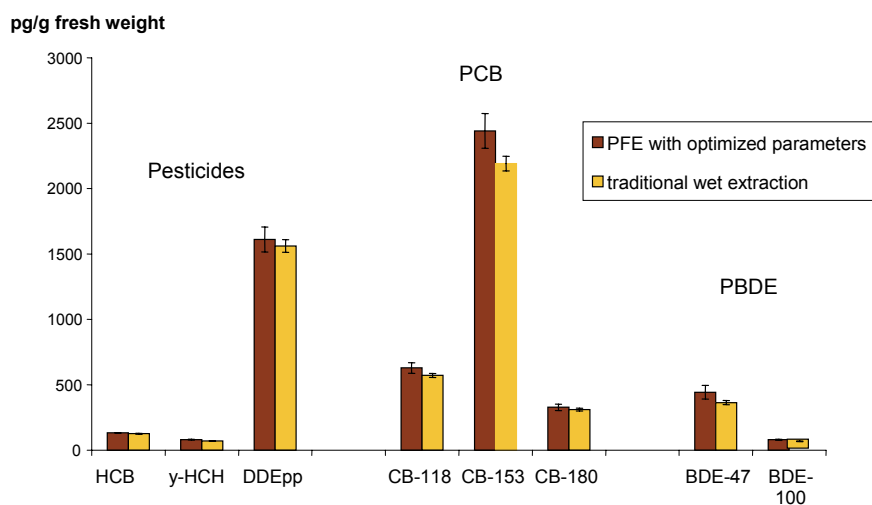

Figure 6. Comparison of the yields of POPs in lipids extracted from cod muscle by PFE and traditional Jensen. HCB (hexachlorobenzene, α- β- and γ−HCH (hexachlorocyclohexanes), p,p´-DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethene), CB 118 (2,3',4,4',5-pentachlorobiphenyl), CB 153 (2,2'4,4',5,5' hexachlorobiphenyl), CB180 (2,2',3,4,4',5,5'-heptachlorobiphenyl), BDE-47 (2,2',4,4'-tetrabromodiphenyl ether) and BDE-100 (2,2',4,4',6-pentabromodiphenyl ether). The bars indicate the RSD values.

No significant difference between PFE and traditional wet extraction method concerning the extraction yield of polychlorinated biphenyls (PCB), chlorinated pesticides and polybrominated diphenylethers (PBDE) was obtained, with a possible exception for CB 153. The RSD values were slightly larger for PFE, which might partly be because of the smaller sample amount that was extracted in the PFE method, 3g compared to 10g, as seen in Figure 7. In Figure 7 an extraction scheme of the modified Jensen and the developed PFE method is illustrated. Although the last clean up step is a bottleneck both for Jensen and for the developed PFE method, solvent consumption was reduced by 50% per extraction. PFE has the additional advantage of higher

degree of automation and reduced solvent exposure to the operator. With a solvent controller, up to four different solvents can automatically be mixed and delivered to the extraction cell. This decreases the amount of time spend on laborious and error-prone tasks like measuring and mixing solvents.
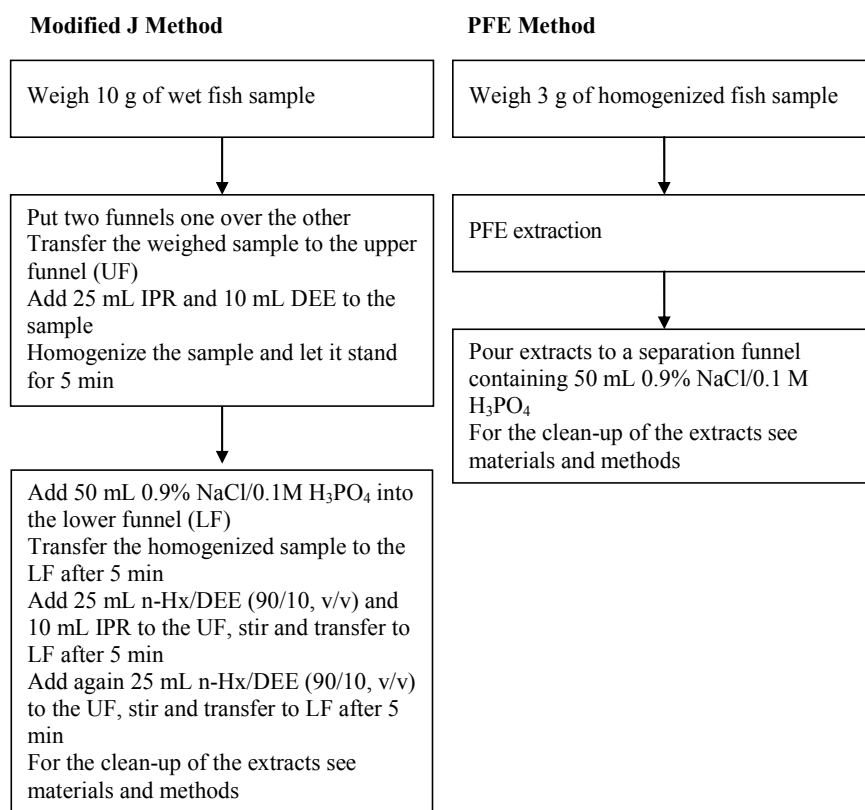
Modified J Method

Weigh 10 g of wet fish sample

↓

Put two funnels one over the other Transfer the weighed sample to the upper funnel (UF) Add 25 mL IPR and 10 mL DEE to the sample Homogenize the sample and let it stand for 5 min

