



CHAPTER I

GENERAL INTRODUCTION

1.1 Problem Definition

Heavy metal is described as a metal having a specific gravity greater than four (4), such as cadmium, lead, copper, silver, nickel, zinc etc. Even though some heavy metals, e.g., cadmium, lead and mercury are highly toxic and cumulative poison to human beings as well as being recognised as an environmental hazard, the quantities of these metals as sustaining in the nature and the ration of their cycles in the ordinary condition lead slightly harm to lives and environment. The growth of agricultural and industrial technology cause substantial use of large quantities of some heavy metals. This usage extensively contaminates environment for example cadmium is reported to be as an irritant to the respiratory tract. Prolonged exposure can cause anosmia and a yellow stain that gradually appear on the necks and the teeth. Nevertheless cadmium and cadmium compounds are widely used as a protective coating for iron, steel and copper by the reason that it is highly corrosion resistant. Cadmium is also utilized in electrodes of alkaline storage batteries, and as a neutron absorbed in nuclear reactors. Various compounds are used as insecticides, catalysts, pigments, paints glass and photographic industry.

Lead, the second heavy metal that is toxic to lives, its poisoning can cause severe mental retardation or death. It is now known that lead interferes with the blood-forming process, kidney function and the neurological process. It also causes headache, aching bones and muscles, fatigues and decreases physical fitness. Most lead contamination starts out as airborne pollution and is distributed by atmospheric transport, however; it eventually settles onto the land and water. Food crops grown on lead-contaminated soil absorb some lead, public water supplies show an increase in lead content. Lead is released into the environment from natural, such as volcanic dust, causes also from human industrial activity. The industrial activity that adds lead is iron smelting, lead smelting, zinc smelting, copper smelting and coal burning. The chief source of lead contamination is lead alkyls used in gasoline. Metallic lead is used for lining tanks, piping and other equipment where pliability and corrosion resistance are required such as in the chemical industry in handling corrosive gases and in petroleum refining.

Nickel is the third toxic heavy metal. The route by which most people in the general population receive the largest portion of daily nickel intake is through food. It is reported that vegetables are contaminated by nickel at the higher rate than animal product. Skin sensitization is the most commonly seen toxic reaction to nickel and nickel compounds and is seen frequently in the general population. Nickel and its compounds are also irritants to the conjunctiva of the eye and the mucous membrane of the upper respiratory tract. Elemental nickel salts are probably carcinogenic, producing and increased

incidence of cancer of the lung and nasal passages. Effects on the heart muscle, brain, liver and kidney have been seen in animal studies. Elemental nickel is used in electroplating, casting operations for machine parts and in coinage in the manufacture of acid-resisting, surgical and dental instruments, and nickel-cadmium batteries. It is used as a catalyst in the hydrogenation of fats, oils and other chemicals, in synthetic coal oil production, and as an intermediate in the synthesis of acrylic esters for plastics.

Copper is another heavy metal. The routes of entry of copper to lives are by inhalation of dust or fume, ingestion or skin or eye contact. Industrial exposure too copper occurs chiefly from fumes generated in welding copper-containing metals. The fumes and dust cause irritation of the upper respiratory tract, metallic taste in the mouth, and nausea. Inhalation of dusts fumes and mists of copper salts may cause congestion of nasal mucous membranes. Copper salts act as irritants to the intact skin causing itching, erythema and dermatitis. In the eyes, copper salts may cause conjunctivitis and even ulceration and turbidity of the cornea. Metallic copper is an excellent conductor of eletricity and is widely used in the electrical industry in all gauges of wire for circuitry, and coils. It is made into castings, tubing and wire and is used in water and gas piping, roofing, materials, cooking utensils and coinage. Copper compounds are used as insecticides, algicides, mordants, pigments, and catalysts.

Silver is also classified as a heavy metal. Silver is extremely cumulative once they enter body tissues, and very little is excreted. The route by which most people receive silver intake is by inhalation of fumes or dust, ingestion of solutions or dust, or eye and skin contact. The only local effect from metallic silver derives from the implant of small particles in the skin of the workmen. Silver nitrate dust and solutions are highly corrosive to the skin, eyes and intestinal tract. The dust of silver nitrate may cause local irritation of the skin, burns of the conjunctiva and blindness. The dust is also deposited in the lungs and may be regarded as a form of pneumoconiosis although it carries no hazard of fibrosis. Silver alloyed with copper, aluminum, cadmium, lead, or antimony is used in the manufacture of silverware, jewelry, ornaments, coins, scientific instruments, automobile bearings and grids in storage batteries. Silver is used in the application of metallic films on glass and ceramics to increase corrosion resistance to sulfuric acid, in photographic films, plates and paper, as a bactericide for sterilizing water, fruit juices, and vinegar. Silver compounds are used in photography silver plating, inks, dyes, coloring glass and porcelain, and in the manufacture of mirrors.

