Chapter 1

Introduction
1.1 Role of geocycle in the introduction of metals into our environment

Air, water, earth and life are strongly interconnected as shown in Figure 1.1, which constitute the major segments of the environment- atmosphere, hydrosphere, geosphere and biosphere. Biogeochemical cycles of matter that involve biological, chemical and geological processes and phenomena manifests the strong interaction among living organism and various spheres of the abiotic (nonliving) environment. Water and air are involved in weathering rocks, producing mineral formations and forming climate; which have profound effects on the geosphere and interchange matter and energy with it. Living systems (biosphere) which largely exist on the geosphere in turn have significant effects on it. However, for better or for worse, the environment in which human must live have been affected irreversibly by technology and industrial activities. Metals in the environment may be present in the different states as solid, liquid or gaseous state or in varying forms as individual elements, organic and inorganic compounds. The movement of metals between environmental reservoirs may or may not involve changes of state. Gaseous and particulate metals may be inhaled and solid and liquid (aqueous-phase) metals may be ingested or absorbed, thereby entering the biosphere [1-3].

Figure 1.1 Interaction of human with the environment

Heavy metals are included within the category of environmental toxins: “Materials which can harm the natural environment even at low concentration, through
their inherent toxicity and their tendency to accumulate in the food chain and/or have particularly low decomposition rates”. The redistribution of many toxic metals into the environment, caused by the gradual increase in industrial activity, has increased the possibility of human exposure. Among the various toxic elements, heavy metals like cadmium, lead, and mercury are especially prevalent in nature due to their high industrial use. These metals serve no biological function and their presence in tissues reflects contact of the organism with its environment. They are cumulative poison and are toxic even at low dose [4,5]. The indication of their importance relative to other potential hazards is their ranking by the U.S. Agency for Toxic Substances and Disease Registry, which lists all hazards present in the toxic waste sites according to their prevalence and severity of their toxicity. The first, second, third and sixth hazards on the list are heavy metals: lead, mercury, arsenic and cadmium, respectively [6]. The other metal ions that pose potential dangers to human lives include chromium, copper, zinc, nickel, cobalt, iron and manganese. Herein, sources and effects of these metal ions are discussed.

**Lead**

Lead is a highly toxic cumulative poison in humans and animals. Its cumulative poisoning effects are serious hematological damage, anaemia, kidney malfunctioning, brain damage etc. In humans, chronic lead poisoning is manifested by abnormalities such as encephalopathy, nervous irritability, kidney disease, altered heme synthesis and reproductive functions. Such poisoning is associated with low to intermediate levels of chronic exposure to lead. The principle risk to children from lead is interference with the normal development of their brains. A number of studies have found small but significant neuropsychological impairment in young children due to environmental lead absorbed either before or after birth. In particular, lead appears to have deleterious effects on children’s behavior and attentiveness, and possibly also on their IQs. [7].

Environmental contamination of lead is widespread; the main anthropogenic source of this element is burning of leaded gasoline. Lead is used as a construction material for equipment used in sulfuric acid manufacture, petrol refining, halogenation, sulfonation, extraction and condensation. It is used in storage batteries, alloys, solder, ceramics and plastics. It is also used in the manufacture of pigments, tetraethyl lead and other lead compounds, in ammunition, and for atomic radiation and x-ray protection. Lead is used in aircraft manufacture, building construction materials (alloyed with copper, zinc, magnesium, manganese and silicon), insulated cables and wiring, household utensils, laboratory equipment, packaging materials, reflectors, paper industry, printing
inks, glass industry, water purification and waterproofing in the textile industry. The primary sources, for low to intermediate levels of chronic exposure to lead, are food, water and air. About 50% of lead is absorbed with inhalation of dusts, 10–15% absorbed orally, out of which 90% is distributed to bones [8]. In natural water its typical concentration lies between 2 and 10 ng mL$^{-1}$, whereas, the upper limit recommended by WHO is less than 10 ng mL$^{-1}$ [9].

**Copper**

Excess copper interferes with zinc, a mineral needed to make digestive enzymes [10]. Physical conditions associated with copper imbalance include arthritis, fatigue, adrenal burnout, insomnia, scoliosis, osteoporosis, heart disease, cancer, migraine headaches, seizures, fungal and bacterial infections including yeast infection, gum disease, tooth decay, skin and hair problems and female organ conditions including uterine fibroids, endometriosis and others. Mental and emotional disorders related to copper imbalance include depression, mood swings, fears, anxiety, phobias, panic attacks, violence, autism, schizophrenia, and attention deficit disorder [11]. Copper imbalance in children is associated with delayed development, attention deficit disorder, anti-social and hyperactive behavior, autism, learning difficulties and infections such as ear infections.

Copper is primarily used as a metal or an alloy (e.g., brass, bronze, gun metal). Copper sulfate is used as a fungicide, algacide and herbicide. Copper particulates are released into the atmosphere by windblown dust; volcanic eruptions; and anthropogenic sources, primarily copper smelters and ore processing facilities [12]. Copper particles in the atmosphere will settle out or be removed by precipitation, but can be resuspended into the atmosphere in the form of dust. Copper is released into waterways by natural weathering of soil and rocks, disturbances of soil, or anthropogenic sources (e.g., effluent from sewage treatment plants) [12]. Another source of copper is drinking water that remained in copper water pipes, or copper added to your water supply.

**Zinc**

Zinc is an essential trace element of great importance for humans, plants and animals. It plays an important role in several biochemical processes and its compounds have bactericidal activity. Zinc phosphide, which is used as an active ingredient in rodenticide, reacts with water and acid in the stomach to release phosphine gas which in turn causes cell toxicity with necrosis of the gastrointestinal tract. Oral zinc increases faecal excretion of copper and blocks the absorption of ingested minerals. In this case, a
series of complex zinc-copper relationship and metabolism resulted in the inhibition of copper absorption and increased faecal loss of copper through saliva, gastric juices and biliary secretions. Breathing large amounts of zinc (as dust or fumes) can cause a specific short-term disease called metal fume fever. Inhalation of fumes may result in sweet taste, throat dryness, cough, weakness, generalized aching, chills, fever, nausea and vomiting. Zinc chloride fumes have caused injury to mucous membranes and pale gray cyanosis. Ingestion of soluble salts may cause nausea, vomiting and purging [10,13,14].