↓

Add 50 mL 0.9% NaCl/0.1M H_3PO_4 into the lower funnel (LF) Transfer the homogenized sample to the LF after 5 min Add 25 mL n-Hx/DEE (90/10, v/v) and 10 mL IPR to the UF, stir and transfer to LF after 5 min Add again 25 mL n-Hx/DEE (90/10, v/v) to the UF, stir and transfer to LF after 5 min For the clean-up of the extracts see materials and methods

PFE Method

Weigh 3 g of homogenized fish sample

↓

PFE extraction

↓

Pour extracts to a separation funnel containing 50 mL 0.9% NaCl/0.1 M H_3PO_4 For the clean-up of the extracts see materials and methods

Figure 7. Comparison of extraction schemes of the modified Jensen and the developed PFE method for the gravimetric determination of total lipid content. (**Paper V**).

In another study performed in collaboration with NFA, although not presented in the thesis, pesticides from very high water containing vegetables like iceberg lettuce (water content 96%) were extracted using ethyl acetate, which is the traditional solvent at NFA. Thirty nine pesticides of varying physical and chemical properties were tested at extraction temperatures ranging between 40 and 100°C, but the recoveries were disappointingly low, between 30-80%, probably due to low diffusion rates of ethyl acetate through the stagnant water layer in the lettuce, although hydromatrix as drying agent was mixed with the iceberg lettuce in proportions (67/33, w/w). Low recoveries were also obtained when the iceberg lettuce was freeze-dried

before extraction because of losses of pesticides during the drying step. However, when the iceberg lettuce was spiked after freeze drying, the extraction yields were good, between 80-100%.

Another interesting aspect with PFE is the possibility to choose nearly any solvent, apart from very low or high pH solvents. In one collaborative study with an oil concern, although not presented in this thesis, the objective was to predict the amount of process chemicals that could migrate into the sea if there would be an oil discharge. By using a water solution of the same salinity as sea water, oil samples containing added process chemicals were extracted at different temperatures and times. The results can be used to predict what would probably happen in a real world situation. This type of investigation is of course more environmentally relevant, than a study performed with traditional extractions based on organic solvents.

3.3.2 The hotter the better?

Besides the selection of an extraction solvent, temperature can be considered as the second most important parameter in PFE. The advantages of using higher temperatures are described in every article published in PFE, and is thoroughly described in Chapter 2.2 and 3.1. As a general rule, it can be said that higher temperature gives better extraction yield. It needs to be remembered that the range of solvents applicable in PFE is much wider than that of Soxhlet extraction. A poor solvent for Soxhlet extraction can however be a good solvent in PFE due to the higher extraction temperatures used [5].

Optimization studies in **Paper III,** and optimization studies using experimental design in **Paper I, V,** and **VI** showed that higher temperatures in general gave higher extraction yields.

However, other factors might hinder the use of the highest temperature even if it was found as being optimal in an experimental design. For example, in **Paper I** the sample matrix (granules of LLDPE) started to dissolve/melt and block the lines of the instrument depending on solvent and temperature, e.g. at temperatures above 75°C using tetrahydrofuran as a solvent. In **Paper IV**, where pesticides from rape seed were extracted, the results from the screening study showed that the interpretation of the chromatograms was difficult due to the large number of co-extracted compounds. It was obvious that higher temperatures gave more matrix peaks [75], which to a large extent most likely were derived from co-extracted lipids. In **Paper V,** the optimal temperature found with homogenized cod tissue, gave lower yield when extracting intact wet cod muscle. Most probably this was because the proteins in the sample denatured /coagulated to a hard pellet during the extraction, making the diffusion of the solvent into the matrix not efficient enough to extract all the lipids.

In another study, in **Paper VI**, where squalene was extracted from olive biomass, the thermostability of the analyte was a limiting factor in the choice

of extraction temperature. To determine the concentration of squalene in the olive biomass, an optimization study using experimental design was performed for the variables; extraction temperature, extraction time, and concentration of acetone in a mixture of acetone and 2-propanol. Significant effects were determined to be temperature, extraction time, the interaction between temperature and extraction time and the square of the extraction time. This study showed that at temperatures above 100°C the yield of squalene decreased with extraction times longer than 12-15 min, which indicated that squalene either decomposed or reacted with other sample components or with the solvent.

From the study in **Paper VI** it was also interesting to note that at lower temperature, i.e. at 100°C, acetone seemed to be the most appropriate solvent compared to 2-propanol and ethanol, as seen in Figure 1, **Paper VI,** but at temperatures at 190°C the difference in solvating power between ethanol and acetone was practically eliminated. Therefore the choice of solvent is not of the same importance at higher extraction temperatures, which makes it possible to choose a less costly and a more environmentally and health friendly solvent.

3.3.3 Speed in PFE

A survey of chemists' working time in laboratories declares, that chemists spend approximately 60% of their working time preparing samples for analysis [53]. One of the biggest advantages of using high diffusion fluids is generally short extraction times, which is explained in Chapter 2.2. Comparative studies between traditional Soxhlet extraction and high diffusion fluid extraction techniques have been performed by many research groups [38,56,76,77]. Vandenburg et al.[56] made a comparison study where they extracted additives from different polymer matrices using Soxhlet, sonication, MAE, SFE and PFE. The Soxhlet method used 6-24 hours, the sonication method used 40-60 minutes, the SFE method used 20 minutes to 2 hours, MAE used 30-60 minutes, and the PFE method used only 15 minutes. These results are comparable to other equivalent studies, where techniques using high diffusion fluids clearly demonstrate the generally high speed of extraction, and PFE has proved to be the fastest [25,38,56].