The permissible concentrations of these heavy metals in water at the maximum contaminant levels established by the United State Environmental Protection Agency (U.S. EPA) are silver, cadmium, copper, lead, and nickel of 50, 10, 1,000, 50, 13.4 $\mu\text{g/L}$, respectively.

For water analysis, atomic absorption is considered to be a good method of analysis. The object of analysis is to determine trace priority metals pollutants (i.e., cadmium, lead, nickel, copper, and silver). A direct analysis of water of low hardness usually allows the elements (Fe, Cu, Ni, Co, Mo, Zn, Pb, Sr, Li) to be determined at a level of 50-100 $\mu\text{g/L}$. However this detection limits of atomic absorption spectrometer are not always sufficient, therefore ; enrichment methods are employed in this study.

1.2 Literature Review

For determination of trace amounts of heavy metals in water, a preconcentration step prior to atomic absorption spectrometric (AAS) analysis is inevitably required. Conventional, liquid/liquid extraction or solvent extraction is mostly popular used for preconcentration technique. This method is useful for compounds that are soluble in organic solvent and can be selectively extracted. Soluble derivatives may also be prepared for compounds that are soluble in organic solvent if reaction is quantitative and does not give multiple products. Ionizable compounds can often be extracted into organic solvent in the presence of a counter-ion as ion-pairs or at the isoelectric point by pH adjustment (2). To convert a metal ion in aqueous solution into an extractable species, the charge has to be neutralized and waters of hydrating have to be displaced. Qualitatively, it is to be expected that if metal ions are effectively surrounded by hydrophobic ligands that are able to bring about charge neutralization and occupy all the positions in the coordination sphere

of the metal ion, distribution will strongly favor organic solvent (3). The most common methods are based on extraction of metal complexes formed with dithiocarbamates in a suitable solvent. If a multielemental analysis is attempted will be made to extract all of the trace elements, except the major matrix elements. The most common chemical reagents are sodium dithiocarbamate, ammonium pyrrolidine dithiocarbamate (APDC) and hydroxy-8-quinoline. The sample solution is buffered to a suitable pH and agitated with water immiscible solvent, the most common of which are methylisobutylketone (MIBK), ethylacetate, diisobutylketone and n-butylether. The solvent must be compatible with the flame combustion (4). The determination of trace amounts of cobalt, nickel, copper, zinc, cadmium and lead in water, which chelation (APDC) and extraction (MIBK) are used, are significantly published in the Report of the International Organization for Standardization (ISO) (5). ISO recommends that for the preconcentration method, the samples of water to determine the trace amounts of heavy metals should be as showed in Table 1.1.

Table 1.1 The concentrations of the elements that can be determined according to ISO 8288-1986(E).

Element to be determined	Range of determination $\mu\text{g/L}$
Cobalt	1 to 200
Nickel	1 to 200
Copper	1 to 200
Zinc	0.5 to 50
Cadmium	0.5 to 50
Lead	5 to 200

The popular liquid/liquid extraction has had many practical and technical limitations, such as: requirements for large volume of immiscible solvent; involved sample handling, which is labor-intensive and ultimately can affect recovery and precision; and sample loss that can occur due to emulsion formation and degradation during evaporation.

In mid-1970s, a simple technique was introduced and it is known as solid-phase extraction (SPE). This technique is used to isolate and preconcentrate. SPE is one of the fastest growing methods. These popular uses are sample clean up, sample concentration and matrix removal. The principles and methods of solid-phase extraction are significantly published (2,6-16).

Bidlingmeyer (17) studied flow rate effects and recovery effect on SPE cartridge. To demonstrate this effect, riboflavin was trace-enriched from 10 ml of an aqueous solution.

SPE is a method to be used in environmental, petrochemical, pharmaceutical, food & cosmetic, biological and clinical samples (18-20).

The study of SPE in environmental branch has been reported as follows:

Lopez-Avila and co-workers (21) used disposable cartridges containing 1 g of Florisil for cleanup of extracts obtained from various environmental matrices. Elution patterns and recoveries are determined for 22 chlorinated hydrocarbons and 16 phthalate esters in the presence of interferences such as corn oils, diesel hydrocarbons, organochlorine pesticides, and chlorinated phenols. Hexane, hexane/diethylether(1:1), hexane/acetone(9:1), and various combinations of hexane/methylene chloride are used as eluents.

