COMPARATIVE STUDIES OF SUBCRITICAL WATER EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN MUNICIPAL SEWAGE SLUDGE

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ABSTRACT

The quantitative determination of organic contaminants in sewage sludge has received little attention so far, as compared to the voluminous amount of information that is available for the analysis of inorganic compounds in sludge. Many organic chemicals, termed as ‘priority pollutants’ due to their potentially toxic effects, are known to occur in sludge. In the specific context of wastewater and sewage sludge, polycyclic aromatic hydrocarbons (PAHs) are generally regarded among the most critical in terms of toxicity. Land application of sludge as fertilizers is a way of disposal and recycling of sludge. However, public concern has arisen due to the fact that organic contaminants in sludge may ultimately enter the food chain. Hence the need to analyse the organic contaminants such as PAHs in sludge. Therefore, in this study, subcritical water extraction (SWE) was utilised as a viable extraction method for the analysis of PAHs from municipal sludge samples. Various extraction procedures, such as pure SWE, surfactant-modified SWE and SWE coupled with membrane discs were attempted. Several factors were investigated for the pure SWE procedure coupled with SPE extract clean-up, which yielded the best recovery in comparison with the other methods. Recovery studies of PAHs from spiked sludge samples were performed using gas chromatography with flame ionisation detection. Although a recovery range of $38.84 - 56.06\%$ was obtained using the above mentioned method, the results indicated the usefulness of the technique as an alternative to Soxhlet extraction for the analysis of PAHs in sludge samples.
ABSTRAK

Penentuan kuantitatif pencemar organik dalam sampel enapcemar kurang mendapat perhatian, berbanding dengan maklumat yang sedia ada untuk analisis pencemar tak organik dalam enapcemar. Terdapat banyak pencemar organik, diistilah sebagai pencemar utama akibat daripada kesan toksik bahan tersebut yang wujud dalam enapcemar. Dalam konteks spesifik air sisa dan enapcemar, hidrokarbon aromatik polisiklik (PAH) merupakan salah satu pencemar yang paling kritikal dari segi ketoksikannya. Aplikasi enapcemar ke atas tanah sebagai baja adalah salah satu cara pelupusan dan penggunaan semula enapcemar. Namun, kebimbangan orang ramai telah timbul kerana pencemar organik dalam enapcemar ini akhirnya akan masuk ke dalam rantai makanan. Oleh itu, adalah perlu untuk menganalisis pencemar organik seperti PAH. Dalam kajian ini, pengekstrakan air sub-genting (subcritical water extraction, SWE) telah digunakan sebagai kaedah pengekstrakan untuk PAH dari sampel enapcemar. Beberapa prosedur pengekstrakan, seperti SWE tulen, SWE yang diubahsuai dengan surfaktan dan SWE yang diganding dengan cakera membrand telah dicuba. Beberapa faktor telah dikaji untuk prosedur SWE tulen dengan pembersihan ekstrak SPE, iaitu kaedah yang telah memberikan hasil pengembalian analit yang terbaik berbanding dengan kaedah-kaedah yang lain. Kajian pengembalian semula analit telah dijalankan dengan sampel pakuan menggunakan teknik kromatografi gas dengan pengesan pengionan nyala. Walaupun pengembalian analit dalam julat 38.84 – 56.06 % telah diperoleh dengan menggunakan kaedah di atas, keputusan ini mencadangkan bahawa teknik ini boleh digunakan sebagai alternatif kepada Soxhlet untuk analisis PAH dalam sampel enapcemar.
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<td>SWE</td>
<td>Subcritical Water Extraction</td>
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<tr>
<td>PAH</td>
<td>Polycyclic Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated Biphenyls</td>
</tr>
<tr>
<td>OCP</td>
<td>Chlorinated Pesticides</td>
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<tr>
<td>US EPA</td>
<td>United States Environmental Protection Agency</td>
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<tr>
<td>PFE</td>
<td>Pressurized Fluid Extraction</td>
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<td>ASE</td>
<td>Accelerated Solvent Extraction</td>
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<td>SPE</td>
<td>Solid Phase Extraction</td>
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<td>SPME</td>
<td>Solid Phase Microextraction</td>
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<td>FID</td>
<td>Flame Ionization Detector</td>
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<tr>
<td>CD</td>
<td>Cyclodextrin</td>
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<tr>
<td>SFE</td>
<td>Supercritical Fluid Extraction</td>
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<tr>
<td>CHClF₂</td>
<td>chlorodifluoromethane</td>
</tr>
<tr>
<td>N₂O</td>
<td>nitrous oxide</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>ε</td>
<td>dielectric constant</td>
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<tr>
<td>g/L</td>
<td>gram per liter</td>
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<tr>
<td>CE</td>
<td>Capillary Electrophoresis</td>
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<td>CZE</td>
<td>Capillary Zone Electrophoresis</td>
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<tr>
<td>EKC</td>
<td>Electrokinetic Chromatography</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>LLE</td>
<td>Liquid-Liquid Extraction</td>
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<tr>
<td>mg/kg</td>
<td>milligram per kilogram</td>
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<tr>
<td>mL</td>
<td>milliliter</td>
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<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulfate</td>
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d - diameter
ppm - parts per million
eV - electron volt
μA - micro Ampere
μL - micro liter
NR - Not Reported
DBS - dodecylbenzene sulfonic sodium salt
CMC - Critical Micelle Concentration
SDB - Styrene divinylbenzene
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CHAPTER I

INTRODUCTION

1.1 Background

Agriculture plays an important role in waste recycling thereby reintroducing substances as plant nutrition elements into natural cycles [1]. For example, sewage sludges contain appreciable amounts of sodium, phosphorus, potassium and magnesium and have significant inorganic fertilizer replacement value for these major plant nutrients. Crop productivity can also be increased by improving soil physical properties through application to soil of organic matter contained in sludge and biowaste. Thus the application of sewage sludge as fertilizer to agricultural lands represent an economical way to use the high amounts of sludge produced by wastewater treatment plants [2].

However, besides beneficial plant nutrients, these waste matrices also contain hazardous heavy metals and organic pollutants which enter food chains and are of great concern to the public. For sewage sludge and compost, limit values for heavy metals have been established as a quality criterion. These inorganic compounds are analysed on a routine basis. However, the characterization and long term observation of organic contaminants in sludge has received little attention so far [3], possibly because the methods of analysing these compounds are laborious and often complicated [4].
The range of organic compounds known to exist in sludge is extensive and diverse and is potentially transferred to sludge-amended agricultural soils. Sewage sludge is known to be contaminated with a wide array of xenobiotics (organic pollutants) [4] which has negative impact on soil organisms. Among these organic contaminants, non polar, persistent compounds exhibiting a high accumulation potential are important.

Typical representatives are polycyclic aromatic hydrocarbons (PAHs), chlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs). However OCPs are present only in extremely minute concentrations and generally does not pose a danger. The chemical-physical properties of the compounds mentioned are similar. They show high octanol-water distribution coefficients, are highly lipophilic and therefore tend to adsorb to solid particles of sewage sludge.

Monitoring the levels of these pollutants in sludge matrices is very important as introduction into the food chain can occur through grazing animals, direct plant intake and through leaching into ground water [5]. There are four main pathways through which a chemical from the soil can enter plants: (i) root uptake and subsequent transport in the transpiration stream; (ii) foliar uptake of vapour from the surrounding air; (iii) uptake by external contamination of shoots by soil and dust, followed by retention in the cuticle or penetration through it; and (iv) uptake and also transport in oil channels which are found in some oil containing plants such as carrots [6].

Concern about the environmental effects of recycling sewage sludge on agricultural land would appear to be legitimate given that many of the organic chemicals designated as ‘priority pollutants’ due to their potentially toxic effects, are known to occur in sludge [3]. Considering these problems, it is necessary to control the pollutant levels. Germany and the USA have indicated maximum levels for several pollutants in sludges and soils. Other countries, such as Sweden are opposed to the use of the sludges as fertilizer in agricultural soil [6].
1.2 Criteria in Re-Use and Disposal of Sludge

Sludge is defined as the by-product of liquid sewage treatment. It is any solid material containing large amounts of entrained water collected during wastewater treatment. If not handled carefully, it can pose detrimental effect to the human environment.

Certain regulations have therefore been made regarding the re-use and disposal of sewage sludge [7]. Of paramount interest are the chemical and biological characteristics of the sludge, as these factors influence its application. Parameters such as organic content, toxic organics, nutrients, pathogens and hazardous metals have to be taken into account before it can be distributed for re-use. Some of these hazardous elements are:

i. Potentially Toxic Elements (eg.: zinc, copper, nickel, cadmium, lead, mercury, chromium, arsenic and selenium)

ii. Organic Micro-Pollutants (eg.: PAHs, dioxins, OCPs, and PCBs)

iii. Pathogenic Organisms (eg.: viruses and human/ animal pathogens)

Some of the more desirable materials in sludge also need to be monitored [7], for the most beneficial reuse of the sludge. These parameters include:

i. Water and plant nutrients (nitrogen, phosphorus)

ii. Organic matter (natural) and non-toxic trace elements

iii. Microorganisms (for microbial diversity)

The above mentioned parameters, together with other important considerations needs careful attention when deciding on the best alternative for the re-use or disposal of sewage sludge.
1.3 Management of Sludge

Desludging in sewerage facilities was done on a need to basis and only less than one percent of the total sludge volume was given due attention. In actual fact, the sludge levels in existing systems keep building up and overflows with the effluent into the receiving water courses.

This clearly speaks for the fact that why most of our rivers systems are polluted with domestic sewage. It has been reported that over 70 percent of the rivers in Malaysia are classified as polluted and the major source of pollutant has been identified as human wastes. The problems were getting serious by the day and this has directly posed a major threat to our water resources.

1.4 Current Sludge Issue in Malaysia

The annual sludge volume produced in Malaysia was estimated to be at 3 million cubic meters [8]. This equates to filling the twin-tower at KLCC to the 78th floor in the first year and requires some 600,000 tanker trips to transport the sludge to designated treatment and disposal sites. By the year 2020, the volume is estimated to increase to 7 million cubic meters which will require about double the KLCC twin tower to fill, or almost 1.4 million tanker trips to manage. These alarming figures indicate the need for better disposal or reuse or recycling of sludge in Malaysia.

1.5 Beneficial Uses of Sludge

The beneficial use of sludge is receiving considerable attention [9] because of the decline in available landfill, and the interest in using the beneficial nutrient and soil conditioning properties of sludge.
1.5.1 Distribution and Marketing

In Europe, sludge that is distributed and marketed is used as a substitute for topsoil and peat on lawns, golf courses, parks and in vegetable gardens. Usually the sludge used for these purposes is composted. The rates of application of sludge may be limited based on whether it is used for food or non-food crops [3].

1.5.2 Chemical fixation

In Europe, the chemical fixation process has been applied to the treatment of industrial sludge and hazardous wastes to immobilize the undesirable constituents [3]. The process has also been used to stabilize municipal sludge for use as landfill cover and for land reclamation projects. The chemical fixation process consists of mixing treated or untreated liquid or dewatered sludge with stabilizing agents such as cement, sodium silicate and lime so as to chemically encapsulate the sludge.