Zinc has many commercial uses as coating to prevent rust, in dry cell batteries, and mixed with other metals to make alloys like brass and bronze. Zinc compounds are widely used in industry to make paint, rubber, dye, wood preservatives, and ointments [14]. Also used for galvanizing sheet iron; as ingredient of alloys such as bronze, brass, Babbitt metal, German silver, and special alloys for die-casting; as a protective coating for other metals to prevent corrosion, for electrical apparatus, especially dry cell batteries, household utensils, castings, printing plates, building materials, railroad car linings, automotive equipment; as reducer (in form of the powder) in the manufacture of indigo and other vat dyes, for deoxidizing bronze; extracting gold by the cyanide process, purifying fats for soaps; bleaching bone glue; manufacture of sodium hydrosulfite; as reagent in analytical chemistry, e.g. in the Marsh and Gutzeit test for arsenic; as a reducer in the determination of iron. Some zinc is released into the environment by natural processes, but most comes from activities of people like mining, steel production, coal burning, and burning of waste.

**Cadmium**

Cadmium may cause renal injuries and may interfere with the renal regulation of calcium and phosphate balance. It was seen that exposure to abnormal levels of cadmium can result in its accumulation in the renal cortex, which causes a series of adverse subclinical reactions such as hypercalciurium, renal stones and renal tubular dysfunction besides probable development of carcinogenic activity in organisms [15]. The toxicity of cadmium may involve its binding to key cellular sulfhydryl groups, its competition with other metals (zinc and selenium) for inclusion in metalloenzymes, and its competition with calcium for binding sites on regulatory proteins such as calmodulin. The lack of an effective elimination pathway is responsible for cadmium’s biologic half-life of 10-30 years. Chronic effects of cadmium exposure are dose-dependent and include anosmia, yellowing of teeth, emphysema, minor changes in liver function, microcytic hypochromic anemia unresponsive to iron therapy, renal tubular dysfunction characterized by
proteinuria and increased excretion of $\beta_2$-microglobulin and (with prolonged poisoning) osteomalacia leading to bone lesions and pseudo fractures [6].

Cadmium is an industrial waste or by-product, which has a great environmental concern. Cadmium is used in many industrial processes, such as a constituent of easily fusible alloys, soft solder, electroplating and deoxidizer in nickel plating, engraving processes, electrodes for vapor lamps, photoelectric cells, and nickel-cadmium storage batteries [16]. It's wide technological use in fertilizers, mining, pigments, as well as its delivering from oil and coal burning and residues incineration; bring about an extensive anthropogenic contamination of soil, air and water.

Nickel

Nickel is a moderately toxic element as compared with other transition metals. However, it is known that inhalation of nickel and its compounds can lead to serious problems, including respiratory system cancer. Moreover, nickel can cause a skin disorder known as nickel-eczema. The most common adverse health effect of nickel in humans is an allergic reaction. People can become sensitive to nickel when things containing it are in direct contact with the skin, when they eat nickel in food, drink it in water, or breathe dust containing it. Less frequently, allergic people have asthma attacks following exposure to nickel. Lung effects, including chronic bronchitis and reduced lung function, have been observed in workers who breathed large amounts of nickel. Headache, dizziness, shortness of breath, vomiting, and nausea are the initial symptoms of overexposure; the delayed effects (10 to 36 h) consist of chest pain, coughing, shortness of breath, bluish discoloration of the skin, and in severe cases, delirium, convulsions, and death. [10]. Long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation. High levels of Ni in the diet may be associated with an increased risk of thyroid problems, cancer, and heart disease [17,18]. Less frequently, allergic people have asthma attacks following exposure to nickel. Lung effects, including chronic bronchitis and reduced lung function, have been observed in workers who breathed large amounts of nickel.

Major sources of exposure are: tobacco smoke, auto exhaust, fertilizers, superphosphate, food processing, hydrogenated-fats-oils, industrial waste, stainless steel cookware, testing of nuclear devices, baking powder, combustion of fuel oil, dental work and bridges. Humans are exposed to it through breathing of air or smoking of tobacco containing nickel, eating of food containing nickel, as well as drinking of water.
contaminated with nickel and handling of coins and touching of other metals containing nickel [19].

**Arsenic**

Once arsenic is in the body, it binds to hemoglobin, plasma proteins, and leukocytes and is redistributed to the liver, kidney, lung, spleen, and intestines. Arsenic produces cellular damage through a variety of mechanisms. Arsenic binds to enzyme sulfhydryl groups and forms a stable ring, which deactivates the enzyme. The process of deactivating the enzyme causes widespread endothelial cell damage, vasodilation, and leakage of plasma. Massive transudation of fluid into the bowel lumen, mucosal vesicle formation, and tissue sloughing may result in large gastrointestinal fluid losses. Arsenic binds to dihydrolipoic acid, a pyruvate dehydrogenase cofactor, blocking the conversion of pyruvate to acetyl coenzyme A and inhibiting gluconeogenesis. Arsenic competes with phosphates for adenosine triphosphate, forming adenosine diphosphate monoarsine, causing the loss of high-energy bonds. In some forms, arsenic is caustic, exerting a direct toxic effect on blood vessels and large organs. Long-term exposure results in nerve damage and may lead to lung, skin, or liver cancer. Once inhaled, arsine gas combines with hemoglobin in RBCs, causing severe hemolysis and anemia. Patients develop hemoglobinuria and hematuria within several hours of exposure. One of the early warning signs of arsenic poisoning is a "pins and needles" sensation in hands and feet. Long-term oral exposure to inorganic arsenic can result in skin changes including darkening of the skin and the appearance of small "corns" or "warts" on the palms, soles, and torso. Arsenite (should be arsenate) (+5) undergoes biomethylation in the liver to the less toxic metabolites methylarsenic acid and dimethylarsenic acid; biomethylation can quickly become saturated, however, and the result is the deposition of increasing doses of inorganic arsenic in soft tissues [20].

Elevated arsenic (As) levels in the environment are attributable to both natural and anthropogenic sources, including geothermal discharges, industrial products and wastes, agricultural pesticides, wood preservatives and mine drainage. It is also used in drugs, war gases and as a homicidal and suicidal weapon. Other uses of arsenic compounds are in alloys, manufacturing of arsenic compounds (arsenic oxides) and certain glass. Copper and lead ores contain small amounts of arsenic. Arsenic is also a major ingredient of Fowler's solution and continues to be found in some folk remedies [14].
**Mercury**

Mercury was recognized as causing neuro-developmental disabilities including dyslexia, attention deficit hyperactivity disorder, intellectual retardation, and autism [21]. Inhalation of very high concentrations causes acute pulmonary edema and interstitial pneumonitis, which may be fatal. In non-fatal cases dyspnea and coughing may persist. Kidney effects may occur at exposure levels lower than those causing central nervous system effects. Also, mercury vapor may cause “Kawasaki” disease, which seems to be immunologically mediated and is similar to Pink disease. Mercury intoxication also causes reproductive effects. Contact dermatitis from mercury amalgam fillings and mercury sensitivity in dental students has been reported. Repeated or prolonged exposure to mercury vapor is highly toxic to the central nervous system [20]. Exposures to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, and developing fetus. Embryo toxicity and teratogenicity of organic mercury compounds have been reported in many test systems. Exposure to methyl mercury is worse for young children than for adults, because more of it passes into children’s brains where it interferes with normal development [22-24].