Werkhoven-Goewie and co-workers (22) studied on-line trace enrichment of moderately polar compounds on short precolumn prior to their separation by means of high performance liquid chromatography. In order to correlate chromatography retention data with sorption behavior from aqueous solutions in preconcentration techniques, several octadecylalkyl-modified silica and different types of carbon materials were selected as sorbents, and a series of chlorophenols was used as model compounds. Special attention was devoted to the

particle characteristics of the sorbent materials. From the experimental data a method has been derived for prediction of the sorption of a compound from a purely aqueous solution onto C₁₈ modified-silica sorbent base on the retention observed on that material in a single reversed-phase system using an isocratic organic/aqueous mobile phase. As an application, the trace enrichment and HPLC separation of highly chlorinated phenols from spiked water samples are described.

Ozretich and Schroeder (23) developed an extraction procedure utilizing sonication with acetonitrile and cleanup using aminopropyl and/or C-18 bonded-phase column to prepare marine sediments and animal tissues for priority organic pollutant analysis. Recoveries from standards reference and interlaboratory comparison sediments and tissue preparations compared favorably to established mean values. The recovery efficiencies of the procedures were determined by spiking marine sediments and a marine animal-tissue homogenate. Mean recoveries of 22 priority organic pollutants from the sediments ranged from 0 % to 84 % with a median recovery of 71% and an average percent relative standard derivation (% RSD) of 9%. Mean recoveries of 13 priority organic pollutants from the tissue homogenate ranged from 7 % to 76 % with a median recovery of 64 % and an average % RSD of 5%. They also discussed about the effects of sediment type and storage method on the spike recoveries.

Wells and Michael (24) illustrated the successful application of solid-phase extraction to aqueous environmental samples for trace enrichment of herbicide residues. Because retention and elution are

confounded during solid-phase extraction method development, it is difficult to optimize one before the other. They are also discussed about a plan for developing single-residue analytical protocols and illustrated the influence of pH, sample concentration and volume, sorbent mass, and solvent strength and volume on the removal of trace organic from water.

The usage of solid-phase extraction for determination of pesticides in water is widely studied.

Brooks and co-workers (25) presented a method for the simultaneous analysis of the pesticides chlorpyrifos, isofenphos, carbaryl, iprodione, and triadimefon in groundwater. The method involves the extraction of the pesticides onto C₁₈ columns and then elution with methylene chloride. After the solvent exchange to hexane, the extracts are analyzed by gas chromatography using nitrogen-phosphorus detection. Recoveries average higher than 90 % with a detection limit of 1 ppb for carbaryl, iprodione, and triadimefon, and 0.1 ppb for chlorpyrifos and isofenphos.

Vinuesa and co-workers (26) studied about the extraction and enrichment of organophosphorus pesticides based on the use of Sep-Pak C₁₈ cartridges. Ten of organophosphorus pesticides was studied about the influence of the elution solvent, pH, salinity and volume of water filtered. 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.

Johnson, Fendinger and Plimmer (27) developed a multiresidue analysis for trifluralin, simazine, atrazine, propazine, diazinon, parathion-methyl, alachlor, malathion, parathion, chlorpyrifos, pendimethalin, methidathion, and DEF in water that utilizes liquid-solid extraction with octadecyl-bonded silica cartridges (C₁₈ BSCs). And then it was followed by gas chromatography/mass spectrometric analysis. Recoveries of most pesticides were greater than 80 % with C₁₈ BSCs from fortified water at concentration levels from about 1 to 500 ppb. Recoveries with C₁₈BSCs, from an optically adjusted humic acid solution (10 ppm dissolved organic carbon) made to simulate a natural water with a high dissolved organic content, ranged from 29 to 153 % and in general were lower than recoveries obtained from pure water. ¹⁴C-labeled diazinon and parathion were recovered from the humic acid solution at levels of 57 and 68 %, respectively, with C₁₈ BSCs; the remainder of the labeled pesticides was found in the cartridge eluents. Partition coefficients with humic acid were calculated based on recovery of ¹⁴C-labeled pesticides from the C₁₈ BSCs.

Marble and Delfino (28) reported the solid-phase extraction technique as applied to cleanup and analysis of organochlorine and organophosphorus pesticides present in sediment and fish tissue. They evaluated several experimental variables, including the selection of an optimum SPE column packing material; the optimization of sample extract volume, column elution solvent, and elution solvent volume; optimization of the extraction technique; sample matrix; sample storage time and temperature; and comparison of pesticide recoveries from fish and sediment using the same extraction method.

