CHAPTER I

INTRODUCTION

1. Problem Definition

1.1 Sample Preparation Problem in General

Sample preparation in modern instrumental analysis is often required for two reasons : cleanup and concentration. The sample matrix frequently interferes with measurement. In gas and liquid chromatography, the life of columns may be drastically shortened by impurities. In these cases, sample cleanup is necessary. In many instances, the analyte concentration falls below the sensitivity range of the analytical method chosen. Only with concentration can the analysis be brought into practical range. In addition, the analytical techniques require minimum detection limits. This is the second reason for pretreatment.

In the cleanup step, the liquid-liquid extraction (LLE) has traditionally been used for these purposes. It requires labor-intensive manipulations of large volumes of water and organic solvents. In many cases, the separation of the organic and aqueous phases is complicated by the formation of emulsions. Another factor that often limits the rate at which samples can be processed is the time required to reduce a relatively large volume of organic extract to a small final working volume. So that, it is tedious and costly. A simpler method was introduced in the mid-1970s : solid-phase extraction (SPE). Similar to low pressure liquid chromatography, it involves the use of small, disposable extraction columns, filled with one of a wide variety of sorbents. The primary limitation of the SPE cartridges is the tendency for fine particulate matter to plug the frits holding the adsorbent in place (1-2). SPE medium was released in a membrane or disk format in 1989 and had advantages over the cartridge format including higher sample flow rates and less back pressure due to the high cross-sectional area of the disk. Compared to cartridge formats, disks have improved mass transfer characteristics due to their smaller sorbent particles. In addition, bed

channeling is eliminated for disks because the sorbent particles are immobilized in a Teflon mesh (3).

Although disks have many advantages, they are costly. In addition, disks can not be reused because of poor reproducibility. The researches using SPE disks are expensive so that we should purpose the cost-effective technique.

1.2 Significance of PAHs' Problem

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants. They are emitted from a variety of sources including : industrial combustion and discharge of fossil fuels, residential heating (both fossil fuels and wood burning), motor vehicle exhaust, and tobacco smoke (4-6). The wide occurrence, mutagenicity, carcinogenicity, and toxicity of these compounds make them serious organic pollutants (7-11). The United States Environmental Protection Agency (U.S. EPA) lists sixteen PAHs as " priority pollutants" of the environment : acenaphthene (Acent), acenaphthylene (Acenl), anthracene (Ant), benzo[a]anthracene (BaA), benzo [b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[g,h,i]perylene (Bghi), benzo[a]pyrene (BaP), chrysene (Chry), dibenzo[a,h]anthracene (Dah), fluoranthene (Flt), fluorene (Flu), indeno[1,2,3-cd]pyrene (Ind), naphthalene (Naph), phenanthrene (Phen) and pyrene (Pyr). The structure of these compounds show in Figure 1.1. PAHs have been measured in a variety of environmental matrices including air (12-13), water (14-16), soil (17-18), sediment (19-20), and airborne particulate matter (21). Moreover, PAHs can find in some food samples such as charcoal-grilled meat (22).

1.3 PAHs' Analysis Problem

There are many researches concerning PAHs. In many cases, the sample pretreatment procedure is the critical step in achieving reliable quantitative results. PAHs concentration and cleanup are increasingly being performed by SPE. However, SPE of PAHs in aqueous samples is rather difficult. The solubility of PAHs in water is very low because of their hydrophobicities (23). PAHs have a tendency to adsorb on the surface that they contact leading to the loss of PAHs during transport, storage, and

SPE step (24-26). For these reasons, the results are irreproducible. The addition of some organic solvents as organic modifier or surfactants as solubilizer into the aqueous samples are the ways to increase the solubility of PAHs. The examples of organic solvents and surfactants were dichloromethane, methanol, 2-propanol, acetonitrile, Triton X-100, polyoxyethylene lauryl ether (Brij-35), cetyl trimethylammonium chloride (CTACl), and sodium dodecyl sulphate (SDS). In many papers, the preferred organic modifier and solubilizer were 2-propanol and Brij-35 because they gave the best results over the others (27-33).