An organic mercury compound, namely methyl mercury, is produced mainly by small organisms in water and soil. Metallic mercury is used to produce chlorine gas and caustic soda and also in thermometers, amalgams (dental fillings), and batteries. Mercury salts are used in skin-lightening creams and as antiseptic creams and ointments. Mercury is used in scientific and electrical equipments, in the electrolytic production of chlorine and sodium hydroxide; and as a catalyst in polyurethane foam production [25]. Inorganic mercury (metallic mercury and inorganic mercury compounds) enters the air from mining ore deposits, burning coal and waste, and from manufacturing plants. It enters the water or soil from natural deposits, disposal of wastes, and the use of mercury-containing fungicides. Breathing of contaminated air or skin contact during use at workplace (dental, health services, chemical, and other industries that use mercury) represents occupational exposures. Inhalation of mercury vapor is the most important route of uptake of elemental mercury [26].

**Chromium**

Epidemiological studies have consistently shown that human exposure to Cr (VI) compounds is associated with a higher incidence of respiratory cancers [27]. Acute toxic effects occur when one breath very high levels of chromium (VI) in air. It can damage and irritate nose, lungs, stomach, and intestines. People who are allergic to chromium
may also have asthma attacks after breathing high levels of either chromium (VI) or (III). Long term exposures to high or moderate levels of chromium (VI) causes damage to the nose (bleeding, itching, and sores) and lungs and can increase risk of non-cancer lung diseases. Ingesting very large amounts of chromium can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death. Skin contact with liquids or solids containing chromium (VI) may lead to skin ulcers [11,13].

Stainless steel welding, particularly the manual metal arc welding method, may well be the most common source of occupational exposure to Cr(VI), given the fact that there are millions of stainless steel welders worldwide. Chromium is used in manufacturing chrome-steel or chrome-nickel-steel alloys (stainless steel) and other alloys, bricks in furnaces, and dyes and pigments, for greatly increasing resistance and durability of metals and chrome plating, leather tanning, and wood preserving. Handling or breathing sawdust from chromium treated wood, manufacturing, disposal of products or chemicals containing chromium, or fossil fuel burning release chromium to the air, soil, and underground water [28].

**Manganese**

Exposure to atmospheric Mn at high concentration is a risk factor in humans that can manifest as neuronal degeneration resembling Parkinson's disease (PD). Although the underlying mechanism of Mn and dopamine (DA) interaction-induced cell death remains unclear, however, Mn exposure alone to mesencephalic cells for 24h induced minimal apoptotic cell death [29]. Increased manganese intake impairs the activity of copper metallo-enzymes. Excess manganese interferes with the absorption of dietary iron. Long-term exposure to excess levels may result in iron-deficiency anemia. High manganese levels indicate problems with calcium and/or iron metabolism [11,13]. Symptoms of toxicity mimic those of Parkinson's disease (tremors, stiff muscles) and excessive manganese intake can cause hypertension in patients older than 40 [30]. Symptoms of increased manganese levels include psychiatric illnesses, mental confusion, impaired memory, loss of appetite, mask-like facial expression and monotonous voice, spastic gait and neurological problems. Manganese toxicity can cause kidney failure, hallucinations, as well as diseases of the central nervous system.

Uses of Mn include: (i) iron and steel production; (ii) manufacture of dry cell batteries; (iii) production of potassium permanganate and other Mn chemicals; (iv) oxidant in the production of hydroquinone; (v) manufacture of glass; (vi) textile bleaching; (vii) oxidizing agent for electrode coating in welding rods; (viii) matches and
fireworks; and (ix) tanning of leather [31]. Organic compounds of Mn are present in the fuel additive, methylecyclopentadienyl manganese tricarbonyl (MMT), fungicides (e.g., maneb and mancozeb), and in contrast agents used in magnetic resonance imaging. The primary anthropogenic sources of Mn in ambient air include emission of Mn from industrial sources such as ferroalloy production plants, iron and steel foundries, power plants, and coke ovens and re-entrainment of soils containing Mn [32]. Well water rich in manganese can be the cause of excessive manganese intake and can increase bacterial growth in water. Manganese poisoning has been found among workers in the battery manufacturing industry.

**Cobalt**

Cobalt metal particles, when inhaled in association with other agents such as metallic carbides (hard metals) or diamond dust, may produce an interstitial lung disease termed "hard metal disease" or "cobalt lung". Acute toxicity of cobalt may be observed as effects on the lungs, including asthma, pneumonia and wheezing, that have been found in workers who breathed high levels of cobalt in the air. The International Agency for Research on Cancer (IARC, USA) has determined that cobalt is a possible carcinogen to humans. Studies in animals have shown that cobalt causes cancer when placed directly into the muscle or under the skin [11,13,33].

Vast applications of cobalt in various arrays of products and processes such as its use in alloys, batteries, catalysts, pigments and coloring, make this element to be considered as an important metal in various industries [34]. Cobalt enters the environment from natural sources and from the burning of coal and oil. Workers may be exposed to cobalt in industries that process it or make products containing cobalt [35].

**Iron**

Excessive iron leads to tissue damage as a result of formation of free radicals [36]. Long term over consumption of iron may cause hemosiderosis, a condition characterized by large deposits of the iron storage protein hemosiderin in the liver and other tissues. Iron overload is most often diagnosed when tissue damage occurs, especially in iron-storing organs, such as the liver. Infections are likely to develop because bacteria thrive on iron rich blood. Ironically, some of the signs of iron overload are analogous to those of iron deficiency: fatigue, headache, irritability, and lowered work performance. Other common symptoms of iron overload include enlarged liver, skin pigmentation, lethargy, joint diseases, loss of body hair, amenorrhea, and impotence. Untreated hemochromatosis
aggravates the risks of diabetes, liver cancer, heart disease and arthritis. In cases of iron overload the natural storage and transport proteins are overwhelmed and the iron spills over into other tissues and organs, such as the muscle, spleen and liver [37], proving to be toxic [38].

