CHAPTER I

GENERAL INTRODUCTION

1.1 Problem Definition

Phthalate esters (PEs) are aromatic dicarboxylic esters such as dimethylphthalate (DMP), diethylphthalate (DEP), di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl)phthalate (DEHP), and di-n-octylphthalate (DOP). The PEs, in most cases liquids with very high boiling points and very low vapor pressures, are moderately persistent and lipophilic. The PEs are widely used as plasticizers in polyvinylchloride (PVC) for food packing, medical products, refrigerator gasketing, luggages, handbags, coated cloth, vinyl floor coverings, waterproof boots and shoes, electrical insulation, industrial hose and tank liners. Besides PVC, the PEs are used to plasticize polyvinyl acetate, polyvinylidene chloride, polystyrene, ethyl cellulose, cellulose nitrate, acetate and acetate butyrate, chlorinated rubber, high styrene-butadiene protein compounds, shellac, acrylic-type resins, polyamides, polyesters, epoxy alkyds, phenolic alkyds, polyurethane, nitrile and neoprene rubber, and chloroethylene resins. As PVC is brittle, PEs plasticizers are added to give the desired flexibility and softness, and may account for as much as 20-40% of the final weight of the material. DEHP is one of the most widely used plasticizers, particularly for vinyl plastics. Minor applications of PEs are in varnishes, cosmetics, lubricants, insect repellents and as pesticide carriers (1-3). Because these PEs

are not chemically bonded to the polymer, they can migrate from plastics to environment under suitable conditions (41). PEs have indeed been found world-wide in water (4-12, 25-28, 33-34), air (5-6,17,24,31,33), fish (6,10,13,42), soil (7,17), sediments (5,10,15, 22,26,30), sewage sludge (48), biota (5-6,43), food (10,19), blood (6,16,20), plasma (14,21), intraveneous solutions (18,23), placenta(25), and parenteral solutions (22). Sources of these environmental PEs are often the wastewaters from plastic and plastic material products factories. Pesticides that contain PEs carriers may release them into air, soil and water. Volatillization and leaching of plasticizers from PVC is another source of undetermined magnitude (6). Several investigators have reported that blood and blood components can leach PEs plasticizers from plastic blood bags and medical tubing and the extent of PEs of blood stored in plastic bags increase with time. Transfusion of blood will transfer substantial amounts of PEs to patients (14,16,18,20-23).

Six PEs were included by the Environmental Protection Agency (EPA) in the list of priority pollutants: DMP, DEP, DBP, BBP DEHP and DOP (7). The PEs toxicity manifests itself chiefly by the influence of hydrolytic products formed. Generally PEs affect the central nervous system and DEP exerts stimulating effect while other PEs act mostly as depressants. Only at higher concentrations can they have an effect on the mucous membranes of respiratory system, on the digestion tract, eventually upon the skin (35). Although most PEs have a very low acute toxicity, there are many reports that chronic exposure to PEs have resulted in testicular atrophy, lung hemorrhage,

hepatomegaly, cytotoxicity, mutagenicity, carcinogenicity, teratogenicity and decreased human platelet function (37-40). There is also evidence that the PEs can disturb ecosystems and can provide a hazard to aquatic organisms like daphnia's already in ppb concentration; besides, it is known that water insects and several types of fish can accumulate DEHP 90-4000 fold, the actual value of the accumulation factor depends on the concentration of the ester in water. Stalling, Mayer and their colleagues (1972) reported that specimens of fish, crustaceans, and water collected throughout North America contained DBP and DEHP. They also showed that the species studied could concentrate DBP and DEHP from water; for example, after 7 days the PEs concentrations of the organisms varied from 350 to 3900 times of that in water. These data imply that DBP and DEHP can accumulate in the food chain. The literature data sum to suggest that water organisms are more susceptible to PEs than are mammals such as rats and mice (2, 6, 15). PEs are degraded by a number of soil and aquatic bacteria, but degradation is slow and may not keep pace with the increase in environmental contamination (36). For the protection of human health, the EPA established the permissible concentrations of these PEs in water are 313, 350, 34, 15 mg/L for DMP, DEP, DBP and DEHP, respectively (37).

In Thailand, there are several manufactures of PEs plasticizers reported by Business Informations & Research Company Limited and Department of Industrial Works as shown in Table 1.1 (78-79). There is no well documented information on the fate of PEs in aquatic environments, nor have water quality been established for them.

Table 1.1 Names of manufacture of PEs plasticizers and amounts of the production a year.