Due to the very short extraction times using PFE, the amount of co-extracted material is relatively low, which results in fewer clean-up step, and therefore drastically shortens the total extraction and analysis time.

The general decrease in extraction time compared to Soxhlet is obvious in all the studies in this thesis. In **Paper I**, the extraction time was 45 minutes compared to a described Soxhlet extraction of 6-72 hours. **Paper I** also demonstrated that no further clean up step was needed before analysis. In **Paper II**, where labile phosphorus compounds were extracted from lake sediment in a sequential extraction using two different aqueous solvents, the

extraction time was 90 minutes compared to one to two days for the tradi-tional liquid extraction method. In **Paper III,** the PFE extraction of CPs from household waste was performed in less than 30 min, compared to the three hours required for Soxtec, besides PFE gave cleaner extracts relatively free from interferences. Instead of five clean-up steps, only the last step was needed for the PFE extracts. The total improvement in analysis time includ-ing extraction and clean-up steps is shown in Figure 5 and in Figure 7, illus-trating method development in **Paper IV** and **Paper V**, respectively.

3.3.4   Particle size matters

It is obvious that the extraction rate, independent of what kind of extraction technique is used, depends on diffusion of the solvent into the matrix, fol-lowed by diffusion of the analytes out from the matrix. Therefore, the ex-traction rate can be greatly increased by decreasing the particle size, which also is a very common procedure [5,56,58,78]. The greater the surface area that can be exposed in a sample, the faster the extraction will occur. On the other hand, homogenization or grinding of the sample may increase the risk of degradation or oxidation of the analytes or the matrix. There is also a risk of loosing volatile compounds [78]. Another concern is that valuable time can be saved, if the homogenization procedure can be minimized.

  As expected, better and more reproducible yields were obtained in several of the studies presented in this thesis, when the sample was grinded. This is obvious in Table 2, **Paper III**, where the recovery of CPs increased to over 90% compared to 75% before homogenization. In **Paper V**, where total lipid content of wet cod muscle was investigated, a high yield of total lipids was obtained when the sample was homogenized (0.68 weight%, RSD 3.7%). When intact tissue was instead extracted, the yield was lower; 0.36 weight% (RSD 3.7%) or 0.51 weight% (RSD 18.0%) depending on which solvent mixture was used. Purely mechanical factors such as formation of inclusion complexes can decrease the extractability of lipids by limiting the accessibility of the solvents [79]. Another reason for the low yields obtained by PFE when using intact wet cod muscle might be due to the large differ-ence in particle size leading to a more inhomogeneous diffusion path distri-bution through formation of channels [80]. However, the increased extraction efficiency when the cod muscle was homogenized was probably due to that finely divided particles are more easily extracted as a result of their large surface area to volume ratio.

  Interestingly, in **Paper I**, the grinding step of LLDP granules was omit-ted, but instead the extraction time was increased and in this way equal yields were obtained. Significant labour time could thus be saved with only a slightly longer extraction time and without losses in yield.

3.3.5 Pressure has little effect

In PFE higher pressure is mainly used to keep the solvents as liquids during the extraction process. The effects of pressure are discussed in more detail in Chapter 3.1. In none of the pre-studies, in **Paper I - VI** did a change in pressure affect the yields. Hence, in all studies reported in this thesis constant pressure was used throughout all experiments.

3.3.6 Selective extractions – by adsorbents or solvent choice

Selective extractions are preferably optimized by changing the solvent or the solvent power. Under ambient conditions (room temperature and atmospheric pressure) the solvent power of a liquid is essentially constant regardless of extraction conditions. In a supercritical fluid, however, the solvating power can be changed depending on the pressure and temperature, as discussed in Chapter 2.3.1. One of the advantages using SFE is that selective extractions can be performed with ease and precision [81]. By using PFE on the other hand, selectivity can only be obtained by varying the nature of the solvent or the solvent mixture [9,82]. Reighard et al. [37] pointed out that a potential disadvantage with PFE is that the extraction tends to be exhaustive, therefore leading to nonselective extractions. Hawthorne et al [38] compared the difference in color of the extracts, when soils contaminated with PAH were extracted by Soxhlet, SFE, PFE and SWE. The Soxhlet and the PFE extracts were much darker due to more extracted compounds i.e. the extractions were less selective.

However, by using adsorbents in the extraction cell as an in-line clean-up step, more selective PFE extractions can be obtained. J. Ezzel et al. [83] employed activated acidic alumina to adsorb fat, when PCBs were extracted from spiked freeze-dried fish tissue, and achieved in this way cleaner chromatograms. S. Sporring et al. [84] used sulphuric acid impregnated silica to adsorb the lipids from fat-containing food and feed when extracting PCBs, and in this way obtain lipid-free extracts.

The effectiveness of activated alumina was tested in **Paper IV**, to eliminate co-extracted lipids, when a multi-method for determination of pesticide residues in rape seed was developed. The lipids adsorbed well on alumina, as seen in Figure 2, **Paper IV**, but since almost all target analytes tested were also strongly adsorbed, alumina was abandoned in this particular study. Instead, a lower extraction temperature was chosen, in combination with gel permeation chromatography (GPC) as a final clean-up step, in order to obtain extracts clean enough for final analysis, as seen in Figure 5.