1.6 Disposal of Sludge

Final disposal of sludge usually involves some sort of land disposal. Ocean disposal is prohibited and is being phased out because of water pollution control regulations. There are various methods of ultimate sludge disposal, which can be carried out in the form of liquid or dried sludge. The methods that will be employed here in Malaysia are [8]:

1.6.1 Agriculture/Forestry Land Improvement

Sludge utilization on agricultural and forestry land has proven to be most resourceful. Sludge contains most of the organic loads from the sewage, which can
help farmers reduce their fertilizer requirements and improve soil fertility. Sewage sludge contains significant proportion of nitrogen and phosphorus and can supply a large part of the requirements of most crops. The organic content of sludge can also improve the water retaining capability and the structure of certain soils. It is also a very useful product for reforestation. In Malaysia, sludge has been used as fertilizer in several plantations in Johor [10].

1.6.2 Land reclamation

Sludge cake can be very effective in improving disturbed soils or providing a growing media where no soil exists. For example, soil is normally stripped and stockpiled prior to mineral extraction for reinstatement on completion of the operation. When reinstatement takes place, the stockpiled soil is generally structurally damaged and the addition of sludge cake provides extra organic matter, improving both the physical and hydraulic properties of soil. In areas where no top soil exists, sludge cake can be used as a soil forming material providing a cheap alternative as top soil. In Malaysia, land reclamation techniques will be most suited to ex-mining lands.

1.6.3 Landfilling

Landfilling of sewage sludge with domestic refuse is the most common method of sludge disposal. The basic procedure is to construct a series of clay sided cells or lagoons which are capable of being filled to an average depth of 3 meters with sewage sludge. Thickened sludge is pumped into the lagoons and allowed to stand for a period of time after which any surface water can be decanted of and additional sludge pumped in. Once the maximum volume of sludge has been passed into the lagoon it is again allowed to stand for a period of time to remove water.
At this point, dry solid wastes are tipped into the lagoon and this absorbs most of the remaining moisture of the sludge. Additional solid waste are then deposited on top of this, up to the final and agreed contour levels. Ground compaction is done and final restoration of the site takes place.

1.6.4 Composting

Composting of sewage sludge is new to Malaysia and maybe considered in the future if the economics are favourable. Liquid or dewatered sewage sludge can be stabilized by mixing it with a bulking agent such as wood chip, straw or municipal waste, provided that non-degradable materials such as metal, plastic and glass is removed.

1.7 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic Hydrocarbons (PAHs) are a group of organic compounds that are principally formed during the incomplete combustion of organic matter such as coal, petrol and oil [11]. They are ubiquitous environmental pollutants which present a potential health concern because of the toxicity, mutagenicity and carcinogenicity of these substances in animals [12]. PAHs have a high persistence in the environment, low biodegradability and high lipophilicity, some of them being highly toxic. Several PAHs have a natural source such as bacterium or terrestrial superior plants. Other have their origin in coal, petroleum and their derived products.

Wastewater catchments receive PAHs from two main sources: industrial and domestic fossil fuel spillages and urban runoff inputs that flush the hydrocarbons deposited on the ground surface from vehicles or heating systems. As a result of their very low aqueous solubility, PAHs are efficiently removed from the water during sedimentation in the wastewater treatment process. During treatment of sewage, most of the PAHs (almost 90%) are removed from the waste and is concentrated on
the sludge due to the insolubility and adsorption capacity of the PAHs [13]. This results in the formation of sewage sludges that typically contain between 1 and 10 mg/kg of each individual PAH [4, 11, 14].

The determination of PAHs in environmental samples represents an area of analysis where strict US government controls now exists in order to regulate the production, usage and disposal of this groups of materials [15]. A significant proportion of the generated sewage sludge is applied to land as an organic fertilizer or amendment. Because some PAHs are known or suspected carcinogens [16, 17], the fate of these compounds in the soil environment is critical in assessing their potential hazard risk.

1.7.1 PAHs of Common Occurrence in Sludge

The occurrence of alkyl- and nitro- substituted as well as other heteroatom-containing PAHs are most often reported. Parent PAHs are the basic polycyclic aromatic compounds and their carcinogens has mainly been observed for tri-, tetra-, penta-, and hexacyclic compounds [18, 19]. However, the most abundant and routinely monitored PAHs are the 16 listed in Method 610 by the United States Environmental Protection Agency (US EPA) and are also included in the Priority Substances List under the Canada Environmental Protection Act (CEPA) [20].

The 16 PAHs are naphthalene (Naph), acenaphthylene (Ac), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Anh), fluoroanthene (Fluo), pyrene (Pyr), benzo[a]anthracene (BaAn), chrysene (Chr), benzo[h]fluoroanthene (BkFl), benzo[a]pyrene (BaPy), dibenzo[a,h]anthracene (DiAn), benzo[g,h,i]perylene (BePe) and indeno[1,2,3-cd]pyrene (InPy), and have been selected by the US Environmental Protection Agency (US EPA) as “Consent Decree” priority pollutants (Figure 1.1)[18]. These compounds typically contain two or more benzene rings. The carcinogenicity of the PAHs are suspected to arise when four or more benzene rings are present in the compound [16].
Figure 1.1: Structures of the 16 PAH Termed as Priority Pollutants by the United States Environmental Protection Agency
1.7.2 General Characteristics of Common PAHs

In determining and understanding the extractability of a PAH, the most important parameters are its solubility and also its boiling point. Some of the general characteristics of the common PAHs are listed below in Table 1.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Mass</th>
<th>Boiling point (°C)</th>
<th>Water Solubility (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>128</td>
<td>218</td>
<td>32</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>142</td>
<td>245</td>
<td>29</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>142</td>
<td>241</td>
<td>25</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>152</td>
<td>270</td>
<td>4</td>
</tr>
<tr>
<td>Fluorene</td>
<td>166</td>
<td>297</td>
<td>2</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>340</td>
<td>1.3</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178</td>
<td>340</td>
<td>0.073</td>
</tr>
<tr>
<td>Fluorantheine</td>
<td>202</td>
<td>393</td>
<td>0.26</td>
</tr>
<tr>
<td>Pyrene</td>
<td>202</td>
<td>394</td>
<td>0.14</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>228</td>
<td>438</td>
<td>0.014</td>
</tr>
<tr>
<td>Chrysene</td>
<td>228</td>
<td>436</td>
<td>0.002</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>252</td>
<td>496</td>
<td>0.0038</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>276</td>
<td>500</td>
<td>0.00026</td>
</tr>
</tbody>
</table>

The other common characteristics of PAHs are that they are highly hydrophobic and lipophilic, show high octanol-water distribution coefficients and due to their hydrophobicity, they also tend to adsorb on to organic particles. These compounds are known to be persistent and also have high accumulation potential [1]. As pure chemicals, PAHs generally exist as colorless, white, or pale yellow-green solids.
1.7.3 Sources and Exposure to PAH

The primary sources of exposure to PAHs are inhalation of the compounds in tobacco smoke, wood smoke, and ambient air, and consumption of PAHs in foods. For some people, the primary exposure to PAHs occurs in the workplace. PAHs have been found in coal tar production plants, coking plants, bitumen and asphalt production plants, coal-gasification sites, smoke houses, aluminum production plants, coal tarring facilities, and municipal trash incinerators [23].

Workers may be exposed to PAHs by inhaling engine exhaust and by using products that contain PAHs in a variety of industries such as mining, oil refining, metalworking, chemical production, transportation, and the electrical industry. PAHs have also been found in other facilities where petroleum, petroleum products, or coal are used or where wood, cellulose, corn, or oil are burned. People living near waste sites containing PAHs may be exposed through contact with contaminated air, water, and soil [23].

1.8 Extraction of PAHs from Sludge

In comparison to inorganic pollutants, the determination of organic contaminants in sewage sludge is a field that has received little attention, possibly because no legal limit values have been implemented.

The other more probable reason is the fact that the determination of organic compounds are often complicated and laborious. Analytical methods for analysing these compounds include extraction, one or several clean-up steps, separation and finally the quantification of the analytes. These traditional methods require large amounts of organic solvents and several recentration and clean-up steps before final quantification [1]. Some of the common extraction procedures for the analysis of PAHs are described below.
1.8.1 Soxhlet Extraction

Soxhlet extraction is a conventional approach that is effective in its extraction but requires large quantities of toxic solvents that are hazardous to work with and dispose of. It is a labour-intensive process and can take up to 18 hours to complete an extraction.

Extraction efficiencies vary according to the solvent that is used. In a study that employed toluene, cyclohexane and dichloromethane for the extraction of PAH from sewage sludge, toluene exhibited the highest percentage of recovery [24]. There have been several recommendations for the best Soxhlet extraction of solid environmental samples. Among these, acetone, benzene and cyclohexane are all said to be highly efficient in the extraction of PAHs [25]. Soxhlet extraction has also been utilised in this laboratory for the analysis of PAHs and OCPs [26], where approximately 10 g of freeze dried sludge sample was subjected to Soxhlet extraction for 16 hours using a mixture of hexane and dichloromethane.

Some Soxhlet extractions are further coupled with saponification techniques to enhance determination of PAHs [11]. Saponification with bases is applied to increase the availability of contaminants in soil by hydrolysis and solvation of organic matter [27]. Codina et al. [11] demonstrated that Soxhlet plus saponification techniques has proven to be yield higher recoveries of PAHs as compared to pure traditional Soxhlet techniques. This method was further improved by using silica gel clean-up and fluorescence detection, in preference over UV detection.

The Soxhlet apparatus can be modified for automation purposes. This results in faster, safer and more economic operating procedures. The modified apparatus is known as Soxtec [28]. Comparison of Soxhlet extraction to other extraction techniques for environmental solids have been described [20, 21].

Soxhlet extraction, when not suitable for extraction of PAH compounds with substantially high molecular weights, is often replaced with pressurized fluid extraction (PFE) [29], also known as Accelerated Solvent Extraction (ASE).
1.8.2 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) is a new method for the extraction of organic compounds from soils, sludges and sewage wastes. It uses organic solvents at high pressures and temperatures above the boiling point. With ASE, a solid sample is placed in a cartridge and different solvents are used to extract the sample statically under elevated temperatures (50-200°C) and pressures (7-20 MPa) to increase the speed of the extraction process with low solvent consumption [30].

The applications of ASE for the determination of contaminants in various environmental samples e.g. the determination of chlorinated pesticides from contaminated soils, the determination of PAHs in heap material and the determination of polychlorinated dibenzo-p-dioxins and dibenzofurans from fly ash samples have been investigated [30].

In a study conducted by Schantz et al. [29], PFE has been evaluated and found suitable as an alternative to Soxhlet extraction for use in the extraction of PAHs, PCB congeners and chlorinated pesticides from natural matrix environmental conditions. It was also demonstrated that Soxhlet extraction with methylene chloride is not quantitative for higher molecular weight PAHs from diesel materials (diesel particulate matter); therefore PFE is the preferred technique for the certification of these materials.

In another study by Popp et al. [30], comparison of Soxhlet extraction (18 h) and ASE (2 x 5 min), both studies employing toluene, was performed. The 16 PAHs listed as priority pollutants by the US EPA were analysed. With exception of phenanthrene and benzo[a]anthracene, ASE provided higher extraction yields and was shown to be more effective than the Soxhlet extraction.

ASE was found to meet the demand for increased productivity, faster analyses, more automation and reduced solvent usage [29]. ASE is considered equivalent to standard US EPA extraction methodology in terms of recovery and precision [30]. Comparison of PFE to other extraction techniques such as Soxhlet,
supercritical fluid extraction as well as subcritical water extraction have been described [20, 21].