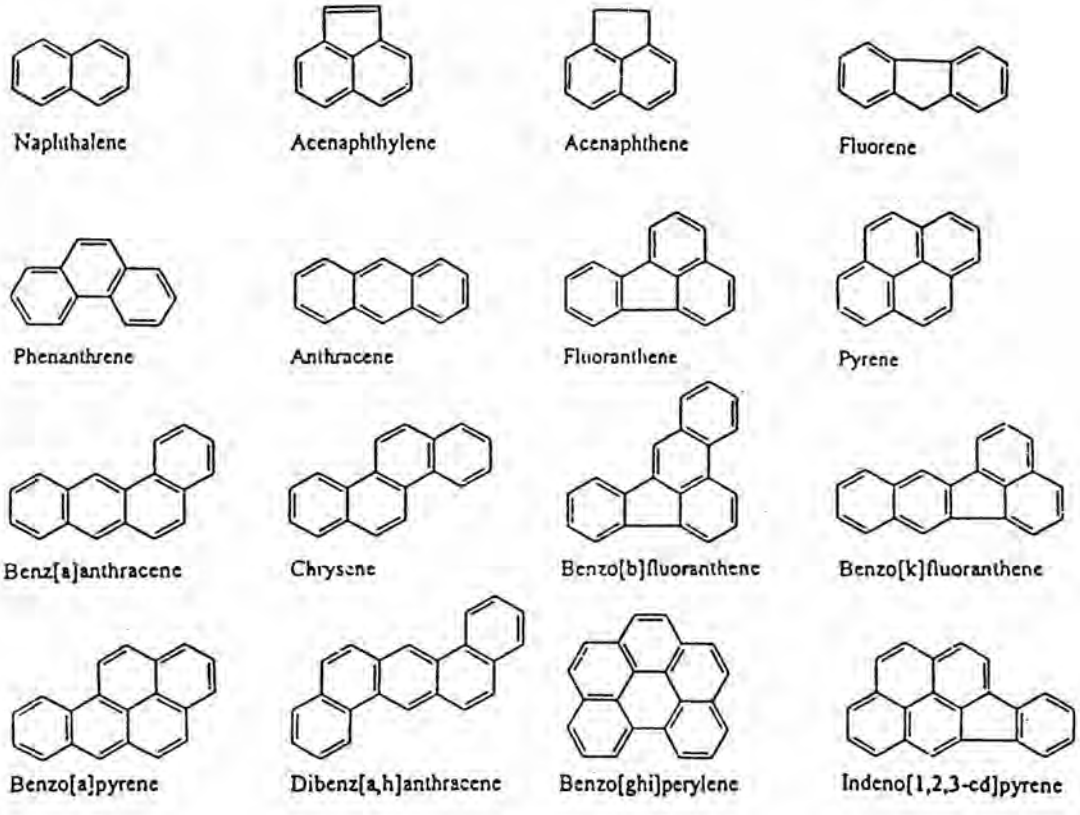

Figure 1.1 Structure of the 16 PAHs including in the EPA

2. Literature Review

Literature Review of Sample Preparation by SPE

Recently, the replacement of conventional LLE for isolating environmental pollutants with SPE techniques has been shown (34). The SPE method has been compared with the LLE in some studies (35-38). The results showed that the SPE method was higher recovery and precision.

The extractions of several compounds using cartridge SPE has been reported.

Trehy, Gledhill, and Orth (39) determined linear alkylbenzenesulfonates and dialkyltetralinsulfonates in water and sediment samples using C_8 SPE columns and detected them by GC/MS. Wells, Riemer, and Wells-Knecht (40) used C_{18} Mega Bond Elut columns for determination of the pesticides in agricultural runoff water. Nakamura and Yamada (41) used many kinds of cartridges for extraction of the agricultural chemicals in water. In addition, Krzyszowska and Vance (42) presented the separation and concentration of the herbicides by using aminopropyl cartridges for dicamba and octadecyl (C_{18}) cartridges for picloram from water and soil samples.