In **Paper III**, adsorbents such as XAD-2, XAD-4, and XAD-16 were tested without success to eliminate interfering compounds, when extracting CPs from household waste, but interestingly clearer extracts and cleaner chromatograms were obtained when the drying agents Hydromatrix and

sodium sulphate were used as shown in Table 2, **Paper III**. These results are in agreement with Ramos et al. [9,38].

In an additional study, although not included in the thesis, squalene and alpha-tocopherol were selectively extracted from olive oil without coextraction of triglycerides when utilizing amberlite XAD-16 as adsorbent. A liquid chromatogram of the olive oil before and after the PFEextraction is illustrated in Figure 8.
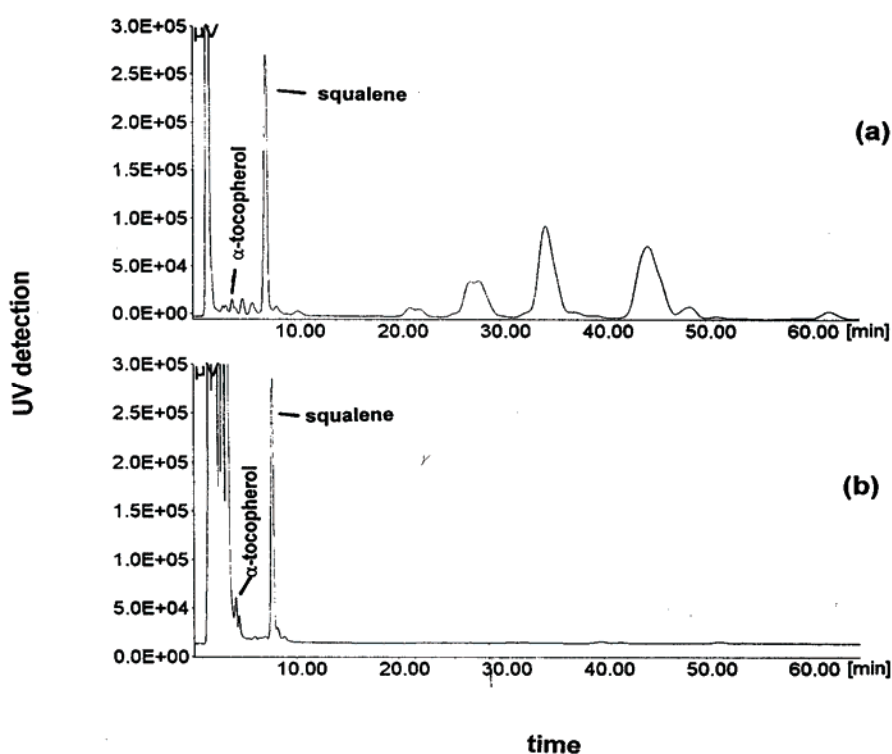

Figure 8. Liquid chromatogram of (a) olive oil diluted with ethanol and acetone; and (b) a PFE extract of olive oil, obtained using XAD-16 as adsorbent and methanol extracting solvent.

3.3.7 Recovery and precision

As mentioned above several studies comparing traditional Soxhlet with PFE, MAE, SFE and other extraction techniques have been published [23-25]. For example, Saim et al. [76] compared the extraction of sixteen individual PAHs from contaminated native soil using Soxhlet, pressurized and atmospheric MAE, SFE and PFE. The highest recoveries of individual PAHs in this study were obtained by Soxhlet extraction. However, the RSD-values obtained by the PFE method were equal or less than the values obtained by

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Soxhlet extraction. In contrast, Heemken et al. [77] compared PFE with SFE, Soxhlet, sonication and methanolic saponification, when extracting about forty different compounds of PAHs from certified sediment and marine particulate matter, and they found that the obtained recoveries and precision using PFE compared well with the other methods investigated. Helaleh et al.[25] compared the techniques Soxtech, Soxhlet, and PFE, when extracting 17 organochlorine pesticides in sewage sludges. The highest recoveries were obtained using PFE (79.5% – 95.8%) and Soxtech (78.8% - 96.7%) compared to Soxhlet (75.8% – 94.3%). On the other hand, looking at precision expressed as RSD, Soxhlet turned out to be the best (1.6 - 3.9%) followed by PFE (2.1% - 5.5%) while Soxtech ranged from 2.2 to 6.8%. In another study, Hawthorne et al. [38] extracted PAHs-containing soil and air particulate matter, comparing the techniques Soxhlet, PFE, SFE and SWE. The quantities of representative PAHs extracted by each method yielded similar extraction efficiencies, but slightly higher total PAH concentrations were shown for the Soxhlet and PFE extraction. The authors interestingly concluded that the higher yield obtained by Soxhlet and PFE might be due to co-extracted artifacts.

When comparing yields, the whole analytical chain has to be taken into account, not only the extraction process, but also the clean-up steps before analysis. The study in **Paper III** showed higher yields of CPs and cleaner chromatograms compared to the traditional Soxtech ™, but in **Paper IV** the recoveries of the pesticides yielded an overall recovery of about 91% compared to 96% using the traditional LLE and with high precision (RSD 1-4%) for both methods, see Table 2,3 and 4 in **Paper IV.** In **Paper V,** the 10% higher yield is due to that more polar lipid-like compounds were extracted.

One should always keep in mind that there is no technique or method that can solve all the problems and challenges in chemical analysis. However, in summary, the recovery and precision of the PFE technique, looking both in the literature and to the results in this thesis, compared well with other extraction techniques like Soxhlet, Soxtech, MAE and SFE.