Loconto (29-30) studied of the feasibility of using a multimodal, multistep SPE approach for isolation and recovery of priority pollutants from water and soil. He introduced the concept of propagation error between the extracted sample (which contains the analytes whose recoveries are to be measured) and the control, or reference sample. Taking this error into account increases the accuracy of percent recoveries and results in relative standard deviations (RSDs) that are somewhat larger but that more correctly estimate the variation in the mean percent recovery for replicate solid-phase extractions. Although this method works when the sample matrix contains no measurable amount of the target analyte before spiking, it is not as useful when the matrix contains a measurable background concentration of the analyte.

Sherma, Dryer and Bouvard (31) 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.

Junk and Richard (32) used the octadecyl (C-18) bonded to porous silica for the solid-phase extraction of organic compounds from water. Excellent performance was deduced from average recovery of > 85% for pesticides and polycyclic organic materials present in contrived water samples at 1-10 ng/mL. Extraction results showed effective performance of the SPE when 1-100 ml of water was passed through small columns containing 40-100 mg of 40 μm C₁₈ bonded porous silica at flow rates as high as 250 bed volumes/min. The adsorbed compounds were removed by collecting 60-100 μL of either ethyl acetate or benzene

evaluate. The unique feature of this research is the combination of small water volumes, fast flow rates, small columns and small eluate volumes that obviate solvent removals prior to GC analysis, while still retaining all the other advantageous features of SPE. Excellent performance was also confirmed in tests of environmental waters where the results based on SPE agreed with those based on accepted classical extractions.

Rostad, Pereira and Ratcliff (33) presented a procedure for isolation of hazardous organic compounds from water for GC/MS analysis and applied to creosote- and pentachlorophenol- contaminated groundwater resulting from wood treatment processes. This simple procedure involved passing a 50-100 mL sample through a bonded-phase extraction column, eluting the trapped organic compounds from the column with 2-4 mL of solvent and evaporating the sample to 100 μ L with a stream of dry nitrogen, after which the sample was ready for GC/MS analysis. Representative compounds indicative of creosote-contamination were used for recovery and precision studies from the cyclohexyl-bonded phase. Recovery of these compounds from n-octyl-, n-octadecyl-, cyclohexyl- and phenyl- bonded phases was compared. The bonded phase that exhibited the best recovery and least bias toward acidic and basic compounds was the n-octadecyl phase. Detailed compound identification is given for compounds isolated from creosote- and pentachlorophenol- contaminated groundwater using the cyclohexyl-bonded phase.

Junk, Avery and Richard (34) characterized procedural blanks from commercial solid-phase extraction cartridges. Alkanes, alkenes, plasticizers, and antioxidants were identified as possible interference compounds by extracting components of the cartridges and by analyzing these extracts by GC/MS. Most of the compounds identified in the extracts were also observed in the procedural blanks from use of the SPE cartridges.

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

Marko, Šoltés, and Radová (35) studied polar interactions between basic drugs (pentacaine, propranolol, and stobadin) and C₁₈ silanized silica along with hydrophobic interactions by using elution profiles of the drugs obtained with six organic solvents. The elution ability of the liquids towards basic drug depends positively on their proton acceptor properties and the negatively on their dipole interaction properties. Addition of triethylamine, a strong proton acceptor, to the liquids improves their elution ability, resulting in practically total recovery in all instances. The shapes of the elution profiles indicate the presence of multiple types of interactions between C₁₈ silica sorbent and basic drugs, ranging from those that originate from hydrophobic interactions to those originating from solute-sorbent ionic forces.

Patel and co-workers (36) compared a poly (styrene-divinylbenzene) sorbent that contained both C₁₈ and sulfonic acid sites with a silica-based sorbent that also demonstrated mixed-mode behavior.

Valerophenone was used as a test probe for reversed-phase interaction, and phenylpropanolamine was used to measure cation-exchange interactions. Applications include the isolation of amphetamines from commercial cough suppressants by cation-exchange chromatography; the isolation of barbiturates from urine by reversed-phase chromatography; and the preconcentration of triazine herbicides from water by cation-exchange chromatography. Pheniramine was used to test for reusability of the polymeric sorbent. Polymeric mixed-mode sorbents have much higher reversed-phase and cation exchange capacities than silica-based mixed-mode sorbents. The polymer-based sorbents are also stable from pH 0 to 14 and are suitable for isolating organic amines with high pKa values by cation-exchange chromatography.

Patel and co-workers (37) studied the retention and elution of organic acids commonly found in the environment using simple pH adjustments. Examples of isolation of organic acids from biological and environmental samples are shown. Data on reusability of Polysorb MP-2 polymer for an environmental application are also presented.

SPE is also applied to inorganic ion in water (18-19,38-39).