Junk, Avery, and Richard (43) found alkanes, alkenes, plasticizers and antioxidants were extracted from the polymeric column and frit components of the cartridge. These compounds were identified as possible interference compounds. The extracts were analysed by GC/MS. The interference compounds varied depending on the solvent used to elute the cartridge and the supplier of the cartridge. Besides, the level of interference present in the cartridges could vary significantly from different lot numbers.

The comparison between extraction cartridges and extraction disks showed that the recovery and precision of the SPE disks were better than the SPE cartridges for pesticides, nitroaromatic, nitramine, and semi-volatile compounds (44-46).

Moreover, the speed of routine analysis could be increased considerably by using the extraction disks instead of the extraction cartridges (47). So that, disks were preferably used for SPE.

Studies of the environmental samples by SPE disk technique are widely reported as follows :

Senseman et al. (48) compared the stability of various pesticides in water at 4 °C with their stability on C_{18} SPE disks under three storage methods : 4°C, -20°C, and 4°C for 1 day and then -20°C for the remainder of the storage period. Disk storage methods involved using the disk to extract the chemicals from water, removing the disk and placing it in a plastic bag and then storing it. The storage periods included 0, 3, 30, 90, and 180 days. Results indicated that the pesticides have equivalent or greater stability on SPE disks compared to their storage in water at 4°C and freezing the disk after pesticides loading was the most favorable storage option.

In order to determine the organochlorine contaminants, McDonnell, Rosenfeld, and Rais-Firouz (49) used C_8 and C_{18} disks for sample preparation of natural water and analysed by GC/ECD. Analytes were eluted from the 47 mm disk by placing the disk in a beaker and leeching the disk with 5 mL of diethyl ether for 20 min. the disks were then washed with two aliquots of 2.5 mL diethyl ether. All eluates were combined. For the 90 mm disk the volumes was increased to 20 mL of diethyl ether for leeching the disk and to 5 mL for the subsequent washing steps. These data suggested that a smaller disk was considered preferable as this would minimise the volume of solvent needed for elution of the analytes.

Crespo, Marce, and Borrull (50) showed a method for the determination of a group of pesticides in water by GC/MS with electron impact ionization. C_{18} and polystyrene-divinylbenzene (PS-DVB) disks were used to preconcentrate 500 mL of water. The low-μg/L levels of pesticides were determined with more than 85%

recoveries. The limits of detection were between 0.06 and 0.2 μg/L in the full-scan mode.

Hodgeson, Collins, and Bashe (51) used 47 mm PS-DVB disk to extract chlorinated acid herbicides in acidified water samples. The analytes were eluted with a mixed methanol-methyl *tert.*-butyl ether solvent. After extract, the analytes were esterified with diazomethane and analysed by gas chromatography with electron-capture detector. Method detection limits were 0.13-1.23 μg/L and the relative standard deviation were 5-30%. The recoveries for fortified reagent water, dechlorinated tap water, and high humic content surface water were 59-97%, 86-150%, and 59-124%, respectively.

Salau et al. (52) used C_{18} extraction disks for the isolation and trace enrichment of several fungicides from drinking, river and estuarine water. The water samples were prefiltered using 0.45 μm PTFE fiberglass filters before extraction to eliminate particulate matter. The combined methods (GC-ECD and LC-DAD followed by GC-MS and LC-MS confirmation) were used for the determination of these compounds. The limits of detection at S/N = 3 were 5, 10, 100, and 500-2000 ng/L when GC-ECD, GC-MS, LC-DAD and LC-TSP-MS were used, respectively.