1.8.3 Solid Phase Extraction

In recent years, solid-phase extraction (SPE) has become an increasingly popular procedure in environmental analysis. SPE is a popular procedure that is used to preconcentrate components to be analyzed and clean-up matrices from sample for analysis [18]. Recently, C_{18}-bonded silica has been introduced as extraction discs in which they are enmeshed in a network of PTFE fibers to form strong porous membrane. These discs have been studied for their suitability to field extractions and storage mechanisms [31].

Another approach for simple extraction method is to couple subcritical water extraction with standard SPE discs (Empore discs). The SPE disc quantitatively collects analytes from the water during the subcritical water extraction step. Empore SPE discs (C_{18}, styrene/divinyl benzene and polymer-based anion exchange resins) are stable under 250°C. Therefore, the SPE discs can be placed in the cell during the heating step to collect extracted organics from the extractant water [32].

Prepacked SPE cartridges provide users of SPE with a variety of stationary phases to selectively separate and concentrate analytes for detection. However since PAHs have low polarity, SPE of pollutants from samples is usually carried out on a bonded octadecyl-silica stationary phase [18]. The combination of the small particle size and high surface area of C_{18} bonded porous silica ensures contact of the dissolved organics with the adsorbents even when very rapid flow rates are employed. Methanol and acetonitrile are the recommended solvents for the elution of compounds sorbed on to alkyl-bonded porous silica, though many hydrophobic compounds such as PAHs have low solubilities in these two solvents [33].

In contaminated soil, besides the PAHs that are analyzed, other compounds may interfere with the determination. In addition, some PAHs in the extraction
solution from the soil cannot be determined directly because of their lower concentrations [18]. Therefore, SPE as a clean-up procedure is often required for the determination of PAHs in soil samples, while aqueous samples need preconcentration [34].

1.8.4 Solid Phase Microextraction

Recently, solid phase microextraction (SPME) which is fast, solvent free and exhibits excellent performance has been studied as the substitution or alternative to SPE. SPME has been successfully employed to analyze a wide range of pollutants including PAHs [5]. Whereas the SPE technique has been primarily designed for use with liquid matrices and exhaustive extraction, SPME can be used in liquid (aqueous) or gaseous matrices and primarily aims for partial or equilibrium extraction of the analyte [35]. There are two basic types of SPME: direct immersion (DI) and headspace extraction. In the DI extraction mode, the coated fiber is inserted into the sample medium, where some level of agitation is sometimes necessary to enhance transport of the analytes from the bulk of the solution into the vicinity of the fiber.

Although SPME coupled with gas chromatography is a powerful approach for the rapid extraction and analysis of non-polar and moderately-polar organics from water, its use for the analysis of organic pollutants from solid samples has been limited to compounds that can be readily vaporized from solids and show strong partitioning to the SPME phase [36]. In a study employing FID as the detector, FID responses of PAHs increased slightly with increasing water volume for DI-SPME, and increasing the working temperature also increased the extraction efficiencies [37]. A method for the determination of PAHs by SPME coupled with cyclodextrin (CD)-modified capillary electrophoresis using UV detection has also been developed [12], where with 30 kV applied potential, separation of the 16 PAHs was successfully achieved in less than 15 minutes.
Headspace SPME (HSSPME) is a good supplement to conventional SPME because the fiber is not in contact with the sampling medium. This approach serves primarily to protect the fiber coating from high molecular-weight species and other non-volatile contaminants present in the liquid sample matrix. This headspace mode also allows modification of the matrix, such as a change of the pH, without damaging the fiber [35]. Therefore, the background is much cleaner and the useful life of the fiber is prolonged.

HSSPME has been proven to be effective on the analysis of volatile organic compounds [37]. The feasibility of HSSPME for the determination of high-ring PAHs (4-6 rings) in water and soil samples have been studied [18, 37]. In this study, 100-\(\mu\)m PDMS showed the highest extraction efficiency compared to 85-\(\mu\)m and 30-\(\mu\)m PDMS fibres. Also, the extraction efficiency decreased with increasing molecular weights of the PAHs. HSSPME with a 90 minute extraction time has been proven to be effective for determination of PAHs in water and soil samples. HSSPME at room temperature was successfully applied to the analysis of low ring PAHs. A working temperature of 80 °C provided significant enhancement in sensitivity of high ring PAHs [37].

1.8.5 Supercritical Fluid Extraction

The analytical scale supercritical fluid extraction (SFE) was introduced in the late 1980s and started a new field of research. SFE paved the way not only for reduction in use of organic solvents but also for automation of analytical procedures [38]. High repeatability, accuracy and high selectivity were among the most important advantages of SFE in residue analysis. Extracts with low amounts of co-extractants from the soil matrix were achieved, allowing extracts to be pooled and concentrated with little or without further clean-up steps. Thus, the limited volume of extraction thimbles of the SFE apparatus used could be compensated and insufficiently high limits of determination could be improved [39].
SFE equipment typically consists of an extraction cell that can be heated and pressurized. This cell (containing the sample) is placed in an oven and connected to a high-pressure pump supplying the supercritical fluid. Pumping of the extraction fluid can be done in two different ways, either continuously via a reciprocating pump or by filling of a syringe pump which then delivers the fluid. Analyte collection can also be done in basically two different ways; in solvents or on solid-phase traps [38].

When a supercritical fluid is used as an extractive solvent, it is possible to separate a multicomponent mixture by capitalizing on both the differences in component volatilities and the specific interaction with the solvent. The application of supercritical fluids is based on the observation that many gases exhibit enhanced solvating power when compressed to condition above the critical point [40]. The advantages of using supercritical fluids are that they possess a very low surface tension, low viscosity, and high diffusivity, which translates into fast mass transfer when compared to liquid solvents [5].

The comparison of supercritical chlorodifluoromethane, CHCIF₂ (Freon-22), nitrous oxide (N₂O) and carbon dioxide (CO₂) as the supercritical media for the extraction of PAHs and PCBs from environmental solids have been described [21]. The supercritical parameters of carbon dioxide (which is non toxic and non flammable) are easily accessible (72.8 bar, 31.1°C), and CO₂ can be modified with methanol, acetonitrile or acetone to improve its polarity [21]. Freon-22 too has recently been shown to yield higher extraction efficiency than N₂O and CO₂. However Freon-22 has ozone depleting potential and the advocacy of freon as a SFE solvent is a step backward in environmental protection [20].

Application of SFE for determination of PAHs in sludge samples have been conducted by Berset and Holzer [1] where the procedure was divided into two discrete steps; in the first step, the more volatile PAHs were extracted using pure CO₂ at a rather low density and pressure. In the second step the less volatile PAHs were removed at a higher density and temperature and by adding modifiers. SFE has also been coupled with liquid chromatography employing fluorescence detection for the analysis of PAHs in sludge [24]. However, as have been previously reported [1], most of SFE based studies have been focused on soils and sediments [14, 20, 21, 39].
viscosity of the water at higher (up to 300°C) temperatures. Under these conditions, organic compounds generally considered insoluble in water show dramatic increases in solubility [36]. For example, the PAHs such as anthracene, chrysene and perylene each have solubilities ca. 20000 fold higher in water at 200°C, than at 25°C [21].

Subcritical water has several potential physiochemical as well as practical advantages over supercritical water and supercritical carbon dioxide. As shown in Table 1.2, the polarity (solvent strength) of subcritical water can be controlled over a much broader range than either supercritical water or carbon dioxide. By simply controlling the temperature with enough pressure to maintain the liquid state, a very wide range of solvent polarity can be achieved with pure water, as demonstrated in Figure 1.3 [42].

![Figure 1.3: Temperature Influence on Physical Properties of Water](image)

In addition to changing the polarity of water, increasing the temperature also lowers the viscosity and surface tension of the water- both factors that enhance water’s ability to extract organic compounds from contaminated solids (Figure 1.3). Additional comparisons of subcritical water with supercritical water and carbon dioxide is shown in Table 1.2 below.
Table 1.2: Typical Characteristics Related to Organic Pollutant Extractions for Subcritical Water, Supercritical Water and Supercritical CO₂

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Subcritical Water</th>
<th>Supercritical Water</th>
<th>Supercritical CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range</td>
<td>&gt;25 to 300°C</td>
<td>&gt;374°C</td>
<td>&gt;32 to 150°C</td>
</tr>
<tr>
<td>Pressure required</td>
<td>2 to 50 atm</td>
<td>&gt;218 atm</td>
<td>&gt;72 atm</td>
</tr>
<tr>
<td>Density range</td>
<td>0.8 to 1g/mL</td>
<td>0.1 to 0.6 g/mL</td>
<td>0.3 to 0.9 g/mL</td>
</tr>
<tr>
<td>Polarity range</td>
<td>15 to 85</td>
<td>5 to 15</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Corrosivity</td>
<td>Low to moderate</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Organics solvated</td>
<td>Polar to non-polar</td>
<td>Polar to non-polar</td>
<td>Non-polar</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Potentially high</td>
<td>Non-selective</td>
<td>Some selectivity</td>
</tr>
</tbody>
</table>

In contrast to the dependence on pressure commonly reported for SFE with supercritical carbon dioxide, the efficiency of subcritical water extractions depends primarily on the water temperature, as long as sufficient pressure is applied to maintain the liquid state (typically < 40 bar). The least polar organics are the easiest organics to extract with pure CO₂. In direct contrast, subcritical water prefers the more polar analytes, i.e., PAHs can be efficiently extracted from air particulates at 250°C [21].

The dynamic mode is the common approach to performing subcritical water extraction. Quantitative extraction of PAHs using the dynamic mode have been reported by several authors [21, 41]. Lately, a prototype extractor was developed for automated extraction in the dynamic mode (Figure 1.4) [41].

It consists of a stainless steel cylindrical extraction chamber, with closed screws at the end to permit the circulation of fluids through them. Both screw caps contain stainless steel filter plates to ensure that the sample remains in the extraction chamber. A pump with digital flow rates and pressure readouts is used to impel the extractant through the system.
Figure 1.4: Schematic diagram of the prototype extractor [41]

HPP=High Pressure Pump, PH=Preheater,
CS=Cooler System, EC=Extraction Cell,
TC=Temperature Controller, DV=Diverting Valve

Static subcritical water extraction is relatively simpler to perform. In this case, the sample is simply placed in the vessel with an appropriate amount of extractant (water) and heated to the desired temperature for the needed duration. After cooling, the extractant is removed and analysed. Good recoveries were also obtained for PAHs and PCBs using this method of extraction [36, 43].

Another approach to subcritical water extraction is the micelle formation extraction methodology [41] that has been applied to the extraction of a wide range of analytes including PAHs, vitamin A, vitamin E, estrogens, progesterones and proteins from aqueous solutions. The analytical interest of the micelle formation is a consequence of the (a) ability to concentrate a variety of analytes; (b) safety and cost benefits; (c) easy disposal of the surfactant; (d) compatibility of the surfactant-rich phase with micellar liquid chromatography; and (e) preclusion of the analytes losses during the evaporation of the solvent used in traditional liquid-liquid extraction [41]. Research has also shown that higher recoveries of PAHs were obtained in the
presence of sodium dodecyl sulfate as the surfactant compared to extraction with pure water.

1.9 Extract Clean-Up

The determination of PAHs in soil, sediment and sludge samples require a good clean-up, while aqueous samples need concentration due to their low concentration levels [34]. As individual PAHs are present in soils in the ng/g level or below, few samples can be analyzed directly without serious interference.

Efficient extraction, preconcentration and clean-up of PAHs from the samples are essential prior to the determination of the PAHs. The two most commonly used techniques for sample clean-up are the conventional column chromatography as well as the SPE clean up.