Company	PEs Plasticizers	amounts of the production a year (tons)
1) TOA Plasticizer[1] Industries Co., Ltd	DEHP	6,300
	DBP	60
	DINP	3,240
2) Sand-Polychemical [1] Co.,Ltd	DEHP	1,600
	DINP	1,600
3) Eternal Chemical Industry Co.Ltd[2]	DEHP	7,200
	DBP	7,200
4) Thai Chemical Corp., Ltd.[2]	DEHP	24,000
5) Siam Sira Co.,Ltd[2]	DEHP	No data

Remark: (1) Registration and Statistical Section, Factory Control Division, Department of Industrial Works, Ministry of Industry.

(2) Business Information & Research Company Limited.

DEHP = Di(2-ethylhexyl)phthalate

DBP = Di-n-butylphthalate

DINP = Diisononylphthalate

In view of the world wide distribution of PEs, their unknown environmental fate, and adverse health effects have promoted great interest in the development of analytical methods for their detection and determination.

1.2 Literature Reviews

Analysis of trace amounts of these PEs in water usually requires a preconcentration step before determination by gas chromatography (GC). There are several extraction and preconcentration methods which can be used in the determination of the PEs e.g., closed-loop stripping (44), purge and trap (44-45), liquid-liquid extraction (2, 5, 7-8, 12, 25-26, 28, 32, 35, 47-49, 50-52), and solid phase extraction (9, 11, 52-55, 59). Each method has advantages and disadvantages which relate to equipment requirements, detection limits, the sample matrix, the sample volume, the analysis speed and complexity. The PEs are low volatiled compounds, so they are not suitable to be analyzed by closed-loop stripping and purge and trap methods. Low recoveries for the analysis of PEs by these two methods were observed (44-45). In addition, these methods necessarily required complex equipment to separate the organics from water and brought about serious problem drawback to the qualitative and quantitative analyzes e.g., the loss of the organic constituent, the interference of impurities in adsorbent trap or stripping gas, poor resolution and peak tailing caused by a too long stripping time, the large amount of water passing through the adsorbent and time consumption (84-85). Liquid-liquid extraction can be classified into two categories: macroextraction and microextraction.

The use of the microextraction method for the determination of PEs has also resulted in low recoveries (47,49). The EPA recommends the method for the determination of trace organic priority pollutants in water be macroextraction method (37, 60). There are serveral shortcomings associated with this method including: the transport of large quantity of water sample, the intensive labor, the use and exposure to large volumes of hazardous solvents, time consuming and costly, and the sample loss that may be associated with emulsion formation and concentration procedures (54, 61).

Another technique, solid phase extraction (SPE) is an emerging chromatographic sample preparation technology that reduces the analysis time, the costs, the labor, and the solvent consumption relative to those obtained from traditional alternatives. This method was introduced in the mid-1970s and has been applied in a variety of areas not only to sample clean up but also to fractionation, solvent changeovers, and compound concentration (62-63). The use of SPE as a tool for isolating organic compounds from complex matrices has grown significantly. The principle and description of SPE technologies have been described (61-73). The technique of SPE has a wide-range application in environmental, pharmaceutical, biological and clinical, petrochemical, food, and cosmetic samples (74-76).

Studies of environmental sample by SPE technique are widely reported as follows :

Junk and co-workers (46) studied the efficiency of porous

polymer resins by the extraction of organic solutes onto a column of clean XAD-2 resin followed by elution with diethyl ether. This eluate was then concentrated by evaporation and the organcis were determined by gas chromatography. Alcohols, aldehydes and ketones, esters, polynuclear aromatics, alkyl benzenes and pesticides were used as model compounds. The study on a large numbers of model compound added to water in the 10 to 100 ppb range (20 parts per trillion for pesticides) demonstrated that this method was accurate and reliable. An extensive studies of the procedure revealed several critical steps where proper technique and conditions were essential to avoid serious error. These included resin purification and handling, preparation of standard samples containing organic impurities, and the technique and apparatus used in concentrating the XAD column eluate.

Gough and Gesser (53) used porous polyurethane foam to remove some phthalate esters from water at the parts per million level. A variety of foams was tested, as well as coated foams, under flow and under static conditions. Phthalate esters were removed from water by absorption, depending on the size of the phthalate ester and the structure of the polymer, this may be rate controlled or equilibrium controlled.