Iron is included in the quality control of industrial and commercial products such as petroleum, alloys, foods, beverages etc [20]. Occasionally, iron pipes may also be a source of iron in water. Water percolating through soil and rock can dissolve minerals containing iron and manganese and hold them in solution.

1.2 Significance and characteristics of preconcentration

Determination of toxic metal ions in environmental samples, wastewater, various natural water bodies and biological fluids is necessary for environment monitoring, assessment of occupational and environmental exposure to toxic metals and its impact on the ecosystem.

The low concentrations of the metal ions and the strong interference of matrices present in association with it, in real samples, poses difficulty in its direct determination despite the availability of sophisticated instrumental methods, with excellent sensitivity and multielemental analysis capability [39-46]. A radical way to eliminate matrix effects is a preliminary separation of macro components by a relative, or absolute, preconcentration of trace metals.

Preconcentration can be of two types: absolute and relative preconcentration. **Absolute preconcentration** involves the transfer of trace elements from a large mass of sample into a small mass, e.g. by evaporation or by solvent extraction into a small volume of an organic phase.

**Relative preconcentration** involves at least partial separation of the components when their concentrations differ very much. Its main aim is often to exchange the matrix for a suitable collector (generally of smaller mass) to prevent its interference in the determination. In some cases, it is difficult to define a boundary between absolute and relative preconcentration. Relative preconcentration increases the mass ratio of trace elements to main components (the solvent is not considered as a major component in this case).

Depending on the purpose, trace elements can be concentrated selectively or in groups and either separation of the matrix or separation of the trace components can be used. Removal of the matrix is reasonable if used in combination with multi element
determination techniques, e.g. spectrochemical analysis, but only if the matrix is of simple composition. Matrix removal is used especially in analysis of high-purity metals. If the matrix contains several elements forming complex compounds (geological and biological materials), it is better to separate the trace elements. Sometimes, there is no need to remove the matrix completely; the process is then called "enrichment". However, it is usually more profitable to change to another matrix which better meets the demands of the subsequent determination, simplifies a calibration, etc. Several such collectors are suitable for determinations by different techniques. For instance, carbon powder can be analyzed by a spectrographic method or by flameless atomic-absorption spectrophotometry [47].

Some quantitative characteristic features used for description of preconcentration are listed in Table 1.1

<table>
<thead>
<tr>
<th>Table 1.1</th>
<th>Characteristic features of preconcentration</th>
</tr>
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<tbody>
<tr>
<td>Recovery (R)</td>
<td>( R = \frac{Q_T}{Q_{T^0}} ), where ( Q_T ) and ( Q_{T^0} ) are respectively the quantities of trace element in the concentrate and in the sample. It is usually expressed as a percentage.</td>
</tr>
<tr>
<td>Concentration coefficient (K)</td>
<td>( K = \frac{(Q_T/Q_M)}{(Q_{T^0}/Q_{M^0})} ), where ( Q_{M^0} ) and ( Q_M ) are respectively the amounts of matrix before and after preconcentration. If ( R = 100% ) then ( K = Q_{M^0}/Q_M ).</td>
</tr>
<tr>
<td>Separation coefficient (S)</td>
<td>( S = \frac{(Q_M/Q_T)}{(Q_{M^0}/Q_{T^0})} = 1/K )</td>
</tr>
<tr>
<td>Preconcentration factor</td>
<td>Maximum volume of sample / minimum eluent volume that gives quantitative recovery of analyte</td>
</tr>
<tr>
<td>Preconcentration limit (µg L(^{-1}))</td>
<td>Minimum concentration up to which preconcentration (maximum volume) is feasible.</td>
</tr>
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</table>

Of the analytical techniques for preconcentration and separation, SPE has been preferred over conventional solvent extraction and coprecipitation. The markedly lower quantity of reagents required, the fact that solid phase can be repeatedly used, higher concentration factors, and simplicity in handling and transfer are frequently quoted as advantages [48-51]. Sorption and ion exchange have been studied for different analytical applications [52–60] using various support materials. Many different methods are used for analytical preconcentration [61]. They can be classified according to the nature of the separations (chemical and physical methods) used and the number and nature of the phases involved in the separation process.
1.3 Solid-phase extraction (SPE) as a preconcentration method

It is a separation process that is used to remove solid or semi-solid compounds from a mixture of impurities based on their physical and chemical properties. Analytical laboratories use solid phase extraction to concentrate and purify samples for analysis. The separation ability of solid phase extraction is based on the preferential affinity of desired or undesired solutes in a liquid (mobile phase) for a solid (stationary phase) through which the sample is passed. Impurities in the sample are either washed away while the analyte of interest is retained on the stationary phase, or vice-versa. Analytes that are retained on the stationary phase can then be eluted from the solid phase extraction cartridge with the appropriate solvent. The preconcentration methods utilizing solid sorbents are considered to be superior than the liquid-liquid extraction in terms of simplicity, rapidity, and the ability to obtain a high enrichment factor. Particularly, solid phase extraction has been demonstrated in various procedures to be a very effective preconcentration technique in combination with atomic absorption spectrometry. The main advantage of this technique is the possibility of using a relatively simple detection system with flame atomization instead of a flameless technique, which require more expensive equipment and are usually much more sensitive to interferences from macrocomponents of various natural matrices [39].

1.3.1 History and development of SPE

The history of using solid phases to isolate drugs from biological samples dates back to 1923 when permutite was used [77] and then subsequently silicic acid [78] for extracting adrenaline. In the 1950s, alumina columns were used [79] for extracting
adrenaline and noradrenaline from blood samples. Then successful extraction of catechol bases, adrenaline and histamine from crude extracts of glands, using Amberlite IRC-50 [80] was performed. Later a method was developed to extract catechol amines from tissues using cation exchange resin Dowex 50 [81]. By the mid 1960s more complex samples were being tried. A method using cation exchange paper chromatography [82], which was qualitative in nature, was developed to detect a number of narcotics, tranquilizers, amphetamines and barbiturates from urine samples but the method was more. In the 1970s, the stress was to develop techniques which were more sensitive. Amberlite XAD-2 resin columns were used to quantify narcotic analgesics from urine and could determine as low as 0.6 mg mL of urine [83]. It is noted that, in most of the above cases, the principle of ion exchange was used to separate drugs from biological samples. Subsequently, adsorption phenomenon was tried on charcoal to concentrate a number of drugs (barbiturate, glutathimide, ethchlorvynol, amphetamine, phenothiazine, quinine, morphine, cocaine and its metabolites) from urine and achieved an average detection limit of 1 mg mL\(^{-1}\) of urine [84]. By the late 1970s, HPLC technology had made rapid progress and one of the major developments was the use of silica and bonded silica as the stationary phase. Waters Associates and Analytichem International were among the first to develop the concept of using sorbents, similar to those used in HPLC, packed in miniature columns, to isolate drugs and chemicals from other interfering impurities of test samples. Specifically, small disposable cartridges containing silica, bonded silica and other phases were developed for commercial use and these were called SPE columns. C18 SepPak@ columns (Waters Associates) were evaluated for the extraction of tricyclic antidepressants from biological samples [85].