1.9.1 Conventional Column Chromatography

Silica gel (silicic acid) is often used in column chromatography for the separation of analytes from interfering compounds of a different chemical polarity. It is a regenerative adsorbent of silica with weakly acidic properties, and is produced from sodium silicate and sulfuric acid. It is used deactivated, after heating to approximately 150-160 °C, or deactivated with up to 10 % water [44].

Generally, the standard column chromatography, as compared to SPE cartridges, use larger amounts of adsorbent and therefore, have a greater clean-up capacity. In the standard column clean-up protocol, the column is packed with the required amount of adsorbent, topped with a water adsorbent, and then loaded with the sample to be analyzed. Elution of the analytes is accomplished with a suitable solvent(s) that leaves the interfering compounds on the column. The eluate is then concentrated, if necessary.
adsorbent material for SPE cartridge that is most commonly used are C_{18} and C_{8} bonded porous silica [18, 33, 34] as well as the silica cartridge [26, 41].

1.10 Instrumental Analysis

Various instrumental analytical procedures have been employed for the determination of PAHs. Among the methods, gas chromatography, high performance liquid chromatography and capillary electrophoresis are the techniques most commonly used.

1.10.1 Gas chromatography

The extreme complexity of PAH mixtures demands the greatest resolution possible in their analysis, and in this respect gas chromatography has proven to be efficient. As the number of known carcinogenic polyaromatics have increased considerably, their detection has been carried out by more sophisticated techniques, in particular capillary column gas chromatography (GC) combined with mass spectrometry [45]. This combination (GC-MS) is often favourable for identification of various components in a complex mixture.

Based on structural information, GC-MS is capable of identifying more than 21 PAHs and PAH derivatives present in soil, sludges and sediments [13]. The maximum resolution of mixture components before MS analysis is of utmost importance in providing unambiguous identification of individual compounds. This is especially true in the case of PAHs because the conventional mass spectra of many isomers are identical [25].

The most widely used gas-chromatographic detector for PAHs is the flame ionization detector (FID). This is a result of its universally accepted characteristics of excellent response linearity, sensitivity and reliability [25]. There are various studies
that have employed the use of GC-FID [6, 13, 14, 46]. This method of detection is mostly used for determination of PAHs.

For chlorinated compounds, electron capture detection (ECD) is a better choice. Analysis of PCBs and OCPs usually employs ECD and MS, instead of FID. This is because halogen-containing compounds possess high affinity for thermal electrons and give strong ECD responses [31]. SPME coupled with gas chromatography is a powerful approach for the rapid detection and analysis of non-polar and moderately polar organics from water [36].

1.10.2 Capillary Electrophoresis

Capillary electrophoresis (CE) methods, including capillary zone electrophoresis (CZE) have recently emerged as the most efficient methods available for separation of components in mixtures. However, CZE is limited to the analysis of water-soluble, charged species and is therefore not applicable for the analysis of neutral and water-insoluble PAHs [47].

Analysis of such uncharged species through electrokinetic chromatography (EKC) is feasible through addition of a charged phase to a standard CZE buffer to effect separation based on analyte partitioning into the charged phase [48]. Micellar electrokinetic chromatography employs the same principle as CZE, except that an ionic surfactant is added to the buffer mobile phase. MEKC is able to effect the separation of neutral compounds based on the differential distribution of solutes between an electroosmotically pumped aqueous mobile phase and a slower moving electrophoretically retarded micellar phase [49].

However, of the limitations of MEKC is the need for samples to be reasonably soluble in an aqueous mobile phase. Hydrophobic compounds, such as the PAHs, tend to be completely solubilized by the micelles and co-elute with migration times near to that of the micelles. These problems can be alleviated by silanizing the capillary column walls, coating the walls with polymers, or adding
modifiers to the electrophoretic buffer. Therefore, for the separation of PAHs, which are all neutral, non-ionizable and are of similar hydrophobicity, it is necessary to extend the concept of employing a mobile phase and pseudostationary phase to the utilization of buffer modifiers [49].

Some common buffer modifier that have been used are derivatized cyclodextrins, tetraammonium perchlorate, histidine complex and organic modifiers [49]. One of the most successful schemes for improving the partitioning for PAH separation is the addition of cyclodextrins to the buffer. Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides which consist of $\text{1,4}$-linked glucopyranose subunits. The most common forms are $\alpha$-CD, $\beta$-CD and $\gamma$-CD. They possess a toroidal structure with a nonpolar interior cavity and can form host-guest inclusion complexes with many hydrophobic complexes [47].

Experience with micellar solutions unfortunately, has shown them to be difficult to prepare reproducibly and MEKC separation methods are highly sensitive to operating parameters such as temperature and applied voltage. Thus method development for MEKC is often cumbesome and time-consuming [47].

1.10.3 High Performance Liquid Chromatography

PAHs are commonly analyzed by HPLC with fluorescence detection. Although capillary gas chromatography is known to have much higher resolution than HPLC methods, but today, HPLC can easily separate the 16 EPA priority PAHs [19]. This method of analysis is known to be selective and requires less clean-up procedures as compared to GC-MS due to the use of the guard precolumn in the HPLC [50].

A number of studies have been conducted to determine PAHs using HPLC [11, 24, 30, 41]. Codina et al. [11] have developed a HPLC method with both UV and fluorescence detection to determine PAHs from sewage sludge-amended soil following extraction using Soxhlet plus saponification extraction and silica gel clean-
up. A comparative study of SPE and conventional LLE was conducted using HPLC as the method of analysis [34]. HPLC was also compared to other methods of analysis such as GC and GC-MS for the determination of PAHs and chlorinated pesticides in solid wastes [30].

1.11 Importance of Research

Almost all of sludge based research in Malaysia concentrate on the inorganic aspects of contamination, particularly heavy metals. Little attention is given to the determination of organic pollutants in sewage sludge, although these organic compounds, namely PAHs are toxic and hazardous. Therefore, it is important to develop an analytical method to determine the levels of these compounds in sewage sludge.

1.12 Research Objectives

The objectives for this research are as follows:

i. To compare three subcritical water extraction methods (pure subcritical water extraction, surfactant-modified subcritical water extraction and subcritical water extraction coupled with membrane discs) for the extraction of PAHs from spiked sludge samples.

ii. To perform instrumental analysis of the extracted PAHs using GC-FID.

iii. To perform recovery studies for the PAHs extracted from spiked sludge samples.
CHAPTER II

EXPERIMENTAL

2.1 Introduction

The study was generally divided into two parts. The first part consists of extraction of the chosen PAHs using subcritical water extraction, employing various extraction methodologies. This was then followed by the second part of the study, which is the instrumental analysis of the extracts using GC-FID.

Four of the 16 PAHs listed as priority pollutants by the US EPA, and which are of common occurrence in sewage sludge were chosen for determination. They are naphthalene, phenanthrene, fluorene and fluoranthene. Identification of the analytes extracted for the real sludge samples were affected using GC-MS.

2.2 Chemicals and Materials

The chemicals that were used in the extraction and analytical procedures, as well as the needed materials, apparatus and samples that were used are listed as follows in Section 2.2.1, 2.2.2 and 2.2.3.
2.2.1 Chemicals

PAH standards of naphthalene and fluoranthene were obtained from Riedel-de-Haen (Germany), phenanthrene from BDH Chemicals (Germany), and fluorene from Fluka, Switzerland.

Methanol was obtained from Merck (Germany), whereas GC-Grade hexane and methylene chloride were obtained from Fischer Scientific (USA). Acetone was obtained from Caledon (Canada). All the solvents used were of analytical grade.

Sodium dodecyl sulfate (SDS) was obtained from Merck (Germany) and anhydrous sodium sulfate was obtained from Bendosen Laboratory Chemicals. Nitrogen gas from MOX, Pasir Gudang was used for extract concentration. Double distilled water used in the extraction procedures was prepared in the laboratory and degassed by means of sonication prior to use.

2.2.2 Materials

SPE silica or C_{18} silica cartridges (0.5 g sorbent, 6 mL capacity) were purchased from Supelco (Bellefonte, USA). SPE clean-up was carried out using a 8-port vacuum manifold from International Sorbent Technology, United Kingdom. C_{18} silica membrane discs (d=47 mm) were obtained from Supelco (Bellefonte, USA). 1PS phase separator filter membranes (d=90 mm) were purchased from Whatman. The vortex mixer for the disc extraction was supplied by Thermolyne, Sybron.

2.2.3 Sludge Samples

Blank sludge samples which have been prepared in a previous study in this laboratory [26] were used as received. The sludge samples obtained by the researcher
were subjected to exhaustive Soxhlet extraction for the removal of organic contaminants, therefore enabling it to be used as blank sludge samples.

Real sludge samples were kindly donated by H.W. Chong [26] who collected the samples from 3 Indah Water Konsortium wastewater treatment plants as part of his MSc research project. These plants are located in Kota Tinggi Industrial Park, Datuk Yunus Sulaiman Light Industrial Park (Lima Kedai, Skudai) and in Seri Alam (Plentong, Masai).

2.3 Instrumentation

Extraction vessels for subcritical water extraction consisted of 2 SFE extraction vessels (dimensions 210 mm long x 14 mm I.D. and 90 mm long x 8.5 mm I.D. respectively) with stainless steel end caps, a PTFE vessel (120 mL capacity, 101 mm long x 40 mm I.D.) obtained from CEM Corporation (North Carolina, USA) as well as a PFA vessel (90 mL capacity, 115 mm long x 33 mm I.D.) that was obtained from Berghof (Germany). An unused gas chromatographic oven (Autosystem, Perkin Elmer), designed to work up to 450 °C, was used for heating of the vessel.

GC analysis was performed using a GC-17A gas chromatograph (Shimadzu, Tokyo). An Ultra-2 capillary column (5% cross-linked methyl siloxane) obtained from Hewlett Packard (USA) was used for the chromatographic separation. The capillary column was of dimensions 30 m in length, 0.25 mm in diameter and with film thickness of 0.25 μm.

GC-MS analysis was performed using a gas chromatograph (Model HP 5890 Series II) and mass spectrometer (Model HP 5989 A) obtained from Hewlett Packard. For the determination of the PAHs, a HP 101 capillary column (100% dimethyl polysiloxane) purchased from Hewlett Packard, USA was used. The column dimensions were 25 m in length, 0.2 mm in diameter and with a film thickness of 0.20 μm.
2.4 Procedure

The procedures that are described below comprise of sample and standard solution preparation. The various extraction processes consist of pure subcritical water extraction, surfactant-modified subcritical water extraction and extraction with membrane discs. These extraction methods, as well as the SPE clean up procedure and the analytical and identification methods are described in Section 2.5 onwards.

2.4.1 Preparation of the PAH Standard Stock Solutions

Stock solutions of the four PAH standards were prepared by weighing accurately 0.04 g of each PAH standard into 4 separate 10 mL vial and dissolving it with 10 mL of hexane, hence producing a concentration of 4000 ppm for the individual PAH solutions.

Equal volumes of each solution was then pipetted into a fresh vial, resulting in a mixture containing 1000 ppm of each PAH. The PAH mixture was further diluted so as to prepare standard solutions containing 500 ppm and 250 ppm of each PAH, which were subsequently used for the extraction and analytical procedures. The vials were wrapped in aluminum foil and refrigerated when not in use.