Chladek and Marano (54) developed a cartridge sampling technique for priority pollutants in wastewater using bonded phase silica adsorbents. This technique has been compared to the EPA Method 625 which requires liquid-liquid extraction of the wastewater. The disposable bonded reversed-phase cartridges were evaluated for all the

phenols and 22 neutral pollutants. These compounds were chosen purposely to cover a wide range of solubility and polarity characteristics. The cartridge extractions of spiked water samples yielded recoveries between 50 and 110%, which were better or equal to recoveries on the same samples using Method 625 liquid extraction. The relative standard deviation of overall recoveries for all pollutants was less than 15%. The detection limits achieved were approximately 50 and 10 ppb (in water) for the phenols and neutrals, respectively.

Renberg and Lindstrom (87) used Sep-Pak C_{18} cartridge for trace enrichment of chlorinated phenolic compounds in water. After desorption with acetone, chlorinated phenols, guaiacols and catechols were determined as their acyl derivatives, using quartz capillary column gas chromatography. Different aqueous acetylation methods were compared, the highest yields being obtained with potassium carbonate solution. The procedure described has been applied to natural and industrial waste waters.

Tatarkovicova and Cap (11) used Porapak Q to preconcentration DEHP from potable waters. Elution was performed with an azeotropic mixutre of MeOH and acetone. The eluate was concentrated prior to DEHP determination by gas chromatography with flame ionization detection.

Bardalaye and Wheeler (88) described a method for the determination of trace quantities of triazine herbicides, terbutryn, prometryn and ametryn in water. The procedure involved preconcentration of water samples by sorption on C_8 cartridges and desorption

with 2-propanol. The determinative step was achieved by capillary gas chromatography on Supelcowax-10 fused silica column using a nitrogen-phosphorus detector. The limit of detection was 0.1-10 µg/L

Sherma, Dryer and Bouvard (55) demonstrated the use of solid-phase extraction and quantitative HPTLC on precoated, preadsorbent silica gel plates for the determination of phthalate ester residues in water samples.

Robinson and co-workers (89) studied the isolation of aromatic hydrocarbons, phthalate esters, and cosmetic (FD & C) dyes from aqueous solutions with Chrom-Prep PRP-1 (styrene divinylbenzene copolymer) and Sep-Pak C_{18} cartridges. They used ^{14}C-labelled solutes in monitoring cartridge effluents for the leakage of small quantities of analyte during trace enrichment. Chrom-Prep PRP-1 cartridges showed greater retention of model aromatic hydrocarbons, phthalate esters than Sep-Pak C_{18} cartridges; however, the latter cartridge type demonstrated greater affinity for FD & C dyes, which changed as a function of pH.

Ritsema and co-workers (9) presented a procedure for the determination of six phthalate esters (PEs) in water and suspended particular matter (SPM). Using a solid-phase extraction (SPE), method detection limits (MDLs) of 0.01 to 0.1 µg/L could be achieved for determining PEs in water, while MDLs in SPM, using a liquid-liquid extraction, were 0.01 to 1 mg/kg. The biodegradation of PEs in water was investigated during ten days at two different temperatures, 4°C and 20°C. PEs were measured in extracts from river Rhine and Lake Yssel water and SPM samples by means of gas chromatography with

electron-capture (ECD) and mass-selective (MSD) detection. Because of its superior selectivity, the latter technique is recommended for the determination of PEs in environmental samples.

Kawahara and Hodgeson (52) described a method (U.S. EPA Method 506) for determination of phthalate and adipate esters in drinking water by liquid-liquid extraction or liquid-solid extraction. A measured volume of sample, approximately 1 L, was extracted with a ternary solvent consisting of methylene chloride, hexane and ethyl acetate using a glass separatory funnel. The solvent extract was isolated, dried and concentrated to a volume of 5 mL or less. The extract was further concentrated by gentle use of nitrogen gas blowing to a volume of 1 mL or less. Alternatively, a measured volume of sample was extracted with C_{18} cartridge or disk. The C_{18} cartridge or disk was eluted with methylene chloride. The eluant was then concentrated using a gentle stream of nitrogen or clean air to a volume of 1 mL or less. The analytes in the extract were separated by means of capillary column gas chromatography using temperature programming and the phthalate and adipate esters were then measured with a photoionization detector.

Vinuesa and co-workers (57) studied the extraction and enrichment of organophosphorus pesticides by using Sep-Pak C_{18} cartridges. The influence of the elution solvent, pH, salinity and volume of water filtered was investigated for ten organophosphorus pesticides. The pesticides were determined by gas chromatography with a BP-1 capillary column and a thermionic detector. Recoveries at the

100 and 200 ng/L spiking levels were greater than 85%, except for disulfoton.