Borgerding and Hites (53) used SPE for isolation of the cosmetic dyes and *N*-benzyl-*N*-ethylaniline sulfonic acid (BEASA) from the interfering compounds. Samples were colored wastewater from a municipal treatment plant. C_{18} extraction disk was used to remove the interfering anions before extraction with the strong anion-exchange cartridge. In this cartridge, the analytes and other ionic compounds were trapped. After elution of the trapped compounds with HCl, analytes were isolated from inorganic ions by extracting them onto C_{18} disk, washing the residual acid and other anions off with water, and extracting the analytes with methanol. Continuous flow fast-atom bombardment mass spectrometry was used to analyse BEASA and liquid chromatography with ultraviolet detector was used to analyse the other dyes. The recoveries were 64% for BEASA and 93-97% for the cosmetic dyes.

Poiger et al. (54) determined fluorescent whitening agents (FWAs) used in laundry detergents from sewage treatment plants and rivers. FWAs were extracted from 10-200 mL water samples with C_{18} bonded phase silica extraction disks and eluted with methanol containing tetrabutylammonium ion-pairing reagent. The eluate were detected by HPLC with post-column UV irradiation and fluorescence detector. The recovery of FWAs ranged from 76 to 96% and the relative standard deviation ranged from 1 to 11%. The limit of quantification was less than 30 ng/L.

Studies of smaller disk for the sample preparation are described as follows :

Lensmeyer, Wiebe, and Darcey (55) employed a 11 mm octyl (C_8) extraction membrane (SPEM) for the extraction of tricyclic antidepressant drugs from patients' serum specimens. Serum (1.0 mL or less), which adjusted to pH 5.5 with phosphate buffer was passed through the SPEM secured in a MF-1 microfilter unit. Protein and potential interference retained on SPEM were removed with 0.5 mL acetonitrile-water (35:65). The tricyclic drugs were eluted with 0.5 mL of water-acetonitrile-acetic acid-*n*-butylamine, 600:400:2.5:1.5 (by volume) and the eluate was injected directly into HPLC column. Recovery for all drugs exceeded 90% and coefficients of variation ranged from 2.9 to 8.3% through the concentration ranged of 75 to 300 μg/L.

Schmidt et al. (56) used membrane impregnated with PS-DVB resin beads for extraction of 16 different phenols, including those included in the U.S. EPA list of priority pollutants, from aqueous samples. A macro procedure used 47 x 0.5 mm disk and phenols were eluted with three 3 mL portions of tetrahydrofuran. A small-scale procedure used a thicker disk (3 mm) packed into a conventional SPE column (7 mm I.D.) and phenols were eluted with 0.75 mL of methanol. HPLC equipped with UV detector was used to analyse the extracts from macro scale procedure and GC equipped with flame ionization detector was used to analyse the extracts from micro scale procedure. Good recoveries were obtained for all of the phenols, both from larger volume (500 mL) through 47 mm disks and from smaller volume (20 mL) through membrane-packed tube.

Krueger and Field (57) developed "in-vial elution" technique to elute 13 mm C_{18} Empore disks. This technique was used to isolate linear alkylbenzenesulfonates (LAS) from samples of liquid detergent formulations and primary sewage effluent. Instead of eluting the disks in a conventional manner, the C_{18} disks were removed from their supports and transferred directly to a 2 mL gas chromatograph autosampler vial containing 0.005 M tetrabutylammonium hydrogen sulphate in chloroform. The vial was then placed in an autosampler tray for a 40 min minimum equilibration period in which LAS was eluted from the disk as its tetrabutylammonium ion pair. Upon injection, LAS was derivatised to its butyl esters detected by FID (58).