2.4.2 Spiked Sludge Samples

1 g of the blank sludge sample was accurately weighed into the extraction vessel and spiked with 2 mL of the standard PAH mixture (500 ppm). The sludge sample was then dried by exposing it to a gentle flow of nitrogen gas in order to evaporate off the solvent.
2.5 Subcritical Water Extraction

Subcritical water extraction was performed using three different approaches. The first approach was using pure subcritical water extraction. The second method used was surfactant-modified subcritical water extraction, whereas the third method employed the use of membrane discs.

2.5.1 Extraction with Pure Subcritical Water

3.5 mL of water was added to the spiked sludge sample in the vessel. The vessel cap was then placed on and tightened, and the vessel was heated in the oven. Static extraction was then developed for the desired duration. After the extraction, an equal volume of hexane was added to the water extract and the mixture was shaken for ca. 5 minutes before it was filtered through a phase separator membrane. The organic filtrate was collected whilst the aqueous layer discarded.

2.5.1.1 Effect of Extraction Temperature and Time

The best extraction temperature was determined by performing extractions at 150 C, 200 C and 250 C. The best temperature was then chosen and subsequent extractions were then performed at 15, 30 and 60 minutes to observe the effect of extraction time.

2.5.1.2 Influence of Water Volumes

Water volumes were increased from an initial volume of 3.5 mL and to a volume of 7 mL and 10 mL to observe the influence of water volumes on the extraction efficiency.
2.5.2 Extraction with Surfactant-modified Subcritical Water

When the surfactant-modified extraction procedure was used, the exact same conditions were applied. However, instead of adding just the water, 1 mL of 0.005 M Sodium Dodecyl Sulfate (SDS) was also added to the spiked sludge sample in the vessel. Extraction was then performed, the aqueous extract was collected after cooling. The hexane partitioning step was omitted.

2.5.3 Subcritical Water Extraction Coupled with Membrane Discs

Extractions using membrane discs were performed using C<sub>18</sub> sorbent discs, and the procedure consisted of determining the disc stability, as well as the influence of extraction temperatures and extraction times on the extraction efficiencies.

2.5.3.1 Disc Stability and Efficiency

To test the stability of the discs, 1 x 2 cm rectangles of the disc material were placed in the extraction vessel and exposed to subcritical water at temperatures of up to 250 °C. The influence of disc size and number of disc rectangles placed on the extraction efficiency were also studied. Three different disc sizes was studied (1 cm<sup>2</sup>, 4 cm<sup>2</sup> and 6 cm<sup>2</sup>), whereas the number of disc rectangles were increased from 1 to 4.

2.5.3.2 Extraction Procedure

The sorbent disc were cut into rectangles and briefly soaked in methanol for ca. 20 minutes for activation, and then air dried for 5 minutes just prior to placing in the extraction vessel. One C<sub>18</sub> sorbent disc (1 cm<sup>2</sup>) was then placed in the cell containing the spiked sludge sample. Then, 3.5 mL of water, which had been
previously sonicated to remove dissolved oxygen, was added. The vessel cap was then placed and sealed tightly.

(Safety note: It is imperative that there is a headspace present in the vessel so that the pressure in the cell upon heating is controlled by the steam/water equilibrium. If the cap of the cell is also filled with water to eliminate the headspace, the pressure in the heated vessel could exceed several thousand bar. However, with the headspace, the pressure is reduced significantly [36].)

The vessel was then placed in a gas chromatographic oven, which was previously heated to the desired temperature. After the heating was completed, the vessel was immediately removed from the oven and cooled to room temperature. When the cooling was completed, the vessel was opened and the sorbent disc was removed with tweezers and rinsed with water to remove sample particles. The rinsed disc was then placed in 3 mL of acetone/methylene chloride mixture (1:2) in a 4 mL glass vial and vortex-mixed to extract the PAHs adsorbed on the disc. The aqueous fractions were extracted three times by mixing for about 5 minutes each time with aliquots of methylene chloride.

2.5.3.3 Influence of Extraction Temperature and Extraction Time

Extractions with spiked PAHs were performed to see the best temperature for the disc extraction. Temperatures were set at 150°C, 200°C and 250°C. The best possible temperature was chosen and subsequent extractions were conducted with the chosen temperature for 15 minutes, 30 minutes and 60 minutes.

2.6 SPE Extract Clean-Up

SPE extract clean-up was carried out using a silica SPE cartridge mounted on a SPE apparatus that was fixed to a vacuum pump. Anhydrous sodium sulfate (0.25g) was placed at the top of the column to remove any residual water. The column was first conditioned with 6 x 2 mL of solvent at a flow rate of 2 mL/min.
2.6.1 SPE for Pure Subcritical Water Extraction

For SPE procedure using C18 cartridge, the eluting and conditioning solvent used was methanol (6 x 2 mL). The water extract was applied directly on to the column, and drying time was allowed for 5 minutes. A methanol volume of 6 x 2 mL was then passed through the cartridge at a flow rate of 1 mL/ min for eluting the PAHs. The eluate was reduced to near dryness under a stream of nitrogen gas and then reconstituted with 2 mL of methanol.

A similar experiment was also carried out using the silica cartridge for pure subcritical water extraction. However, in this procedure, the water extract was partitioned into a hexane phase (3.5 mL). The separated organic phase was passed through the column, instead of applying the water extract directly as was performed for the C18 cartridge. The eluting and conditioning solvent used was hexane.

2.6.2 SPE for Surfactant-modified Subcritical Water Extraction

This SPE procedure employed the use of silica cartridge, and the water extract was passed through the column where the micellar phase was retained and the waste discarded. Drying time was allowed for 5 minutes. A hexane volume of 6 x 2 mL was then passed through the cartridge at a flow rate of 1 mL/ min for eluting the PAHs. The eluate was reduced to near dryness under a stream of nitrogen gas and then reconstituted with 2 mL of hexane.

2.7 Gas Chromatographic Analysis

All extracts were analysed using GC-FID. The parameters used for the GC analysis of the PAHs are listed in Table 2.1 as follows.
Table 2.1: Instrumental Conditions for GC-FID

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Helium gas flow rate</td>
<td>1.8 mL/min</td>
</tr>
<tr>
<td>Injection temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Detection temperature</td>
<td>300°C</td>
</tr>
<tr>
<td>Temperature programming</td>
<td>50°C for 2 mins</td>
</tr>
<tr>
<td></td>
<td>50°C – 265°C at 10 °C/min</td>
</tr>
<tr>
<td></td>
<td>265°C for 7 mins</td>
</tr>
<tr>
<td>Total program time</td>
<td>30.5 mins</td>
</tr>
<tr>
<td>Injection mode</td>
<td>Splitless mode (30 seconds)</td>
</tr>
<tr>
<td></td>
<td>Split mode (1:20)</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1 μL</td>
</tr>
</tbody>
</table>

2.8 Analysis of Real Sludge Samples

Real sludge samples that were obtained as mentioned in Section 2.2.3 were extracted using the most favourable extraction procedure, that is the extraction using pure subcritical water (Section 2.5.1). The extraction and analytical procedure is outline briefly in Figure 2.1. However, owing to the low concentration of the PAHs in real sludge samples, the extract was concentrated by evaporation to near dryness and reconstitution with 25 μL of hexane prior to injection into the GC. SPE clean-up was also conducted as described in Section 2.6. GC analysis was then performed for the extracted sample, followed by GC-MS identification procedure.

2.9 GC-Mass Spectroscopy Analysis

GC-MS was employed for identification of PAHs found in the real sludge samples. The GC-MS interface was at 250 °C. An electron impact mode was used at conditions of 176 °C, 70 eV and 300 μA. The temperature profile and injection parameters used is similar to that of the GC-FID analysis (Table 2.1).
3.5 mL H₂O
Blank sludge sample (1g)
Standard mixture in hexane (2 mL)
-solvent evaporated

Heat for 200°C, 15 mins

Filter the heated sample
with a blank SPE cartridge

Add 3.5 mL of hexane
to the filtered water extract

Shake for 1 min
Leave for 5 mins
Separate with phase separator

Inorganic phase
-discard

Organic phase

Evaporate to 1 mL

SPE Silica

Elute with 6 mL hexane

GC-FID analysis

Evaporate to dryness
+ 25 μL hexane

Figure 2.1: Flow Chart of the Analysis of the Real Sludge Sample
CHAPTER III

RESULTS AND DISCUSSION

3.1 Introduction

This chapter will discuss in detail the various extraction methods used in this study as well as the selection of a suitable SPE cartridge for clean-up purposes. This chapter will also attempt to identify and explain the reasons behind the inefficiencies of the methods used. The analysis of real sludge samples using GC-FID and GC-MS will also be presented.

3.2 Separation of the PAH mixture

Four PAHs of common occurrence in sludge were chosen for determination with GC-FID using an Ultra-2 capillary column. They are naphthalene, fluorene, phenanthrene and fluoranthene. All four PAHs were successfully separated, with a total run time of less than 30 minutes. The gas chromatographic resolution of the four PAHs is illustrated in Figure 3.1.

Table 3.1 shows the retention times of the PAHs. It can be observed that the PAHs were eluted according to the increase in their molecular weight as well as their boiling points (Table 1.1).
Figure 3.1: GC Separation of the PAHs using an Ultra-2 Capillary Column.

GC Conditions: Injection port temperature was set at 250°C and the detector temperature was set at 300°C. The oven temperature was programmed from 50°C (held for 2 mins) then ramped to 265°C (held for 7 mins) at a rate of 10°C/min.
Table 3.1 Retention times of the standard PAHs

<table>
<thead>
<tr>
<th>PAH Standard</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>12.08</td>
</tr>
<tr>
<td>Fluorene</td>
<td>18.42</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>21.08</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>24.51</td>
</tr>
</tbody>
</table>

3.3 Selection of the Extraction Vessel

In the selection of a suitable extraction vessel for subcritical water extraction, vessels of various sizes and make of material were considered. Hawthorne et al. [43] used a stainless steel cell (5 mL capacity, 64 mm long x 7 mm I.D) fitted with end caps. The researchers claimed that the use of such a vessel provided efficient heating of the sample.

The vessel was also able to avoid adsorption of the PAHs on to the vessel. In addition to that, the specific volume capacity of the cell would enable only a small headspace to be present and this would efficiently control not only the pressure within the cell, but also the PAHs partitioning between the matrix and the extractant.

However, due to the unavailability of such vessels with appropriate dimensions and time constraints to purchase one, other alternatives were sought. Two stainless steel SFE extraction vessels (dimensions 210 mm long x 14 mm I.D. and 90 mm long x 8.5 mm I.D. respectively) were initially used. The vessels were tested by half filling it with water, and sealing it tightly, coupled with teflon tape. The vessels was then heated in an oven for 30 minutes at a temperature of 150°C. Unfortunately, upon cooling of the vessels, the water was found to be completely evaporated.

A PTFE vessel that was used could withstand the heat that is required of the extraction procedure (250°C) and also did not result in evaporation of the water.
However, once the PAHs were spiked on to the water, they adsorbed on to the inner walls of the vessel very strongly, which was evident from the pale yellow colouration of the walls.

The only other available alternative was a PFA vessel (115 mm long x 33 mm I.D) from Berghof (Germany), which is also used for sample digestion purposes. The vessel was found to not only withstand the heat, but also did not result in the adsorption of the spiked PAHs on to the inner walls of the vessel. However, the only available size was one with a 90 mL capacity, and this would result in a large headspace, when small sample sizes are used. This would therefore render the extraction not fully efficient. However, since this vessel seemed to be the best choice compared to the other alternatives, it was subsequently used in further experiments.