Bagchi and Haddad (38) examined the contamination sources which is resulting from the releasing of ions by the cartridge or filter, or loss of sample component through adsorption effect in the cleanup of samples for inorganic ion analysis. They examined these aspects by using a popular brand of cartridge and filter.

Saari-Nordhaus, Anderson and Henderson (39) reported five different Maxi-clean IC SPE cartridges to eliminate the matrix interference problems. These cartridges are used for cleanup the sample prior to analysis by ion chromatography. These cartridges may also be used for preconcentration of anions or cations.

Donat, Statham and Bruland (40) presented results from an investigation using a C₁₈ solid-phase extraction method for isolating dissolved metal-organic complexes from seawater. The vertical distribution of dissolved organically complexed copper is isolated by this technique in the intermediate and deep waters of the central North Pacific is similar to that observed in North Atlantic. Evidence from voltammetric studies suggests that the C₁₈ SPE technique may isolate the major portion of the organically complexed copper from deep waters. They also studied the fractions of total dissolved cadmium, iron, manganese, and nickel isolated by the C₁₈ SPE technique.

The automate sample preparation using solid-phase extraction was published (41-43).

Yago (42) described the Varian AASP (Advanced Automated Sample Processor) sample preparation system. This system was discussed in detail in the five sections : cassette processing, preinjection purge, isolation elution (injection), valve reset and after injection purge.

Majors, Dimson and Brocato (43) discussed the steps in 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.

Markell, Hagen and Bunnelle (44) described a new technology devices of SPE, which is called SPE disk. They compared the cartridge and disk formats. They also presented experimental techniques that can be used to optimize the application of SPE disks, commercial products based on this new configuration, and examples of environmental, biochemical, and clinical applications.

1.3 Hypothesis

4-(2-Pyridylazo) resorcinol (PAR) is well-known as a complexing agent that usually forms complexes with ligand:metal ion ratio of 1:1 or 2:1. Most importantly, PAR is an unselective complexing agent which forms water soluble complexes with the vast majority of transition metals. Also, the formation constants of most PAR-complexes are high, suggesting that they would be stable under reversed-phase liquid chromatography (RPLC). The study of trace amounts of some heavy metals in water by means of solid-phase extraction is recognized to be a considerably attractive technique for quantitative analysis. It is supposed that the metal of interest (Ag, Cd, Cu, Ni, Pb) can be formed with PAR to be metal-PAR complexes which can be trapped on C₁₈ SPE cartridge and can be eluted with the suitable solvents (organic solvent or acid solution). In this study, nitric acid solution was used as an eluent. It is assumed that the large volumes of trace amounts of heavy metals which are formed metal-complexes (100.0 mL) can be concentrated on C₁₈ SPE cartridge in the retention step and can be eluted by using the small volumes of nitric acid solution (5 mL) in the elution step.

Therefore, the trace amounts of metals in water can be increased the concentration upto twenty times. Hence, the extraction of metal-PAR complexes in water by using SPE seems possible. In this study, Cd-PAR complex and Cu-PAR complex have been employed as the models in order to :

1. evaluate the elution solvent volume and concentration
2. investigate the pH of sample extract
3. assess the flow rate on the step of retention and elution
4. measure the PAR concentration
5. examine of sorbent mass

The suitable conditions have been used for determination of Pb, Ni, and Ag as a single component in water and employed to determine five metals (Pb, Cd, Ni, Ag, Cu) as the mixture components of metals in water.

1.4 The Purpose of the Study

SPE has been developed to analyze trace amounts of some heavy metals such as; lead, cadmium, nickel, silver, and copper in water. The aim of the experiment is to study the effect of variables on the recovery of these metals. In addition, the accuracy and precision of this technique are also studied and evaluated prior to use it in the analysis of these metals in the real water samples. To achieve these objectives, Cd-PAR complex and Cu-PAR complex have been employed as the models for this study. Several experimental variables have been

evaluated :

1. the concentration of nitric acid solution i.e., 0.1, 0.5, 1.0, 2.0, and 4.0 M.
2. the volume of nitric acid solution i.e., 1.0, 2.0, 3.0, 4.0, and 5.0 mL.
3. the pH of extracted sample i.e., 5.0, 6.0, 7.0, 8.0, 9.0, 10.0.
4. the flow rate on the step of retention i.e., 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 mL/min.
5. the flow rate on the step of elution i.e., 0.1, 0.5, 1.5, 3.0, 6.0, and 9.0 mL/min.
6. the concentration of PAR higher than metal concentration i.e., 5, 10, 20, 50, and 100 fold.
7. C₁₈ bulk packing mass i.e., 100, 200, 300, 400, 500 mg.