Solid-phase extraction is now emerging as a very important sample preparation technique. It is preferred over other traditional procedures, such as liquid-liquid extraction (LLE), mainly because it is more efficient and much less time-consuming.

1.3.2 Basic principles

The principle of SPE is similar to that of liquid-liquid extraction (LLE), involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix) and a solid (sorbent) phase. This sample treatment technique enables the concentration and purification of analytes from solution by sorption on a solid sorbent.

The basic approach involves passing the liquid sample through a column, a cartridge, a tube or a disk containing an adsorbent that retains the analytes. After the
entire sample has been passed through the sorbent, retained analytes are subsequently recovered upon elution with an appropriate solvent. The first experimental applications of SPE started fifty years ago [86,87].

1.3.3 Technique

An SPE method always consists of three to four successive steps, as illustrated in Figure 1.2.

**STEP 1:** The solid sorbent should be conditioned using an appropriate solvent, followed by the same solvent as the sample solvent.

**Significance:**

- This step is crucial, as it enables the wetting of the packing material and the solvation of the functional groups.
- In addition, it removes possible impurities initially contained in the sorbent or the packaging.
- Also, this step removes the air present in the column and fills the void volume with solvent.
- The nature of the conditioning solvent depends on the nature of the solid sorbent.
- Care must be taken not to allow the solid sorbent to dry between the conditioning and the sample treatment steps, otherwise the analytes will not be efficiently retained and poor recoveries will be obtained.
- If the sorbent dries for more than several minutes, it must be reconditioned.

**STEP 2:** The second step is the percolation of the sample through the solid sorbent. Depending on the system used, volumes can range from 1 mL to 1000 mL. The
sample may be applied to the column by gravity, pumping, aspirated by vacuum or by an automated system.

**Significance:**

- The sample flow-rate through the sorbent should be low enough to enable efficient retention of the analytes, and high enough to avoid excessive duration.
- During this step, the analytes are concentrated on the sorbent.
- Even though matrix components may also be retained by the solid sorbent, some of them pass through, thus enabling some purification (matrix separation) of the sample.

**STEP 3:** The third step (which is optional) may be the washing of the solid sorbent with an appropriate solvent, having low elution strength, to eliminate matrix components that have been retained by the solid sorbent, without displacing the analytes.

**Significance:**

- A drying step may also be advisable, especially for aqueous matrices, to remove traces of water from the solid sorbent.
- This will eliminate the presence of water in the final extract, which, in some cases, may hinder the subsequent concentration of the extract and or the analysis.

**STEP 4:** The final step consists in the elution of the analytes of interest by an appropriate solvent, without removing retained matrix components.

**Significance:**

- The solvent volume should be adjusted so that quantitative recovery of the analyte is achieved with subsequent low dilution.
- In addition, the flow-rate should be correctly adjusted to ensure efficient elution.
- It is often recommended that the solvent volume be fractionated into two aliquots, and before the elution to let the solvent soak the solid sorbent.

**1.3.4 Mechanism of retention**

Adsorption of trace elements on the solid sorbent is required for preconcentration (Figure 1.3). The mechanism of retention depends on the nature of the sorbent, and may include simple adsorption, chelation or ion-exchange. Active functional groups of the
ligand moiety are responsible for the selective chelation with the metal ions whereas macro porous polymeric support offers large surface area.

**Adsorption**

Trace elements are usually adsorbed on solid phases through van der Waals forces or hydrophobic interaction. Hydrophobic interaction occurs when the solid sorbent is highly non-polar (reversed phase). The most common sorbent of this type is octadecyl-bonded silica (C18-silica). More recently, reversed polymeric phases have appeared, especially the styrene-divinylbenzene copolymer that provides additional pi-pi interaction when p-electrons are present in the analyte [88]. Elution is usually performed with organic solvents, such as methanol or acetonitrile. Such interactions are usually preferred with online systems, as they are not too strong and thus they can be rapidly disrupted. However, because most trace element species are ionic, they will not be retained by such sorbents.

**Chelation**

Several functional group atoms are capable of chelating trace elements. The atoms most frequently used are nitrogen (e.g. N present in amines, azo groups, amides and nitriles), oxygen (e.g. O present in carboxylic, hydroxyl, phenolic, ether, carbonyl, phosphoryl groups) and sulfur (e.g. S present in thiols, thio carbamates and thioethers). The nature of the functional group will give an idea of the selectivity of the ligand towards trace elements. In practice, inorganic cations may be divided into 3 groups:—

1. **Group I-`hard’ cations:** these preferentially react via electrostatic interactions (due to a gain in entropy caused by changes in orientation of hydration water molecules); this group includes alkaline and alkaline-earth metals ($\text{Ca}^{2+}$, $\text{Mg}^{2+}$, $\text{Na}^{+}$) that form rather weak outer-sphere complexes with only hard oxygen ligands.
2. **Group II-`borderline’ cations:** these have an intermediate character; this group contains $\text{Fe}^{2+}$, $\text{Co}^{2+}$, $\text{Ni}^{2+}$, $\text{Cu}^{2+}$, $\text{Zn}^{2+}$, $\text{Pb}^{2+}$, $\text{Mn}^{2+}$. They possess affinity for both hard and soft ligands.
3. **Group III-`soft’ cations:** these tend to form covalent bonds. Hence, $\text{Cd}^{2+}$ and $\text{Hg}^{2+}$ possess strong affinity for intermediate (N) and soft (S) ligands. For soft metals, the following order of donor atom affinity is observed: O<N<S.

Chelating agents may be directly added to the sample for chelating trace elements, the chelates being further retained on an appropriate sorbent. An alternative is to
introduce the functional chelating group into the sorbent. For that purpose, three different means are available:

1. The synthesis of new sorbents containing such groups (new sorbents);
2. The chemical bonding of such groups on existing sorbents (functionalized sorbents); and
3. The physical binding of the groups on the sorbent by impregnating the solid matrix with a solution containing the chelating ligand (impregnated, coated or loaded sorbents).