Marvin and co-workers (90) developed a method to determine trace concentrations of propoxur, carbofuran, carbaryl, propham, captan, chloropropham, barban, and butylate in water. One hundred milliliters of sample water was passed through a disposable SPE cartridge packed with 90 μm sorbent at 10 mL/min. The concentrated analytes were eluted from the cartridge with acetonitrile. The resulting eluate was blown-down under nitrogen, made up in water, and injected into the HPLC. The analyte are separated on a 25 cm C_8 analytical column and determined by UV absorption at 220 nm. The total analytical time was 90 min and the lowest detectable concentrations were in the range of 0.02-0.92 μg/L for the eight pesticides. Recoveries for the eight pesticides ranged from 84% to 93%. The procedure was totally automated and could analyze 30 samples consecutively and unattended.

The SPE study of biological and clinical and pharmaceutical samples are described as follows :

Patel, Benson and Hometchko (91) described a procedure for isolation of amphetamine and methamphetamine from urine by using polymer-based C_{18} extraction cartridges. The eluent from the cartridges was injected directly onto the HPLC column for analysis. The extraction principle involved hydrophobic interaction using ion pairing with hexanesulfonic acid before sample application. The final

extract was clean, with no coelution of pigmented impurities. The absolute recovery of the compounds was > 90% from 5.0 μg/mL to 25 μg/mL. Interassay variation over two weeks was 5.7% for amphetamine and 6.0% for methamphetamine. The extraction was linear between 5.0 and 25 μg/mL The extraction procedure is well suited for detailed pharmacokinetic studies and for routine analyzes.

Timmons, Cox and Brown (92) reported a method for the determination of citral in rodent blood. The method involved isolation of citral from whole blood by solid-phase extraction using a C_{18} Sep-Pak cartridge and acetone as the eluting solvent. The prepared samples were then analyzed by high resolution gas chromatography-mass spectrometry-selected ion monitoring to identify and quantitate any observed citral. Validation of the method included linearity studies of citral response, absolute recovery studies, and estimates of precision, limits of detection, and limits of quantitation.

Patel and co-workers (93) compared a poly (styrene-divinyl benzene) sorbent that contained both C_{18} and sulfonic acid sites with a silica-based sorbent that also demonstrated mix-mode behavior. These sorbents are capable of both reverse-phase and cation-exchange interactions. Polymeric mixed-mode sorbents can be successfully used to isolate basic and neutral compounds from biological and pharmaceutical matrices by cation-exchange and reversed-phase mechanisms, respectively. As a result of the sorbent's reversed-phase and cation-exchange functions, selectivity for samples in complex matrices can be improved. Furthermore, polymeric mixed-mode sorbents have much higher reversed-phase and cation-exchange capacities than silica-based mix-

mode sorbents. The polymer-based sorbents are also stable from pH 0 to pH 14 and are suitable for isolating organic amines with high pK_a values by cation-exchange chromatography.

Mills, Thurman and Pedersen (94) compared silica- and styrene-divinylbenzene-based mix-mode resins that contain C_8, C_{18} and sulphonated cation-exchange groups for their efficiency in isolation of neutral triazine compounds from water and of the basic drug, benzoylecgonine, from urine. The triazine compounds were isolated by a combination of Van der Waals and hydrogen-bonding interactions, and benzoylecgonine was isolated by Van de Waals interactions and cation exchange. All analytes were eluted with a polar organic solvent containing 2% ammonium hydroxide. Larger recoveries (95%) were achieved on copolymerized mixed-mode resins where C_{18} and sulfonic acid are in closer proximity than on "blended" mixed-mode resins (60-70% recovery).

Hsu and Walters (95) studied the effect of extraction recoveries during solid-phase extraction by the volume or type of matrix applied to the column. Using as examples the extration of four basic drugs from biological matrices using cyano and octadecyl solid-phase extraction columns, it is shown that the recovery from 1 mL of plasma can be good while the recovery from water or diluted plasma is poor. Control of sample pH was found to increase recoveries from the cyano column by improving adsorption during sample application. Addition of detergent was found to enhance recoveries from the octadecyl column by moderating retention and allowing the drugs to be eluted more easily.

The SPE technique is also applied to food samples (96-97).

Muccio and co-workers (96) developed a multi-cartridge system in a single step for the extraction and clean-up of organophosphate (OP) pesticide residues from oils and fatty extracts. A solution in hexane containing up to 1.8 g of lipidic material was loaded on to an Extrelut-3 column to which a silica-gel cartridge and a C_{18} silica cartridge have been connected in series. The OP pesticide residues were eluted with 15 mL of acetonitrile. Carry-over of fatty material was in the range 2-5 mg per 1.8 g of different oils, which made the final solution amenable to capillary gas chromatography. Recoveries of 23 OP pesticides were in the range 82-111%. The whole procedure took ca. 20 min. and compared favourably with current procedures.