3.4 Extraction with Pure Subcritical Water

Subcritical water extraction was performed as described in Section 2.5.1. An extraction temperature of 150°C and a heating duration of 15 minutes were chosen for initial experiments. The silica SPE cartridge was initially used for the clean-up.

Preliminary studies using the pure subcritical water extraction method with SPE silica extract clean-up showed that the method was viable and could produce quantitative results. Therefore further efforts were targeted at observing the influence of extraction temperatures, the extraction time as well as the water volume. Factors such as the volume and concentration of the spiked standards were maintained.

3.4.1 Effect of Extraction Temperature

Extraction temperatures were set at three different temperatures of 150°C, 200°C and 250°C, while the extraction time was maintained at 15 minutes to observe
the effect of temperature changes. The recoveries of PAHs with varying extraction temperatures are summarized in Table 3.2.

Table 3.2: PAH Recoveries at Different Heating Temperatures (15 mins)

<table>
<thead>
<tr>
<th>PAH Compounds</th>
<th>Recovery (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150°C</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>9.87 ± 2.93</td>
</tr>
<tr>
<td>Fluorene</td>
<td>40.74 ± 4.61</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>44.04 ± 3.11</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>27.33 ± 4.09</td>
</tr>
</tbody>
</table>

*Based on duplicate analyses

All PAHs showed a significant increase in recovery when the temperature was raised from 150°C to 200°C. However, when the temperature was further raised to 250°C, the recoveries dropped drastically. The recoveries observed for the 250°C temperature was even lower than that of the 150°C temperature. This is contrary to the recoveries reported by other authors [21, 42], where temperatures of 250°C and even 300°C yielded better recoveries of PAHs.

The reason for this observation is unclear. Preliminary heating of the vessel (Section 3.3) demonstrated that evaporation of water did not occur, as the water volumes before and after heating remained the same. The only other remaining possibility is that the vessel is not completely sealed and at higher temperatures, evaporation of the PAHs, especially for the easily volatilizable compounds like naphthalene [18, 34] occur.

Another possibility is one that is suggested by Harrak et al. [19]. The author mentioned that due to the low solubility of PAHs in water, they tend to adsorb on to wall and surfaces with which they come into contact. To prevent the adsorption of PAHs on the wall of the water containers, some authors add an organic solvent such
as methanol or acetonitrile to the sample, while others add surfactants in the aqueous media to increase its solubility. However, with subcritical water extraction, this method would prove to be futile, as the solvents would evaporate into the headspace even before the PAHs.

Since there seemed to be no working alternative to the above problem, a heating temperature of 200°C was chosen for further analyses, as this temperature seemed to produce the most quantitative results with the present conditions used.

3.4.2 Effect of Extraction Time

Using 200°C as the extraction temperature, the next step was to determine the optimum extraction time. Previous studies with subcritical water have employed extraction times from as low as 15 minutes up to 60 minutes [21, 32, 42, 43]. For low-polarity compounds such as PAHs, a longer extraction time is usually required to quantitatively extract the PAHs. Therefore, extraction times were increased from 15 minutes to 30 minutes and 60 minutes. The results of the recovery are summarized in Table 3.3.

Table 3.3: PAH Recoveries at Different Extraction Times (200°C)

<table>
<thead>
<tr>
<th>PAH Compounds</th>
<th>Recovery (%)*</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>38.84 ± 1.07</td>
<td>35.04 ± 5.84</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>49.83 ± 6.45</td>
<td>48.94 ± 5.90</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>56.06 ± 7.90</td>
<td>51.08 ± 7.04</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>50.14 ± 1.55</td>
<td>46.89 ± 2.23</td>
<td></td>
</tr>
</tbody>
</table>

* based on duplicate analyses
An extraction time of 15 minutes was insufficient for the complete extraction, where the recovery achieved was only within 38.84-56.05%. However, an extraction time of 30 minutes did not provide any significant increase in the recovery. This suggests that the insufficient recovery was not due to the heating temperature alone, but perhaps also other contributing factors such as the size of the vessel.

When the duration was increased to 60 minutes, the water in the vessel evaporated completely. This prevented any analyses to be conducted. Since there was no significant difference between the recovery for 15 minutes and 30 minutes, a period of 15 minutes was chosen for the further analyses. Other studies have also reported similar heating times for subcritical water extraction, however only when coupled with headspace and direct immersion SPME [32, 36].

3.4.3 Influence of Water Volumes

A further study was conducted to observe if an increase in water volumes could eliminate the inaccuracy caused by the large vessel. Since the PAHs would finally be reconstantated to its original volume of 2 mL before injection into the GC, the amount of water present for the extraction should not pose a problem with respect to the lowering of PAH concentration once it partitions into the water.

In preliminary experiments, an initial volume of 3.5 mL was used as it was found to adequately immerse 1g of sludge sample. The water volumes that were subsequently investigated were 7 mL and 10 mL. Higher volumes were not investigated due to the absence of a rotating mixer to adequately mix the high volume of samples during the extraction.

Although it was expected that with the reduction of the headspace volume the extraction efficiency would increase, this was not the case in the procedure conducted. There was a no effect on the amount of PAHs extracted. A possible explanation for this may lie in the mixing of the vessel content. A small substance content would not need vigorous mixing, but increasing the content would inevitably
lead to stagnant conditions within the vessel, and this would inhibit the PAH partitioning into the water.

Mixing is of crucial importance during the heating step, especially when higher volumes of substance are present in the vessel. This is why the water volume was increased instead of increasing both the sample amount and water volume by ratio. It was logically assumed that the PAH from the sludge sample (if increased by ratio) would not be efficiently transferred onto the water without being mixed.

This has been reported in a previous study using membrane discs [43], where recovery of the PAHs without mixing only amounted to ca. 45%. In the same study, another similar experiment was conducted, but by attaching the vessel to a rotating rack which was connected by a rod through the GC oven door to an external motor rotating at ca. 60 revolutions per minute. This resulted in continuous mixing of the sample and ensured good contact between the extracted PAHs and the sorbent disc, with simultaneous heating of the sample, and the recovery increased to ca. 85%.

3.4.4 Adsorbent for Extract Clean-Up

Previous methods [18, 33, 34] have suggested the use of C₈ and C₁₈ for clean-up of extracts with pure subcritical water. However, a recent study conducted [26] showed that the silica cartridge also produced quantitative recoveries of the PAHs. Therefore, recoveries studies of a standard PAH mixture was performed on both the silica and C₁₈ cartridge. The subcritical water extraction was omitted in this procedure, as the aim was to determine only the SPE cartridge efficiency.

3.4.4.1 SPE Clean-Up Procedure

The procedure as mentioned in Section 2.6 and 2.6.1 was used. For SPE with the silica cartridge, the partitioning of the extract into hexane was omitted, as the
subcritical water extraction was not performed. Instead, 1 mL of a standard mixture of PAHs (250 ppm) was applied onto the cartridges.

A concentration of 250 ppm was chosen for spiking based on a previous study [26] that performed an analyte breakthrough test for the extraction of PAHs. In the study, a standard PAH mixture of varying concentrations (50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm) was applied onto a silica cartridge and elution was performed. The study showed that recoveries of the PAHs increased with the concentration, but started decreasing when concentration was 300 ppm and above.

At concentrations within the range of 200-300 ppm, only one eluting step (6 x 2 mL of solvent) was required to recover the PAHs. However, at higher concentrations, not only were the recoveries lower, but double eluting steps were required. Therefore, a concentration of 250 ppm was chosen as the optimum concentration with regard to the efficiency of the cartridge.

3.4.4.2 Comparison of C₁₈ and Silica Cartridges

Both cartridges produced quantitative recoveries of the standard PAHs, but silica was evidently more efficient than the C₁₈ cartridge. The results for both the C₁₈ and silica cartridges are summarized in Table 3.4.

Table 3.4: Comparison of C₁₈ and Silica SPE Cartridge

<table>
<thead>
<tr>
<th>PAH Compound</th>
<th>Recovery (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₁₈</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>84.84 ± 4.92</td>
</tr>
<tr>
<td>Fluorene</td>
<td>80.46 ± 2.55</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>82.65 ± 1.39</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>76.56 ± 1.93</td>
</tr>
</tbody>
</table>

* based on duplicate analyses
The silica cartridge, with recoveries ranging from 90.74-96.44 % proved to be more efficient in terms of the recovery compared to the C\textsubscript{18} cartridge, where the recoveries spanned from 76.55-84.84 %. Only single elution steps (6 x 2 mL of solvent) was necessary for both the cartridges.

The better recoveries obtained with the silica cartridge was probably due to the eluting solvent used in the procedure. The C\textsubscript{18} cartridge is non-polar and requires a polar solvent for elution of the retained compounds. Therefore, the elution solvent used was methanol. For the silica cartridge, which is polar, hexane was used as the non-polar eluting solvent. The PAHs are more readily soluble in hexane, and presumably contributed to the higher recoveries obtained.

Also, during the evaporation step, the methanol solution required a reasonably longer evaporation period compared to the hexane solution. This also could have been a contributing factor, as previous studies [18, 34] have also reported that evaporation of the solvent tends to lower the recovery rates. Owing to the above reasons, the silica cartridge was chosen for subsequent analyses.

The recoveries for both the C\textsubscript{18} and silica cartridges are comparable to previous works and are shown in Table 3.3 and 3.4 respectively.

**Table 3.5: PAH Recoveries using C\textsubscript{18} SPE Cartridge**

<table>
<thead>
<tr>
<th>PAH Compound</th>
<th>Recovery (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This study*</td>
<td>Previous study[34]**</td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>84.84 ± 4.92</td>
<td>88.00 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>80.46 ± 2.55</td>
<td>84.00 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>82.65 ± 1.39</td>
<td>83.00 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>76.56 ± 1.93</td>
<td>71.00 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>

* based on duplicate analyses
** based on triplicate analyses
Table 3.6: PAH Recoveries using Silica SPE Cartridge

<table>
<thead>
<tr>
<th>PAH Compound</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This study*</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>90.74 ± 1.61</td>
</tr>
<tr>
<td>Fluorene</td>
<td>96.44 ± 0.59</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>94.58 ± 6.43</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>94.72 ± 2.82</td>
</tr>
</tbody>
</table>

* based on duplicate analyses

** based on triplicate analyses

NR: Not Reported

3.4.5 SWE Operating Conditions

The final temperature and heating duration that was decided on was 200°C and 15 minutes (Table 3.6). These conditions, despite showing low recovery levels (38.84% - 56.06%), yielded the best extraction recoveries for the procedure that was used. Several reasons were identified for the low recovery levels obtained from this procedure, as will be elaborated further in this section.

Before the analyses were conducted for the best procedure, blank runs were performed to determine if the vessel contributed to any additional PAH recovery. Fortunately, the vessels appeared inert at the working temperature, and no peaks were detected when analysed with GC-FID.

However, one problem that was initially faced during the removal of the water extract from the sludge sample, was the complete removal of the water. Filtering through a filter membrane was insufficient as some of the extract still appeared to be mixed within the solid sample. The only other alternative was to filter the sample by vacuum. This was done by filtering the sample over an empty SPE
cartridge as suggested by Koostra et al.[34]. The SPE frits that was used for filtering were of inert material. This method of filtering proved to be efficient and the sludge sample could be extracted to dryness.