Field and Monohan (59) determined the monocarboxylic and dicarboxylic acid metabolites of dacthal in groundwater samples by concentrating the analytes onto 13 mm diameter strong anion exchange (SAX) Empore disks. The carboxylic acid metabolites were then simultaneously eluted and derivatised to their ethyl esters by placing the disk in a 2 mL autosampler vial together with 140 µL of ethyl iodide and 1 mL of acetonitrile and heating for 1 hour at 100°C. GC/ECD was used to detect them. Recoveries of 87.7 (1.1% R.S.D.) and 91.7 (1.0% R.S.D.) were obtained for the monocarboxylic and dicarboxylic acid metabolites from 100 mL samples of blank groundwater. The detection and quantification limits of the method were 0.02 and 0.06 µg/L, respectively.

The disadvantages of off-line SPE such as loss of sensitivity (injection of aliquot), loss due to evaporation or during transfer, and contamination are well known. On-line trace enrichment can eliminate these drawbacks and be automated system. Trace enrichment of the analytes could be done on 10 mm x 2.0 mm I.D. disposable cartridges using the Prospekt, which is an automated programmable sample preparation unit allowing direct elution to the LC column. The cartridges contained adsorbent such as PS-DVB or C_{18} (60-61). Moreover, on-line trace enrichment using membrane extraction disk in an adjustable membrane disk holder could be done. Ten 4.6 mm diameter extraction disks containing different packing materials held in a disk holder coupled on-line to a LC system were used for extraction and determination of polar pollutants in the environmental water (62-65). Kwakman et al. (66)

preconcentrated the organophosphorus pesticides from aqueous samples on three 0.5 mm thick, 4.2 mm diameter extraction disks in a disk holder. The final analysis was carried out by GC with thermionic detector.

Literature Reviews of The Determination of PAHs and Their Metabolites

Boos, Lintelmann, and Kettrup (67) presented the on-line sample processing and the determination of free and conjugated 1-hydroxypyrene in urine by coupled-column high-performance liquid chromatographic system. The method was based on copper phthalocyanine modified porous-glass precolumn packing material. When copper phthalocyanine trisulphonic acid derivative bound to the porous-glass support, it could adsorb selectively compounds having three or more fused rings (68). The adsorption took place in aqueous media, involving 1:1 complex formation between the ligand and the polycyclic compound. The desorption could be done by eluting with organic solvents, most effectively with methanol. This method was selective, enriched trace amounts of 1-hydroxypyrene, and eliminated the residual sample matrix. Moreover, the automated chromatographic system allowed the direct and repeated injection of urine samples. Lintelmann, Hellemann, and Kettrup (69) used this method for the quantification of 1-, 4-, and 9-hydroxyphenanthrene as well as for 1-hydroxypyrene in urine.

Potter and Pawllszyn (70) used solid-phase microextraction (SPME) as a solvent-free alternative method for the extraction and analysis of nonpolar semivolatile analytes such as PAHs and polychlorinated biphenyls (PCBs) from water and detected them by GC-MS. Analytes were extracted into a polymeric phase immobilised onto a fused silica fiber. The fiber was then inserted directly into the injector of a GC, and the analytes were thermally desorbed. This technique allowed sampling directly from the source (lake, drinking fountain, etc.) and therefore, eliminated the loss of analytes through adsorption onto container walls and saved transport costs. Detection limits ranged from 1 to 20 pg/mL and linearity extended from low pg/mL to ng/mL levels. Relative standard deviations ranged from 10% for PAHs to 20 % for PCBs.

Lai and White (71) used Aquapore RP-18 cartridge as precolumn concentration for the analysis of PAHs in drinking water by HPLC. The precolumn used for SPE was placed on-line between the autosampler and the analytical column replaced the sample loop. An autosampler controlled by a customised "Method Development Language". The precolumn concentration methods normally required a switching valve and/or pump in addition to the HPLC system but this study reported a method using no addition valve or pump to the HPLC system. The only switching valve used was the one in the autosampler and the only pump used was the HPLC pump. Analysis of PAHs had been performed using this method with as little as 1.5 mL of sample resulting in good reproducibility, linearity, and sensitivity.