4 Conclusions

In this thesis, the PFE technique has been evaluated as a versatile and environmentally friendly technique in chemical analysis. A number of analytes of different chemical properties have been extracted from different environmentally interesting matrices like polyethylene, lake sediment, source separated household waste, rape seed, fish muscle and olive oil biomass. In addition to similar and improved recoveries and precisions, there are several advantages with the PFE technique compared to many of the commonly used solvent extraction techniques, as described below:

Method development in PFE is rather straightforward, especially compared to the situation with SFE. Oftentimes, knowledge from currently used (or other similar) traditional methods can be utilized as a starting point, as demonstrated in **Paper I, III, IV** and **V**.

In addition, since the extractions are performed at higher temperatures, diffusion rates are faster and solvent power is stronger, which facilitate the extraction process, and make the PFE technique a powerful tool in chemical analysis. PFE is especially suitable for *complex matrices*, which is often the case in environmental applications, like soil, sediment, clay, sludge, and households waste, see **Paper III**.

In all of the studies included in this thesis, **Paper I-VI**, there is an apparent *decrease in solvent consumption* in the range of 50-90% compared to in traditional solvent extraction technique. Furthermore, because of the advantages when performing extractions at higher temperatures, the choice of solvent is of considerably less importance, and thus *traditional solvents can be exchanged* to more environmentally friendly and health-beneficial solvents, giving the same yield and precision, as seen in **Paper VI.**

All studies in this thesis present *very short extraction times*, as well as *shorter overall analysis time.* Due to the shorter extraction times, fewer compounds are co-extracted, and less time is needed for additional sample clean-up, and thus labour-intensive sample preparation steps can be omitted as seen in **Paper I-V**. In addition, when fully automated PFE instruments are used the total time of manual work per sample can be minimized.

The main drawback of the PFE technique is often said to be the non-selective extractions [9]. This thesis shows that some selectivity is possible to obtain, if a thorough investigation of the "best" solvent is chosen, i.e. a solvent with good properties for selectivity, dissolving the analytes at the extraction temperature without extracting matrix components, as seen in **Paper**

I, and to some extent, in **Paper III**. However, in some cases it is necessary to use adsorbents in order to accomplish the desired selectivity [84].

Another potential drawback is the high initial cost of the instrument, but on the other hand, the higher degree of automation, sample throughput, and lower solvent consumption, resulting in lower solvent disposal costs, will outweigh the high initial investment cost.

4.1 Does the PFE technique obey the twelve principles of Green Chemistry?

Is PFE an environmentally sustainable analytical technique obeying the Twelve Principles of Green Chemistry? Is PFE capable of providing the society with ecologically and economically efficient processes?

PFE meets the first principle of Green Chemistry by using smaller amounts of organic solvent, thereby preventing unnecessary waste, since less organic solvent needs to be manufactured and less waste is produced at the manufacturer. In addition, less organic solvent waste is produced at the laboratory, and in total there will be a decrease in VOC emissions, and a reduced risk for photochemical smog formation. Accidents of emissions to municipal waste water plants will do less harm. In general, the health beneficial effects are notable.

By being able to substitute hazardous solvents to more environmentally friendly ethanol and water, the toxicity is reduced with positive effects on both the environment and health in general, which meets with principle 5.

Principle 6 deals with minimizing the amount of energy. Creating high diffusion fluids require a significant amount of energy, which is a disadvantage. But since the extraction times are shorter, minutes compared to hours in Soxhlet and modified Soxhlet methods, and these traditional solvent extraction techniques are usually performed just below the boiling point, a fair amount of energy is needed in these techniques too. A limited LCA would be needed to calculate the overall energy conversions.

The possibility to screen for highly valuable compounds, from renewable feedstock, from forestry or agricultural origin or from by-products from forestry and agriculture industries, by utilizing PFE or PHWE suits well with Principle 7. The compounds of interest should be highly valuable and for the industry interesting compounds. In this way, these compounds are not needed to be chemically synthesized and thus emissions from manufacturing will be omitted. This was shown in **Paper VI** where squalene was extracted from olive biomass.

In summary, this thesis has shown the versatility of the PFE technique, which can be utilized in different fields, and thus give a major contribution to sustainable chemistry.

5 Discussion and future aspects

One of the most important challenges for scientists in general is to set up hypotheses and look for models that describe the reality as close as possible. In addition, chemists need to invent methods, to prove hypotheses. New techniques and methods will hopefully give us as true an answer as possible in all kinds of areas investigated, e.g. human body, other biota, water, soil, sediment and air, regarding which species is present and at what concentration. From these results we may draw conclusions of what is normal and what is deviating. To achieve those results, analytical chemists need to consider the entire analytical chain, from sample preparation to data analysis. From a sample preparation point-of-view, several new questions and ideas have arisen during the course of this thesis work.

For example, there is still a need for more knowledge, to fully understand the eutrophication issue in lakes and seas. There is a lack of consensus, whether nitrogen or phosphorus is the limiting factor in the Baltic sea, but in lakes, phosphorus is considered to be the limiting element. It is essential to understand the causes behind the still so high concentration of phosphorus in lakes and in the Baltic sea in spite of decreased river input [85]. From the results in **Paper II,** where the amount of labile organic phosphorus compounds were extracted under mild extraction conditions from lake sediments, the interesting questions concerned the identification of these groups of labile phosphorus compounds. In a following study, **Paper VII**, different categories of phosphorus compounds were mapped in a lake sediment profile. These results are valuable for understanding the chemistry of the internal loading of phosphorus from anoxic sediments that distribute into the water. The next challenge will be to identify single phosphorus compounds, and to track their origin, and thereby improve the understanding of the still high concentration of phosphorus in the water column in lakes and in the Baltic sea.