As can be observed in the recovery tables, the recovery for naphthalene was always the lowest. This could be associated with its low molecular mass (128) which is vastly different from the other 3 compounds (Table 1.1). Other studies have reported [11, 37] that low molecular mass, low ring PAH structures such as naphthalene (2 rings) tend to be eliminated faster than higher molecular mass structures, and result in losses of PAHs during analysis.

Fluoranthene however, with a four ring structure and a molecular mass of 202 did not produce the highest recovery when extracted. Instead, the recovery of phenanthrene and fluorene was better on two occasions, where the heating temperature was 150°C and 250°C (Table 3.2). The reasons for this is unclear.

It is a possibility that the above recoveries are not fully representative of the extraction efficiency since the reproducibility of the extractions were low, and therefore it would be inaccurate to justify the reasons for the compound recoveries. As can be seen from the recovery tables, the relative standard deviations were rather high (up to 7.89%), and this is due to the irreproducibility of the extractions.

The large variance in the extractions is due to the elaborate extraction procedure that was employed. In such multiple-step procedures, errors are bound to arise from various sources: from the analyst, inconsistency in the transfer of extract from the vessel onto to the SPE cartridge as well as inconsistency in the evaporation step. Also evaporation procedures tend to cause some loss of analyte as have been reported by previous authors [18, 34].

Another reason for the low recovery obtained is the repartitioning of the PAHs from the aqueous phase back into the sludge sample when the vessel is allowed to cool [43, 51]. To avoid this problem, the vessel was only allowed to cool for a short period of time, before the extract was removed. Cooling was allowed to
be in the headspace. However, the benefit of such a precautionary step was not ascertained, as there did not seem to be a significant increase in the recovery.

One other reason that was identified for the inefficiency of the method was the absence of mixing in the procedure. As mentioned in Section 3.4.3, mixing ensures good contact between the PAHs in the sample and the extractant, whether it is a sorbent disc or the extractant water. With the absence of mixing, the recovery of the analytes will also be lower. The final reason is the influence of the large headspace present in the extraction vessel, which affects the equilibrium of the steam/water interface, and hence the required partitioning of the PAHs.

The recoveries of PAHs obtained from this method are hardly satisfactory due to the above mentioned reasons. At present, other extraction methods still seem superior. Table 3.8 summarizes the comparison of recovery levels obtained from this study and also other works using Soxhlet extraction and solid phase extraction.

<table>
<thead>
<tr>
<th>Table 3.7: Comparison of PAH Recoveries with other Extraction Methods</th>
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<tr>
<td>PAH Compound</td>
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<td></td>
</tr>
<tr>
<td>Naphthalene</td>
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<tr>
<td>Fluorene</td>
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<tr>
<td>Phenanthrene</td>
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<td>Fluoranthene</td>
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* based on duplicate analyses
** based on triplicate analyses
NR = Not Reported

Although the compared methods seem to be more efficient than the subcritical water method in this study, it must be stressed that the low recoveries obtained could be due to technical constraints, and does not reflect the efficiency of the method as an established procedure.
3.5 Extraction with Surfactant-Modified Subcritical Water

It has been previously suggested [41] that the addition of a surfactant to the water during subcritical water extraction would result in higher extraction recoveries of PAHs. The hydrophobic nature of the PAHs enables it to be almost totally incorporated into the micelle [49]. This method of extraction has gained interest due to its ability to concentrate a variety of analytes including PAHs as well cost benefits and its easy and safe disposal.

Owing to those reasons, the development of a method to extract the chosen PAHs was attempted (Section 2.5.2). This method employed the use of static subcritical water extraction, with a procedure similar to the previous method. The exception of course, was the addition of the surfactant for the extraction.

3.5.1 Choice of Surfactant

In this study, instead of using pure water to perform the extraction, the water was modified with a surfactant in order to obtain a micellar medium where the PAHs could be easily extracted. Sodium dodecyl sulfate (SDS) was chosen as the surfactant as this choice corresponded with a study by Fernandez-Perez et.al. [41] where different surfactants were studied above their critical micellar concentration (CMC). In the same study, Triton X-100, SDS and DBS (dodecylbenzene sulfonic sodium salt) were studied as non-ionic, anionic and cationic surfactants, respectively. The best results were obtained when the anionic surfactant was used. Another anionic surfactant (Aerosol OT) was also investigated and compared to SDS. SDS was found to be the best surfactant for subcritical water extraction of the PAHs.

3.5.2 Experimental Parameters

The surfactant concentration chosen was 0.005 M. A previous study [41] reported that the efficiency of the extraction increased with concentration but levelled off at concentrations higher than 0.005 M. An extraction time of 15 minutes
and a temperature of 200°C were chosen to maintain similar extraction conditions as the previous method (Section 3.4.5).

3.5.3 Solid Phase Extract Clean-Up

The choice of a suitable bonded sorbent material to retain and separate the analytes from the extract, and the eluent required for proper analyte elution are factors which need consideration. Since the analytes were extracted as micelles, the use of a SPE silica cartridge for clean-up of the analytes was preferred due the ionic nature of the surfactant. Hexane is suitable as the eluting solvent for PAHs and was chosen as the eluent.

3.5.4 Subcritical Water Extraction with Micelle Formation

The extraction was performed as described in Section 2.5.2, and was subjected to SPE clean-up as described in Section 2.6.2.

The micellar extract was applied directly onto the silica cartridge, and was later eluted with hexane. Even though it was necessary to use the silica cartridge since the micelle was of an ionic nature, this choice posed some problems in the recovery of the PAHs. Despite repeated attempts, the recovery of the PAHs were rather low, as reflected in the GC profile as compared to the profile of the direct injection of the standards (Figure 3.2). This observation was attributed to the application of water on to the silica cartridge, which would naturally cause the extraction to be ineffective.

The micellar extract consists of water (H₂O) with OH groups. This would deem the silica cartridge unsuitable for the extract, as the interaction between silica and water would severely deteriorate the extraction efficiency of the cartridge. However, replacement of the cartridge with a non-polar cartridge would not solve the problem as the micelle is ionic in nature.
Figure 3.2: GC Profiles of PAHs with (a) Surfactant-modified subcritical water extraction as compared with (b) PAH standards by direct injection. Extraction time was 15 minutes with extraction temperature of 200°C. GC conditions as in Figure 3.1.
In the study conducted by Fernandez-Perez et al. [41] (the primary reference for this procedure), a silica cartridge was used for the SPE procedure, after extraction was conducted using micelle formation for improvement of the subcritical water extraction. However, the extraction procedure was a combination of static and dynamic mode, and not purely static as attempted by this study. In this method, subcritical water extraction was performed using a prototype extractor as shown in Figure 1.4. The above experiment was therefore aborted as it was clear that the static extraction procedure could not be used as attempted.

3.6 Subcritical Water Extraction Coupled with Membrane Discs

The use of membrane discs in the subcritical water extraction of PAHs from sludge was investigated based on studies made by Hawthorne et al. [32, 43] where the use of C_{18} silica and styrene-divinyl benzene (SDB) discs were reported. However, as the SDB discs were not available within the duration of the study the C_{18} silica disc was used.

Without the use of membrane discs, PAHs which have been extracted by subcritical water might repartition upon cooling of the vessel. However, by using a suitable adsorbent such as the membrane discs, the PAHs will most likely be adsorbed onto to the discs during extraction and will not repartition into the water. In this study, several factors such as the stability of the disc, effects of disc size and disc surface area were considered. The influence of extraction temperature and extraction time on the extraction efficiency were also investigated.

3.6.1 Disc Stability at Various Extraction Temperatures

Initial experiments were performed to determine the stability of the extraction discs under subcritical water conditions since loss of sorptive ability and/ or physical breakdown of the disc material would make the method unusable.
To test the stability of the discs, ca. 1 x 2 cm rectangles of the disc material were exposed to subcritical water (without the presence of the sample and the spike PAH solution) at temperatures of 150°C, 200°C and 250°C for 15 minutes each time. After cooling of the extraction vessel, the discs were removed from the vessel with tweezers and later extracted with 3 mL of acetone/ methylene chloride mixture (1:2). The extract was then concentrated to 1 ml and analysed using GC-FID to observe any impurity peaks which might interfere with the PAHs peaks in the GC chromatogram.

The disc appeared to be both physically and chemically stable at temperatures 150°C and 200°C and no artifact peaks were detected by GC-FID. However, the discs were found to disintegrate once the temperatures was raised to 250°C. Handling with tweezers during removal of the discs from the cooled vessel resulted in the crumbling of the disc at its edges. When the disc was subjected to analyte desorption with the acetone/ methylene chloride mixture using the vortex mixer, the disc was found to further disintegrate. The extract formed a cloudy solution with the disc material suspended in it.

A similar preliminary study as was described above was conducted by Hawthorne et al. [43] to determine the stability of the discs during the application of subcritical water extraction. However, instead of using a C_{18} sorbent disc, a SDB sorbent disc was used. This sorbent disc was reported to be more stable and could withstand temperatures of up to 300°C without resulting in any chemical or physical breakdown.

In this study, since the disc disintegrates when temperatures are increased, extraction could not be performed at the desired temperature (250 – 300°C). Hence, subsequent experiments were performed at 150°C and 200°C. Previous works [21, 32, 43] have suggested that non-polar organics such as PAHs require temperatures of up to 250°C for efficient extraction. This proved to be a serious limitation in the study carried out using membrane disc extraction.
3.6.2 Effects of Disc Size

The influence of disc size on the extraction efficiency was also investigated. A previous study utilised 1 and 2 cm$^2$ as their disc measurement [32]. However, since C$_{18}$ disc was not a suitable sorbent disc for PAH extraction under subcritical conditions, the aim of this investigation was to examine whether increasing the disc size would improve the extraction efficiency of the disc. A previous study showed that 2 x 3 cm (6 cm$^2$) was the optimum size for the sorbent disc [43].

In this study, the disc size was increased from 1 cm$^2$ (1 x 1 cm) to 4 cm$^2$ (2 x 2 cm) and 6 cm$^2$ (2 x 3 cm). Each disc was placed in the extraction vessel, and spiked with 2 mL of PAH in 3.5 mL of water. After heating to 150°C, the disc was extracted and analysed according to the described procedure (Section 2.5.3.2). The aqueous fractions were extracted three times by mixing for about 5 minutes each time with aliquots of methylene chloride.

Recoveries of the PAHs were not calculated due to the extremely low levels detected. Estimation of the effectiveness of the procedure was conducted simply by comparing the profiles of the extracted PAHs to that of the standard profile.

Counter to the previous study mentioned earlier [43], increasing the disc size did not result in improved efficiencies of extraction. From the GC profiles, it was observed that there was hardly any significant difference in the recovery of PAHs between the various sizes used. The recoveries of the PAHs were also low, indicating that some modification had to be made before the method could be used for the analysis of real samples. GC profiles of the aqueous fraction showed that the PAHs were still in the aqueous phase and was not successfully adsorbed onto the discs.

Therefore, instead of increasing the disc size, the number of disc rectangles placed in the vessel were increased. It was assumed that by increasing the number of discs place in the extraction vessel, the surface area of the total discs would be larger and this would result in increased sorption of PAHs on to the disc. The disc size used was 1 cm$^2$, since increasing the size did not contribute to higher recovery of PAHs. The number of disc placed were increased from 1 to 4. Subsequent analysis of the
aqueous extracts were not performed as it was assumed that the PAHs, if not adsorbed on to the disc, would still be in the aqueous phase.