Michor et al. (72) described the analysis of 23 PAHs in natural water at ng/L levels extracted by reversed phase disks with octadecyl silica (C_{18}) enmeshed in PTFE. Experimental variables were investigated including determination of solvent type, extraction number, preconditioning requirements, breakthrough volume, and the use of an in-line drying agent. Moreover, the stability of PAHs stored extracted on disks were compared to samples left unextracted. Disks stored at -19°C for 60 days had higher recoveries than samples left in sampling bottles unextracted at 4°C. In general, less decomposition of the target analytes occurred when extracted and stored on disks. Method detection limits for these PAHs ranged from 9 to 56 ng/L.

The U.S. EPA published many methods for the determination of PAHs in various samples. The methods used LLE for sample preparation such as method 610 (73) for municipal and industrial discharge, method 8310 (74) for ground water and waste, and method 550 (75) for drinking water. Moreover, the U.S. EPA have some methods used SPE for sample preparation such as method 525 (76) used C_{18} cartridges to extract organic compounds including PAHs from drinking water and method 550.1 (77) used C_{18} cartridges or C_{18} disks for the extraction of PAHs in drinking water.

3. Hypothesis

Solid phase extraction membranes or disks are the most devices used for SPE. They have many advantages over other devices and other extraction methods but they are very expensive and require the large amounts of organic solvents for eluting step. From literature reviews, the disk could be folded or even cut into pieces and be removed from their supports. After that, it was placed directly into a vial and soaked with low volume of eluting solvent for analytes elution. Therefore, the small disk can be used for the sample preparation and should be the cost-effective method. Moreover, this method tries to reduce the toxic solvents and hazardous wastes. Thus, the cost for waste treatment is excluded.

The application of small disk for determination of 16 PAHs, U.S. EPA priority pollutants, in aqueous samples should have high percent recoveries, precision, and accuracy. Moreover, this technique should be used as routine analysis. The solubility problem of PAHs tries to solve by adding 2-propanol and Brij-35 into the aqueous samples. Besides, the factors affecting retention and elution process should be optimised to improve the efficiency of the small disk extraction method. The extracted PAHs will be analysed by HPLC followed EPA method 550.1. In EPA method 550.1, ultraviolet and fluorescence detector are used for detection of 16 PAHs. Although fluorescence detector provide higher sensitivity and selectivity but the number of compounds that can be detected is rather limited, acenaphthylene has nearly no response to fluorescence detection, and the confirmatory power of absorbance detection lacks. This is partially solved by the use of diode array detection (65,78). So that, photodiode array detector (PDA) is used in this study. Moreover, the small disk extraction method is feasible for real water samples and expects to the further development of the EPA.

4. The Purpose of The Study

The proposal of cost-effective SPE method used low volume of eluting solvent was aim of this work. SPE used small solid phase extraction membrane (13 mm) was developed for the determination of 16 PAHs i.e. acenaphthene (Acent), acenaphthylene

(Acenl), anthracene (Ant), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[g,h,i]perylene (Bghi), benzo[a]pyrene (BaP), chrysene (Chry), dibenzo[a,h]anthracene (Dah), fluoranthene (Flt), fluorene (Flu), indeno[1,2,3-cd]pyrene (Ind), naphthalene (Naph), phenanthrene (Phen) and pyrene (Pyr). The various parameters affecting the percent recoveries of PAHs were studied and the optimum conditions of the method were determined. The various effects were :

1. The concentration of 2-propanol i.e., 0, 5, 10, 15, and 20%
2. The concentration of Brij-35 i.e., 0, 0.05, 0.11, 0.30, and 0.50 mM
3. The volume of eluting solvent i.e., 0.50, 0.75, and 1.00 mL
4. The elution time i.e., 5, 15, 30, 45, and 60 min
5. The breakthrough volume i.e., 25.00, 50.00, 100.00, and 250.00 mL

In addition, the detection limit, accuracy, and precision of this method were also studied and evaluated prior to use it in the analysis of these compounds in real water samples.