In order to better understand and thus improve the knowledge about nature, many different analytical techniques are frequently applied. The PFE technique is an attractive tool to mimic and/or follow natural occurring processes, as shown in **Paper II**. Another proposed study at our department, where the PFE technique can be utilized, concerns the bonding/adsorption of siderophores, strong iron-chelate complexes, onto podzolic soil particles. Such experiments could result in gained knowledge about the processes of weathering and release of minerals to the forest. We will try to mimic the

natural weathering process by using water or weak acids, resembling the pH in pore water in the forests, and perform extractions at different temperatures.

The amount of POPs in living organisms is commonly specified and calculated based on their total lipid content. In **Paper V**, the PFE technique showed a yield of about 10% more lipids in cod and herring compared to a conventional technique regardless of whether the material was homogenized or not. These extra 10% of lipids are most likely of phospholipids nature. Questions that should be answered are what is the distribution of organic contaminants across various lipid types? Are the partitioning of the different POPs the same? Are there "new" environmental toxic compounds to be found in this extra extracted lipid like phase? If so, are they harmful?

Another challenge would be to *follow the migration* of any hazardous compound and their degradation products in soil, sediment, sludge or biota.

It would also be interesting *to simulate* what could happen in a real case situation (in the environment, in the body or within an industrial process). This can be accomplished using a suitable solvent, similar to the surroundings in question, for example water with the right pH or the right ion concentration (like sea water). Anaerobic conditions can be simulated using a nitrogen purge between sequential extractions. Thus it would be possible to *predict* what would happen if a specific chemical is used by a specific company in a specific environment and in this way obtain detailed information for an environmental regulatory impact analysis.

As a result of fast and efficient extractions and automation there is a possibility to *perform screening* studies, i.e. to extract valuable compounds from plants, biomass waste and other renewable matrices, like in **Paper VI.** Such extracted valuable compounds could be of great interest for pharmaceutical-cosmetic and/or food industries. The extraction of compounds synthesized by plants would promote a more efficient use of natural resources and recycling and thereby good adaptation to several of the twelve principles. Today there are several hundred industrial plants that use supercritical carbon dioxide instead of organic solvent e.g. in the fields of food and natural product manufacturing. A future possibility is to transform the PFE technique to process industry using water, water mixed with ethanol or ethanol, so called green fluids, as solvents. There would be no contamination of the raw feedstock, and the rest products could still be used as e.g. animal food, fuel or biodegradable waste. This idea is presented in Figure 9. Ongoing projects in our laboratory are extraction of the anti-inflammatory compound betulin in birch bark using ethanol at 160°C and 5 MPa as a solvent [86], and extraction of the antioxidant quercetin from onion waste using water at 120°C and 5 MPa as a solvent, in manuscript [87,88].
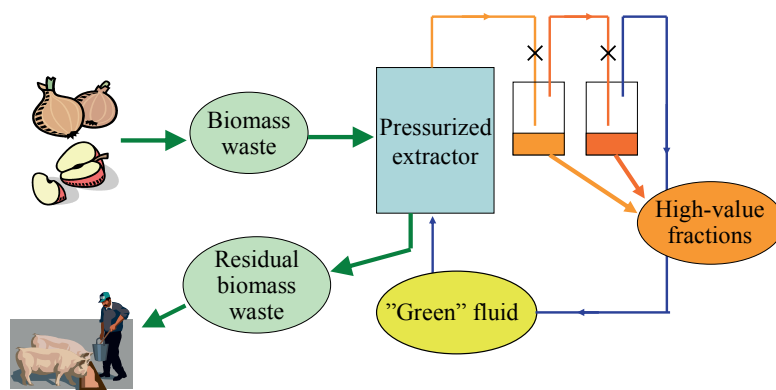
Figure 9. A future PFE plant using plants or biomass waste as raw material and "green" fluid as extraction solvent for the extraction of valuable compounds.

If this "green" industry would become a reality the total emissions in a LCA perspective would decrease by at least one order of magnitude compared to what is emitted in conventional chemical processes, and thus the PFE technique would fully obey green chemistry and a more sustainability engineering.

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…….. att det blev en avhandling…..tusen tack till

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7 Summary in Swedish

7.1 Bakgrund

Brundtlandrapporten, utarbetad av FN, definierade 1987 begreppet hållbar utveckling, som en utveckling som tillfredställer dagens behov, utan att äventyra kommande generationers möjligheter att tillfredställa sina behov. Begreppet hållbar utveckling ratificerades vid det globala mötet i Rio 1992. I samband med mötet i Rio, behandlades helhetsperspektivet på globala miljöfrågor. Genom att fokusera på helheten och hur olika delar påverkar varandra, i stället för att betrakta ett systems enskilda delar, har livscykeltänkandet vuxit fram. Analyser som utreder helheten av en produkt/nyttighet kallas livscykelanalys (LCA). I begreppet *hållbar kemi* ingår att kemin ska anpassa sig till besluten från Riodeklarationen. Forskning och tillämpning inom kemiområdet har till stor del bidragit till vår utveckling med längre livslängd, friskare, bekvämare och roligare liv, men kemiska föreningar har också orsakat och orsakar miljöproblem. Dessa kan förkomma i för höga halter eller inneha egenskaper som t.ex. toxiska, hormonpåverkande, fosterskadande, svårnedbrytbara och/eller bioackumulerbara. Kemister, kemikalier och kemisk industri har fått skulden för många av dagens miljöproblem och därför är utmaningen för dagens kemister att tänka i banor som hållbar kemi vid framställning av nya ämnen, nya produkter eller vid förändring av kemiska processer. Detta kan ske genom att göra bedömningar i ett LCA-perspektiv och/eller försöka anpassa sig till Green Chemistry, grön kemi, och dess tolv principer. Green Chemistry utvecklades i USA i början på 1990-talet och är baserad på att utveckling av nya produkter och ny teknik ska ske på ett så miljöanpassat och kretsloppsanpassat sätt som möjligt.