Unfortunately, this procedure also proved to be futile, as there was no significant difference between the recovery using one sorbent disc and multiple sorbent discs (based on GC profile comparisons). This shows that the surface area of the disc did not play a major role in the extraction, but rather the disc sorbent material itself. It was evident from the above tests that the C18 disc was not capable of extracting the PAHs from the bulk of the solution onto the sorbent disc at the extraction conditions used.

Another possible reason for the low recovery of the PAHs is the size of the extraction vessel. Previous works [36, 43] on subcritical water extraction have emphasized that a small headspace is needed within the extraction cell, to avoid building up of pressure, that can sometimes go up to several thousand bars [43]. However, while it was imperative to maintain that headspace, a large headspace volume could result in severe deterioration of the extraction capacity. The large headspace is said to influence the equilibrium of the steam/water interface, and hence the required partitioning of the PAHs.

### 3.6.3 Influence of Extraction Temperature

Temperatures of the oven in which the extraction vessel was placed were set at 150°C and 200°C for each extraction and heated for 15 minutes. GC profiles of extractions at the various temperatures as well as the profile for the standard PAHs are shown in Figure 3.3. Based on the GC profiles, the recovery for both the 150°C and 200°C (Figure 3.3a) appeared similar, or with insignificant difference. A temperature of 150 °C was subsequently chosen as the extraction temperature of all further analyses.
Figure 3.3: GC Profiles of PAH with subcritical water extraction at (a) 150 °C and 200 °C as compared with (b) PAH standards by direct injection. Extraction time was 15 mins with C\textsubscript{18} membrane disc. GC conditions as in Figure 3.1.
3.6.4 Influence of Extraction Time

With 150°C as the extraction temperature, the extraction time was set at 15 minutes, 30 minutes and 60 minutes. The recoveries for 15 minutes and 30 minutes were similar and the profiles resembled that of the chromatogram in Figure 3.2a (150°C, 15 minutes), where the low levels of recoveries can be observed. When the vessel was heated up to 60 minutes, the water completely evaporated, leaving the vessel dry, and the disc was also in a disintegrated form.

Since this method also did not appear to be feasible, especially with the use of the C\textsubscript{18} membrane discs, further investigations were stopped, and this method was not employed for subsequent experiments in this study.
3.7 Real Sample Analysis

From the three extraction methods investigated, it was clear that the pure subcritical water procedure was the best in terms of recovery, and it was therefore chosen as the method to be used for the analysis of real sludge samples. The primary aim of this research was to develop an extraction method for the purpose of recovery studies, and not to perform quantitative analysis of the PAHs in real sludge samples. Therefore, the standard calibration was not performed. A rough quantification of the PAH amounts was based on relative peak area of the extracts to that of the standards.

The analysis was performed with sludge samples that were previously obtained from three different wastewater treatment plants from Kota Tinggi, Lima Kedai and Seri Alam. Extraction was conducted according to the pure subcritical water procedure, employing an extraction temperature of 200 °C and 15 minute extraction time. Samples were first analysed with GC-FID, and peak confirmations were performed with GC-MS.

3.7.1 Pure Subcritical Water Extraction

The levels of PAHs, if present in the samples, were assumed to be very low, and preconcentration steps were necessary. Therefore, after extraction with subcritical water and clean-up with SPE, the sample extract was reduced to almost dryness under a gentle stream of nitrogen gas, and was reconstituted with 25 μL of hexane, resulting in a concentration factor of 80 times. The concentrated solution was then injected into the GC for analysis.

3.7.2 Analysis with GC-FID

The analysis with GC-FID was performed for all three samples. However, detection of the PAHs was only possible with the sludge sample from Kota Tinggi
(Figure 3.4). The samples from Seri Alam and Lima Kedai (Appendix 1 and Appendix 2) showed many co-extracted peaks, even after SPE clean up, and identification of the peaks was not feasible.

For the sample from Kota Tinggi, two of the four PAHs could be detected according to the similar retention times. The two PAHs are fluorene and phenanthrene (Figure 3.4). The levels however were very low, even after concentration of 80 times. This could be due to two reasons: one, the levels of the PAHs are naturally very low in native sludge samples; and two, the extraction efficiency was not sufficient to quantitatively extract the PAHs.

Figure 3.4: GC-FID Chromatogram of Sludge Sample from Kota Tinggi
3.7.3 GC-MS Identification

It was questionable whether the identified peaks were actually PAHs peaks owing to their extremely low levels. These peaks could easily be any other co-extracted contaminant, or possibly just noise peaks. Therefore, GC-MS identification was carried out to determine the quality of the peaks.

The PAH standards were first analysed to obtain a more accurate identification of the PAHs in the sludge samples. The GC-MS chromatogram for the standard PAH mixture as well as the ion chromatograms for both the standard fluorene and phenanthrene are shown in Figures 3.5, 3.6 and 3.7 respectively.

The ion chromatograms of the standard fluorene and phenanthrene correspond with the actual molecular weight of the PAHs compounds (Table 1.1), with a high probability value of 95% and 94% for the respective PAHs.

![Figure 3.5: GC-MS Chromatogram of the Standard PAH Mixture](image)

1= Naphthalene, 2= Fluorene, 3= Phenanthrene, 4= Fluoranthene
Figure 3.6: Total Ion Chromatogram of Fluorene (standard)

Figure 3.7: Total Ion Chromatogram of Phenanthrene (standard)
The GC-MS chromatogram of the Kota Tinggi sample is shown below in Figure 3.8. This observation is further confirmed by the ion chromatograms of the peaks, as can be seen in Figures 3.9 and 3.10. The probability value for fluorene is 91% (Appendix 3), whereas for phenanthrene it is 93% (Appendix 4). An estimate of the concentration of both the extracted PAHs were calculated by comparing the GC-MS peak areas of the extracts to that of the standards. The concentration for fluorene was 1.36 mg/kg, whereas the concentration for phenanthrene was 2.19 mg/kg.

The concentrations however, does not accurately represent the actual concentrations in the sludge due to the recovery range of the method used (38.84 – 56.06% ). A previous study with the same samples [26] using Soxhlet extraction and SPE silica clean-up reported that concentration of phenanthrene in the Kota Tinggi sample to be 4.09 ± 0.10 mg/kg. The concentration of fluorene was not reported. The reasons for the low recovery have been justified in Section 3.4.5.

There were also a number of other interfering peaks. The most significant of the peaks being the one observed in the GC-FID chromatogram of the Kota Tinggi sample (Figure 3.4). This peak was identified as hexadecanoic acid. The concentration in the sludge sample was very high, and this has been previously observed by another author, from whom the sludge samples were obtained from [26]. The author used Soxhlet extraction, and GC-FID and GC-MS for the analysis.
Figure 3.9: Total Ion Chromatogram of Fluorene (Sample from Kota Tinggi)

Figure 3.10: Total Ion Chromatogram of Phenanthrene (Sample from Kota Tinggi)
CHAPTER IV

CONCLUSION AND SUGGESTIONS

4.1 Conclusion

Coupling subcritical water extraction with SPE as the clean-up procedure was found to provide a simple approach for the extraction of PAHs from spiked sludge samples. However, rather low recoveries were obtained in this study. Various factors have been identified for the low recoveries, the most important factor being the unsuitability of the extraction vessel, and the absence of a mechanical rotator during the heating process.

The pure subcritical water extraction procedure, with recoveries of the PAHs ranging from 38.84 – 56.06% proved to be the best extraction method in this study, as compared to the surfactant-modified method and the membrane disc method. However, due to time constraints, numerous other parameters for those methods were not thoroughly investigated, and this could have contributed to the inefficiencies of the method.

For the SPE clean-up procedure, both the C_{18} and the silica cartridge proved to be workable adsorbents for the determination of PAHs, where the recovery for the spiked PAHs ranged from 76.56 – 84.84 % and 90.74 – 96.44 % using the respective SPE cartridges. It was evident from the preliminary studies that silica was a better adsorbent, and was therefore used in the subsequent experiments.
Analysis of samples from three wastewater treatment plants were conducted to see the effectiveness of the method for actual sludge samples. From the three samples analysed, only the sample from Kota Tinggi was found to contain detectable amounts of PAHs. The sludge samples from Lima Kedai and Seri Alam showed many interfering peaks in the chromatogram and identification of the analytes was not feasible. Two PAH compounds were detected in the Kota Tinggi sample, that is fluorene, with an estimated concentration of 1.36 mg/kg and phenanthrene, with an estimated concentration of 2.19 mg/kg.

4.2 Suggestions

Although unsatisfactory recovery levels were obtained in this study, subcritical water extraction still remains a field that is worth exploring, due to the many advantages that it offers. Little work has been reported on the selectivity that subcritical water offers despite the fact that its potential seems to be greater than with supercritical CO₂, considering the wide range of polarities that can be generated using subcritical water. Therefore, the advantages that are offered should be wisely exploited to develop methods that are both efficient and simple.

A number of technical problems need to be addressed before the method proposed in this study can be efficiently used. Firstly, a suitable extraction vessel must be acquired for the procedure in order to obtain satisfactory results. The ideal vessel would be a stainless steel vessel, with fitting end caps, and which is able to withstand high pressures and temperatures (up to 300 °C). The size of the vessel should also be proportionate to the sample volume, so that a minimum headspace is present. For laboratory purposes (as proposed in this method), a 5 mL capacity is sufficient [43].

Another important criterion for subcritical water extraction is the presence of mixing throughout the heating step. This is attainable by attaching the extraction vessel to a rotating rack which can be connected by a rod through the GC oven door
to an external motor rotating at the desired speed. As have been reported before [43], this step can greatly improve the extraction of the analytes.

For subcritical water extraction with the membrane disc, various types of sorbent material should be tested, to identify the material that best extracts the analytes of interest as well as maintains its stability at high temperatures and pressures. One of the more popular sorbent material is styrene divinylbenzene, which was reported to effectively extract PAHs from the bulk solution [32, 43].

Two other methods of extraction with subcritical water that was not investigated in this study, is the use of online subcritical water extraction and subcritical water extraction with headspace or direct SPME. In the online SWE, the dynamic extraction mode controls the extraction process and a previous study has shown that the dynamic extraction period is more effective than the static period [41]. The SPME method should also be explored as this method of extraction completely eliminates the use of organic solvents, therefore providing an environmental-friendly way of analysing these compounds.

The primary aim of this study was to compare the various SWE extraction methods for the purpose of recovery studies, and not to perform quantitative analysis of the PAHs in real sludge samples. Therefore, estimates of the PAH concentrations in the extracts were calculated by comparing the relative peak area of the extracted sample to that of the standards. Therefore, in further studies, accurate quantification of the PAH concentrations in the sludge samples can be conducted by employing the use of suitable internal standards and the construction of calibration graphs.

In this study, only four of the 16 PAHs listed as priority pollutants by the US EPA were studied. In the future, efforts should be targeted at analysing the other PAHs as they show different extraction capabilities according to the number of rings in the compound and their molecular weight. This would provide a better understanding of the behaviour of PAHs.
The solubility characteristics of these compounds in subcritical water is needed to support fundamental understanding of the chemistry involved. Also, a better understanding of the thermodynamic and kinetic processes which control subcritical water extraction of hazardous organics from solids is needed to support the further development on this process, especially the kinetic alternative.

Despite the fact that the procedure in this study was unable to quantitatively extract most of the PAHs, this method can be used for the removal of the PAHs from sludge samples. Simply by using water at subcritical conditions, almost 50% of the PAH contaminants can be removed, and this provides a cost-effective method of treating the sludge before disposal or re-use.
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