Widmer (97) improved a method for quantitation of limonin in citrus juice based on the use of C_{18} Sep-Pak cartridge. Limonin was eluted with aqueous 70% methanol and the eluate was filtered and analyzed by reversed-phase HPLC on a column of Supelco CN and detection was at 214 nm. Citrus juices were also extracted with chloroform before analysis by normal-phase HPLC on a similar analytical column. Results by the two methods compared well and no interference was observed in the reverse-phase method. Use of the solid-phase extraction column removed contaminating constituents from the citrus extracts. The coefficient of variation (n=8) ranged from 1.2 to 11.8%; recoveries of 0.4 to 19.8 ppm of limonin were 95 to 109%.

Automated sample preparation using solid phase extraction was published (73, 98).

Yago (98) described the Varian AASP (Advanced Automated Sample Processor) sample preparation system. The system comprises five segments such as cassette processing, preinjection purge, isolate elution (injection), valve reset and after-injection purge that was discussed in detail.

Majors (73) discussed the steps in the method development for the conversion of a manual solid phase extraction to an automated method on the AASP. Differences in the two approaches were explained. Conversion to such automated procedure is warrented when large numbers of samples are encountered. In addition to a material and labor savings, other advantages of the automated procedure are a lower sample mass and volume requirement, improved accuracy and precision, and an increase in sample throughput.

The detail and application of SPE disks were published (63, 99-100).

Markell, Hagen and Bunnelle (63) described the type of SPE disks and compare the cartridge and disk formats. Three basic disk configurations may be used in SPE : packing-impregnated PTFE, packing-impregnated polyvinyl chloride and derivatized membranes. They also present experimental techniques that can be used to optimize the application of SPE disks and examples of environmental, biochemical, and clinical applications.

Mc Donnell , Rosenfeld , and Rais-Firouz (100) conducted investigation of sample preparation for natural waters using the

Empore disk. The Empore disk is a new solid-phase sample preparation technology which was developed for rapid isolation of organic contaminants from aqueous matrix. In order to increase the volume of water that could be prepared, it was found that in-line or off-line filtration prior to the extraction step was required. The appropriate filters were identified.

1.3 Hypothesis

The solid phase extraction is considered to be an attractive technique for quantitative analysis of trace amounts of PEs in water. The reason is that this technique has a number of advantages over other sample preparation techniques. For instance, it has lower solvent, reagent, and apparatus costs; greater recoveries as a result of fewer sample transfers, minimal evaporation, and superior selectivity toward compounds of interest; and greater accuracy because there is less cross-contamination. The method is faster because it has fewer operational steps, and it can use batch processing; it features a wide choice of stationary phases available for excellent selectivity; and it prolongs column life time because particulate matter and strongly retained compounds can be removed (61).

SPE is based on liquid chromatographic mechanism. The organic matter originally dissolved in water is partitioned between the sorbent and the water according to the partial distribution coefficients. The factors affecting retention and elution process can be optimized to secure the effeciency and the reproducibility of the entire solid phase extraction method. The various parameters which

affect the efficiency such as concentration of the sample, pH of extracted sample, sorbent mass, and the type and volumes of elution solvent have been studied in this work. The suitable conditions have been applied to determine the PEs (DMP, DEP, DBP, BBP, DEHP, DDP) in real water samples.

1.4 The Purpose of the Study

Solid phase extraction method was developed for determination of some PEs i.e., DMP, DEP, DBP, BBP, DEHP and DOP. This work aimed at the studies of various parameters affecting the percent recovery of the PEs and the determination of the optimum conditions of the method. The various effects are:

1. The concentration of sample i.e., 0.20 and 1.00 ppm
2. The pH of extracted sample i.e, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0
3. C_{18} bulk packing mass i.e., 100, 200, 300, 400, and 500 mg.
4. The eluting solvent i.e., ethyl acetate, methylene chloride totuene, hexane and isooctane
5. The volumes of eluting solvent i.e., 1.0, 2.0, 3.0, 4.0 and 5.0 mL.

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

The procedure concerned with the preconcentration of PEs by

passing the large volume of sample (250.0 mL) through the conditioned C_{18} SPE cartridge. The PEs was then desorbed by elution with the small volume of proper solvent (5 mL). Therefore, the concentration of PEs originally presence in a trace amount of PE_S in water could be increased up to fifty times. The PEs in this eluate was separated by gas chromatography (GC) equipped with flame ionization detection.