Analytisk kemi och kemisk analys måste självklart också anpassa sig till hållbar kemi. Den analytiska kedjan indelas i; provtagning, provupparbetning, separation, detektion och mätvärdes- behandling. Under de senaste 30-40 åren har utveckling av teknik och forskning varit fokuserad på den senare delen av den analytiska kedjan. Dagens krav på analyser är att de ska vara snabba och korrekta, samtidigt som allt fler analyser ska utföras inom alla tänkbara områden i samhället, vilket gör att intresse för forskning inom provupparbetningsområdet ökat markant.

Den vanligaste provupparbetningsmetoden under hela 1900-talet var extraktion. Traditionella vätskeextraktioner kan vara tillförlitliga men de är långsamma, arbetskrävande och kräver ofta stora volymer organiska lösningsmedel, vilket leder till att stora mängder kontaminerade lösningsmedel måste tas om hand. Dessa lösningsmedel kan vara toxiska för dem som handhar dem, men också miljöbelastande, då de vid utsläpp till luft kan orsaka bildning av t.ex. marknära ozon. Genom att kontrollera temperatur och tryck kan lösningsmedlens diffusionsegenskaper och den lösande kraften hos lösningsmedlet avsevärt förbättras, vilket lett till att nya tekniker utvecklats, t.ex. superkritisk vätskeextraktion (SFE), mikrovågsextraktion (MAE) och trycksatt lösningsmedelsextraktion (PFE). Alla dessa tekniker ger överlägset snabbare extraktioner och system som är lättare att automatisera. De ger dessutom bättre eller likvärdiga utbyten, möjlighet till selektivitet samt eliminerar eller kraftigt minskar volymen av hälsovådliga och miljöbelastande lösningsmedel.

7.2 Denna avhandling

Denna avhandling har studerat effektiviteten, tillförlitligheten samt bidrag till miljöpåverkan av PFE- tekniken som provupparbetningsmetod inom kemisk analys. PFE är en relativt nyutvecklad teknik som utnyttjar hög temperatur, högt tryck och små volymer organiska lösningsmedel. Hög temperatur bidrar till att öka lösningsmedlets lösande förmåga samt diffusionen av analyten. Det höga trycket är till för att hålla lösningsmedlet i flytande fas under extraktionen. I den här avhandlingen har många olika analyter i flera olika omgivningar extraherats.

Artikel I. Utveckling av ny metod för att analysera halten av antioxidanten Irganox 1076 i poly-etengranuler.

Artikel II. Utveckling av en mild extraktionsmetod där vatten användes som lösningsmedel, för att uppskatta och bestämma halten i sjösediment av organiska fosforföreningar, som är så pass lättnedbrytbara att de frigörs från sedimenten och bidrar till den ökande övergödningen i sjöar och även i Östersjön.

Artikel III. Utveckling av PFE-metod för att extrahera klorparaffiner ur källsorterat hushållsavfall, som anses vara en komplex matris i extraktionssammanhang, eftersom kemiska sammansättningen hos hushållsavfall kan vara mycket varierande. Den utvecklade PFE-extraktionsmetoden har jämförts med den Soxtech-metod som används vid avdelningen för Tillämpad Miljöanalys, Lantbruksuniversitetet Ultuna, Uppsala.

Artikel IV, beskriver en multimetod för att extrahera pesticider, allt från polära till opolära, i rapsfrön. Svårigheten är att extrahera ut de opolära pesticiderna ur de fettrika fröna, utan att extrahera fett från rapsfröna, då detta

fett stör den slutliga analysen. Den utvecklade metoden har jämförts med den gängse metoden som Livsmedelsverket, Uppsala, använder idag.

Artikel V. Ur ekologisk synpunkt är det mer relevant att uttrycka mängden miljögifter s.k. persistenta organiska föreningar (POPs) per mängd fett i stället för per mängd våtvikt. Därför är analys av total mängd fett viktigt. Åtskilliga jämförande laboratorier har visat att den ursprungliga s.k. Jensen-extraktionsmetoden ger tillfredställande utbyten, när den användes för extraktion av totala mängden lipider i fet fisk. Däremot gav den ursprungliga Jensen-metoden för låga utbyten av totala lipidinnehållet i mager fiskmuskel (där fettinnehåll är <1 %). En PFE-metod har utvecklats för magra matriser, i detta fall torskmuskel, och resultaten har jämförts med traditionell och modifierad Jensen-metod, som används hos ITM, Stockholms universitet

Artikel VI behandlar frågeställningen om det finns det värdefulla föreningar i en restprodukt s.k. biomassa, från olivoljetillverkning, som skulle kunna extraheras och användas för framtida bruk i andra industrier. En PFE-metod utvecklades för extraktion och kemisk analys av skvalen och α-tokoferol ur ovanstående restprodukt. Den utvecklade metoden skulle kunna vara en start för uppskalning till en process helt i linje med Green Chemistry.