The latter remains the most simple to be used in practice. Its main drawback is the possible flush of the chelating agent out of the solid sorbent during sample percolation or elution that reduces the lifetime of the impregnated sorbent. Different ligands immobilized on a variety of solid matrices have been successfully used for the preconcentration, separation and determination of trace metal ions. Chelating agents with a hydrophobic group are retained on hydrophobic sorbents (such as C18-silica). Similarly, ion-exchange resins are treated with chelating agents containing an ion exchange group, such as a sulfonic acid derivative of dithizone (i.e. diphenylthiocarbazone) (DzS), 5-sulfo-8-quinolinol, 5-sulfosalicylic acid, thiosalicylic acid, chromotropic acid, or carboxyphenylporphyrin (TCPP) [89-92].

Binding of metal ions to the chelate functionality is dependent on several factors:

- nature, charge and size of the metal ion;
- nature of the donor atoms present in the ligand;
- buffering conditions which favor certain metal extraction and binding to active donor or groups; and
- nature of the solid support (e.g. degree of cross-linkage for a polymer).

In some cases, the behavior of immobilized chelating sorbents towards metal preconcentration may be predicted using the known values of the formation constants of the metals with the investigated chelating agent [93]. However, the presence of the solid sorbent may also have an effect and lead to the formation of a complex with a different stoichiometry than the one observed in a homogeneous reaction [94, 95]. In fact, several characteristics of the sorbent should be taken into account, namely the number of active groups available in the resin phase [93-96], the length of the spacer arm between the resin and the bound ligand [97], and the pore dimensions of the resin [98].
**Ion-pairing**

When a non-polar sorbent is to be used, an ion-pair reagent (IP) can be added to the sorbent [99]. Such reagents contain a nonpolar portion (such as a long aliphatic hydrocarbonated chain) and a polar portion (such as an acid or a base). Typical ion-pair reagents are quaternary ammonium salts and sodium dodecylsulfate (SDS) [100,101]. The non-polar portion interacts with the reversed-phase non-polar sorbent, while the polar portion forms an ion-pair with the ionic species present in the matrix (that could be either free metallic species in solution or complexes).

**Ion exchange**

Ion-exchange sorbents usually contain cationic or anionic functional groups that can exchange the associated counter-ion. Strong and weak sites refer to the fact that strong sites are always present as ion-exchange sites at any pH, while weak sites are only ion-exchange sites at pH values greater or less than the pKa. Strong sites are sulfonic acid groups (cation-exchange) and quaternary amines (anion-exchange), while weak sites consist of carboxylic acid groups (cation-exchange) or primary, secondary and tertiary amines (anion-exchange). These groups can be chemically bound to silica gel or polymers (usually a styrene-divinylbenzene copolymer), the latter allowing a wider pH range. An ion-exchanger may be characterized by its capacity, resulting from the effective number of functional active groups per unit of mass of the material. The theoretical value depends upon the nature of the material and the form of the resin. However, in the column operation mode, the operational capacity is usually lower than the theoretical one, as it depends on several experimental factors, such as flow-rate, temperature, particle size and concentration of the feed solution. As a matter of fact, retention on ion-exchangers depends on the distribution ratio of the ion on the resin, the stability constants of the complexes in solution, the exchange kinetics and the presence of other competing ions. Even though ion-exchangers recover hydrated ions, charged complexes and ions complexed by labile ligands, they are of limited use in practice for preconcentration of trace elements due to their lack of selectivity and their retention of major ions [102]. Yet, for some particular applications they may be a valuable tool. Hence, iron speciation is possible through selective retention of the negative Fe(III)-ferron complex on an anion-exchanger [103]. Selenium speciation is also feasible by selectively eluting Se(IV) and Se(VI) retained on a anion-exchanger [104].
1.3.5 Mechanism of elution of trace elements from the sorbent

Similar kinds of interactions (as mentioned in the previous section) usually occur during the elution step. The type of solvent must be correctly chosen to ensure stronger affinity of the trace element to the solvent, to ensure disruption of its interaction with the sorbent (Figure 1.3). Thus, if retention on the sorbent is due to chelation, the solvent could contain a chelating reagent that rapidly forms a stronger complex with the trace metal. Elution may also be achieved using an acid that will disrupt the chelate and displace the free trace element. Similarly, if retention is due to ion exchange, its pH dependence enables the use of eluents with different pH to be used, such as acids. Of prime importance is to selectively elute only the target species. So, if they are more strongly retained on the sorbent than the interfering compounds, a washing step with a solvent of moderate elution strength is highly advisable before elution of the target species with the appropriate solvent.

1.3.6 Selection of Solid Sorbents

The nature and properties of the sorbent are of prime importance for effective retention of metallic species. In practice, the main requirements for a solid sorbent are:
The possibility to extract a large number of trace elements over a wide pH range (along with selectivity towards major ions);
Kinetically faster quantitative sorption and elution;
A high capacity;
Regenerability; and
Accessibility

Solid sorbents may be reversed-phase sorbents or normal-phase sorbents.

**Reversed-phase sorbents** usually refer to the packing materials that are more hydrophobic than the sample, which are frequently used with aqueous samples. When hydrophobic supports are used, retention of ionic metal species will require the formation of hydrophobic complexes. This can be achieved through addition of the proper reagent to the sample or through immobilization of the reagent on the hydrophobic solid sorbent.

**Normal-phase sorbents** refer to materials more polar than the sample and they are used when the sample is an organic solvent containing the target compounds.

**Nascent polymeric resins as sorbent**

Nascent styrene–DVB resins such as Amberlite XAD-1180 [105,106], XAD-4 [107], and XAD-16 [108-112] are used directly for enrichment of inorganic species as their halide or thiocyanate complex. The type and quantity of sorbent, hydrophobicity, ionizability of the analytes, sample volume and pH interactively determine the breakthrough volume. Using a styrene–divinylbenzene sorbent, the primary interaction mechanism is via Vander Waals forces; therefore, the more hydrophobic the compound the larger the breakthrough volume will be and the larger the sample size from which quantitative recovery can be expected. This observation can be generalized to other sorbents by stating that regardless of the primary interaction mechanism between the analyte and the sorbent, it holds true that the stronger the interaction, the larger the breakthrough volume will be.