7.3 Sammanfattning

Ett antal anlyter med helt olika kemiska egenskaper har extraherats med PFE-teknik ur mycket varierande matriser. Förutom att utbyten och precision var likvärdiga med vanliga, traditionella lösningsmedelsextraktioner har ett antal fördelar noterats;

- *Metodutveckling* är relativt enkel och generell. Ofta kan kunskap från traditionell lösningsmedels-extraktion överföras och modifieras, se artiklar I, III, IV och V.
- Tack vare att extraktionerna med PFE utförs vid högre temperaturer erhålls bättre diffusions-egenskaper, som gör PFE-tekniken *användbar för särskilt komplexa matriser*. Komplexa matriser är ofta vanliga i miljösammanhang, t.ex. jord, sediment, slam och lera, se artikel III.
- Alla studier i avhandlingen pekar på en *reduktion av organiska lösningsmedel med 50-90 %,* se artikel I-VI. Samtidigt visas också att eftersom extraktionerna utförs vid högre temperaturer, är val av lösningsmedel inte lika kritiskt, som när extraktionerna utförs strax under lösningsmedlets normala kokpunkt. Därigenom kan *mer miljövänliga och mindre toxiska alternativ till lösningsmedel väljas*, se artikel III och VI.
- Alla studier visar också på *mycket korta extraktionstider*, såväl som *totalt förkortade analystider* jämfört med de traditionella extraktionsmetoderna. Kritiken mot PFE-tekniken är oftast beskriven som att den inte är så selektiv som man skulle önska. Trots korta extraktionstider, föreligger risk för extraktion av icke önskade analyter. Denna avhandling visar att ge-

nom att välja lösningsmedel eller blandning av lösningmedel och "rätt" temperatur tillsammans med adsorbenter, kan viss selektivitet uppnås. På grund av att extraktionstiderna är förkortade, från åtskilliga timmar (6-48 tim.) till mellan 5-10 minuter, leder det till färre eller inga upreningssteg, se artikel I och III, IV och V. Detta betyder också att arbetsinsatserna i tid blir mindre och att arbetsmiljön för personalen förbättras, då eventuell exponering av lösningsmedel förkortats drastiskt.

Avhandlingen visar att PFE-tekniken är väl anpassad till Green Chemistry, och hållbar kemi, framför allt genom att volymerna av organiska lösnings-medel minskar så drastiskt samt att mer miljöbelastande lösningsmedel kan bytas ut mot mer miljövänliga såsom etanol och vatten.

Nya frågeställningar och idéer har framkommit under avhandlingens gång:
- För att bättre förstå kemin bakom den ökande övergödningen, som antas bero på att lagrade organiska fosforföreningar från sedimenten frigörs, undersöktes i artikel **VII**, en sedimentprofil från en insjö för att identifiera vilka grupper av organiska föreningar som förekommer på sedimentytan samt på olika djup. I en pågående studie försöker vi identifiera vilka en-skilda organiska fosforföreningar, som frigörs från sediment.
- PFE-tekniken kommer att användas i en planerad studie för att extrahera sidoforer, ett järnkelatkomplex, som finns i podsoljordar i våra skogar. Detta för att förstå och öka kunskapen om den naturliga vittringsproces-sen i skogsmark.
- Resultaten från artikel V visar att med PFE-teknik var utbytet av totallipi-der 10 % högre än vid traditionell extraktion. Dessa 10 % extra extrahe-rade föreningar har lipidliknande egenskaper, frågan är då, vilka POPs som kan finnas i denna fraktion. Är det samma POPs som i "vanliga" li-pidfraktionen? Finns det andra föreningar av POPs än dem vi analyserar idag? I så fall, är de toxiska?
- PFE-tekniken skulle kunna användas för att följa migration av ett förore-nande ämne i t.ex. mark, jord eller sediment och på så sätt vara underlag till miljökonsekvensananlyser.
- PFE-tekniken skulle också kunna användas för att simulera vad som skul-le kunna hända i verkligheten i olika situationer t. ex i människokroppen, i yttre miljön eller vid en industriell process. Detta genom att val av "rätt" lösningsmedel för varje situation kan väljas. Anaeroba miljöer kan skapas genom att blåsa kvävgas genom PFE-utrustningen.
- PFE-tekniken ger också möjlighet att relativt enkelt utföra "screenade" analyser, se t.ex. artikel VI, där värdefulla föreningar som skvalen och α-tokoferol extraherades ur ett avfall från olivoljeframställning. Extraktion av ekonomiskt intressanta föreningar, som syntetiserats av växterna själ-va, skulle utgöra en värdefull råvara i ett kretsloppsperspektiv. Förening-ar som extraheras från en biprodukt från skogsindustrin eller jordbruket,

ha ett högt ekonomiskt värde för t.ex. livsmedel-, kosmetika-, läkemedel-eller övrig industri. Som lösningsmedel skulle vatten, vatten blandat med etanol eller ren etanol användas. Pågående projekt är extraktion av betu-lin (troligen anti-inflammatorisk förening) ur björkbark med etanol som lösningsmedel och extraktion av quercetin (antioxidant, anti-neurodegenerativ förening) ur lökavfall med vatten som lösningsmedel. I figur 9 i avhandlingen illustreras tankegången kring en "grön" och krets-loppsanpassad industri, där miljöbelastningen i ett LCA-perspektiv kom-mer att vara betydligt lägre jämfört med nuvarande framställning av mot-svarande förening i kemisk processindustri. Här skulle PFE-tekniken kunna användas till "hållbar kemi " och Green Chemistry även i process-industrin.

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