**Modification of nascent polymeric resins**

Macroporous hydrophobic resins of the Amberlite XAD series [polystyrenedivinylbenzene (PS-DVB) resins] are good supports for developing chelating matrices. Amberlite XAD resins, as the copolymer backbone for the immobilization of chelating ligands, have some physical superiority, such as porosity, uniform pore size distribution, high surface area and chemical stability towards acids, bases, and oxidizing agents, as compared to other resins. In addition to the hydrophobic interaction that also
occurs with C18-silica, such sorbents also allow π-π interactions. Due to the hydrophobic character of PS-DVB, retention of trace elements on such sorbents requires the addition of a ligand to the sample. The use of surface-modified PS–DVB copolymers with different polar substituent overcomes the following disadvantages suffered by standard silica-based material used for SPE:

- lack of pH stability under acidic or basic conditions,
- low breakthrough for polar analytes,
- they are not wettable by water alone and always need a conditioning step with a wetting solvent, such as methanol.

However, in practice, the resins prepared by impregnation of the ligand are difficult to reuse, due to partial leaching of the ligand (thus resulting in poor repeatability). To overcome this problem, the resin may be chemically functionalized. Chemical modification of PS–DVB copolymers have been carried out by immobilizing varying substituents through different bridging groups.

### Amberlite XAD-2

**Surface modification:** Amberlite XAD-2 resin modified by surface adsorption with oxime

[113,114] 1-(2-thiazolyazo)-2-naphthol [113,115], pyrocatechol violet [113], 4-(2-pyridylazo) resorcinol [114], eriochrome blue black R [114], ammonium pyroldidine dithiocarbamate (APDC) [116], tropolone [117], 1-(2-pyridylazo)- 2-naphthol (PAN) [118], 2-(2-thiazolyazo)-p- cresol [119], calmagite [120,121], and organophosphinic acid [122] were used as solid phase extractant sorbents in off-line or online column preconcentration modes.

**Chemical modification:** Singh et al. chemically immobilized Amberlite XAD-2 with alizarin Red S [123], tiron [124], catechol [125], thiosalicylic acid [126], o-aminophenol [127], chromotropic acid [128], catechol violet [129], salicylic acid [130], and pyrogallol [125] via azo (-N=N-) spacer. A similar synthetic scheme was employed by Jain et al. [131, 132] to chemically immobilize o-vanilline thiosemicarbazone on to Amberlite XAD-2 resin. Dogutan et al. [133] synthesized palmitoyol-8-hydroxyquinoline functionalized Amberlite XAD-2 by a modified procedure described by Suebert et al. [134] through chloromethylation. In 1992, Trojanowicz group [135] chemically immobilized Eriochrome blue black R onto Amberlite XAD-2. Yuan and Shuttler [136] immobilized quinoline-8-ol onto Amberlite XAD-2 and controlled pore glass, and
reported higher enrichment factors with the former as it gave higher flow rates during quantitation of aluminum by FIA-ETAAS.

**Amberlite XAD-4**

*Surface modification:* Surface adsorption of Amberlite XAD-4 resin beads with oxine [137-140], APDC [137, 138], 2[2-(5-chloropyridyl)azo]-5-dimethyl amino phenol (5-CIDMPAP) [139,140], butane–2,3-dionebis(N-pyridinoacetylhydrazone)[141], 2-(5-bromo-2-pyridylazo)-5-diethyl aminophenol (5-Br PADAP) [142-144], 5-phenyl azo-8-quinolinol [145], 1-nitroso-2-naphthol [146], and N-benzoylphenylhydroxylamine [147] were used as solid-phase extractants for the trace determination of inorganics using a variety of detection techniques which include spectral and X-ray techniques.

*Chemical modification:* Azotization was used for immobilization of o-aminobenzoic acid onto Amberlite XAD-4 resin by Cekic et al. [148]. Jain et al. [149] employed similar synthetic scheme for functionalizing Amberlite XAD-4 with o-vanilline-semicarbazone. Amberlite XAD-4 was functionalized with N-hydroxy ethyl ethylene diamine via acylation by Hirata et al. [150] as per the synthesis procedure described by Dev and Rao [151]. Acid chloride was grafted onto Amberlite XAD-4 [152], Yakin and Apak [153] immobilized maleic acid by electrophilic substitution of the Amberlite XAD-4 resin with maleic anhydride by a Friedel-Crafts reaction. Quinoline-8-ol functionalized Amberlite XAD-4 resin was synthesized by Gladis and Rao [154] through acetylation.

**Amberlite XAD-16**

*Surface modification:* Traces of inorganics were enriched on Amberlite XAD-16 resin beads after surface adsorption with a variety of chelates, namely PAN [155], NaDDTC [156,157], 4-(2-thiazoylazo) resorcinol [158,159], N,N-dibutyl-N-benzoylthiourea (DBBT) [160], and di-(2-ethylhexyl phosphoric acid (D2EHPA) [161].

*Chemical modification:* Azotization was employed to functionalize (bis-2,3,4-trihydroxy benzyl)ethylene diamine(BTBED) [162], 2-[[1-(3,4-Dihydroxyphenyl)methyldiene] amino]-benzoic acid (DMABA) [163], 4-[[2-Hydroxyphenyl]imino]methyl]-1,2-benzenediol (HIMB) [164] and Nitrosonaphthol [165] on to Amberlite XAD-16. D. Prabhakaran et. al. immobilized 1,3-dimethyl-3-aminopropan-1-ol onto Amberlite XAD-16 via simple condensation mechanism [166].
Apart from this a number of different solid sorbents have been investigated for the preconcentration of trace metals from an aqueous solution. They include:

- Silica gel (Inorganic based sorbents) [167-172]
- C-bonded silica gel (Inorganic based sorbents) [173-178]
- Other inorganic oxides (Inorganic based sorbents)[179-182]
- Divinylbenzene-vinylpyrroloidone copolymers (polymeric organic sorbents)[183-184]
- Polyacrylate polymers (polymeric organic sorbents)[185-187]
- Polyurethane polymers (polymeric organic sorbents)[188-192]
- Polyethylene polymers (polymeric organic sorbents)[193]
- Polytetrafluoroethylene polymers (polymeric organic sorbents)[194-196]
- Polyamide polymers (polymeric organic sorbents)[197]
- Iminodiacetate-type chelating resins (polymeric organic sorbents)[198-201]
- Propylenediaminetetraacetate-type chelating resins (polymeric organic sorbents)[202]
- Polyacrylonitrile based resins (polymeric organic sorbents)[203-206]
- Ring-opening metathesis polymerization based polymers (polymeric organic sorbents)[207,208]
- Carbon sorbents (non-polymeric organic sorbents)[209-215]
- Cellulose (non-polymeric organic sorbents)[216-218]
- Naphthalene based sorbents (non-polymeric organic sorbents)[219-226]
- Molecularly Imprinted Polymers (MIP) [227,228]

1.3.7 Advantages of SPE

Reduced detection limit

This step is used if the detection limit of the analytical technique is higher than the concentration of trace elements in the sample. Concentration often involves separation of the matrix or the bulk of it, and sometimes a number of interfering minor constituents as well. In a prepared concentrate, the relative concentration of trace elements is usually higher than in the initial sample. Moreover, the possibility of increasing the amount of sample analyzed means that the absolute amounts of elements to be determined can also be increased. As a result, it is possible to reduce the detection limit of trace elements (sometimes very significantly; by a factor of 100 or 1000). This is the main but not the only reason for the widespread use of preconcentration [229].
**Preparation of a representative sample**

Preconcentration is almost essential if trace elements are non-homogeneously distributed in the material. In this case, a representative sample must be quite large; it is difficult to analyze it directly, especially if the method of determination needs a small sample as, for instance, spark-source mass-spectrometry or spectrochemical analysis. In many other cases, preconcentration with preliminary dissolution and production of a small volume of concentrate facilitates the preparation of a representative sample. Samples can be homogenized during other operations also. SPE enjoys superiority over solvent extraction as it is free from difficult phase separation, which is caused by the mutual solubility between water and organic solvent layers [230].

**Facilitates calibration**

Concentration facilitates calibration, especially if there is a lack of standard reference materials. It makes it possible to obtain concentrates with identical matrices in analysis of quite different materials, for example, concentrates on carbon powder in spectrochemical analysis. Reference samples are prepared as concentrates of the same type. There is then no necessity to have standard reference materials for all substances analyzed. Preconcentration with exhaustive removal of the matrix is desirable in the analysis of toxic, radioactive or, if the matrix can be recovered, very expensive materials. Moreover, it is convenient to add elements as internal standards, if necessary, during decomposition of the sample and concentration. Sometimes, preconcentration allows an increase in the number of trace elements which can be determined by a selected technique or makes it possible for the determination technique to be used at all. These advantages of preconcentration make it an important part of trace analysis. In spite of the progress in sensitive instrumental methods of direct analysis, the significance of concentration does not diminish. On the contrary, its possibilities increase, particularly because of new combinations with methods of determination [39,231].

**Other attractive features**

This technique is attractive as it reduces consumption of and exposure to solvents, their disposal costs and extraction time [232]. It also allows the achievement of high recoveries, along with possible elevated enrichment factors. However, different results between synthetic and real samples may be observed [233]; recoveries should be estimated in both cases as far as possible. Its application for preconcentration of trace metals from different samples is also very convenient due to sorption of target species on
the solid surface in a more stable chemical form than in solution. Use of carcinogenic organic solvents is avoided and thus the technique is ecofriendly to nature. Finally, SPE affords a broader range of applications due to the large choice of solid sorbents.

**High preconcentration factor**

The use of SPE enables the simultaneous preconcentration of trace elements, removal of interferences, and reduces the usage of organic solvents that are often toxic and may cause contamination. Upon elution of the retained compounds by a volume smaller than the sample volume, concentration of the extract can be easily achieved. Hence, concentration factors of up to 1000 may be attained [229].

**Preservation and storage of the species**

SPE allows on-site pre-treatment, followed by simple storage and transportation of the pre-treated samples with stability of the retained metallic species for several days [234,235]. This point is crucial for the determination of trace elements, as the transport of the sample to the laboratory and its storage until analysis may induce problems, especially changes in the speciation. In addition, the space occupied by the solid sorbents is minimal and avoids storage of bulky containers and the manpower required to handle them.

**Selective extraction**

SPE offers the opportunity of selectively extracting and preconcentrating only the trace elements of interest, thereby avoiding the presence of major ions. This is crucial in some cases, such as with spectrophotometric detection, since the determination of heavy metals in surface waters may necessitate the removal of non-toxic metals, such as Fe or Zn, when they occur at high concentrations [236]. It may also be possible to selectively retain some particular species of a metal, thereby enabling speciation. For example, salen I modified C18-silica is quite selective towards Cu(II) [237], while chemical binding of formylsalicylic acid on amino-silica gel affords selectivity towards Fe(III) [238]. This high selectivity may also be used to remove substances present in the sample that may hinder metal determination, such as lipid substances in the case of biological samples [239]. The chelating resin method is an economical method since it uses only a small amount of ligand and extraction solvent and this also increases the sensitivity of the system.
**Automation and possible on-line coupling to analysis techniques**

SPE can be easily automated, and several commercially available systems have been recently reviewed [239]. In addition, SPE can be coupled online to analysis techniques. On-line procedures avoid sample manipulation between preconcentration and analysis steps, so that analyte losses and risk of contamination are minimized, allowing higher reproducibility [240]. In addition, all the sample volume is further analyzed, which enables smaller sample volume to be used. However, in the case of complex samples, off-line SPE should be preferred due to its greater flexibility, and the opportunity to analyze the same extract using various techniques.

1.3.8 Challenges

Some of the challenges of preconcentration are as follows:

- Preconcentration increases the analysis time and complicates the analysis for large volume. However, this can be minimized with online systems using flow injection (FI) techniques because of their potential for automation, minimization of reagent and/or sample consumption, and reduced risk of contamination. It offers all essential prerequisites for trace analysis [241].
- It may also lead to losses or contamination of trace elements to be determined.
- Special working procedures, reagents of high purity, specially equipped laboratories and special materials for equipments are necessary.

1.3.9 Common SPE applications

The common SPE application includes:

- Metal ions in various complex real samples,
- Pharmaceutical compounds and metabolites in biological fluids,
- Drugs of abuse in biological fluids,
- Environmental pollutants in drinking and waste water,
- Pesticides and antibiotics in food/agricultural matrices,
- Desalting of proteins and peptides,
- Fractionation of lipids, and
- Water and fat soluble vitamins

1.4 Types of analytical techniques coupled with preconcentration method
Some frequently used analytical techniques in combinations with preconcentration may be described as follows:

**1.4.1 Adsorptive stripping Voltammetry**

In trace analysis, mainly of heavy metal ions, Anodic Stripping Voltammetry (ASV) is popular because of the low limit of determination – ranging to sub ppb concentrations, its accuracy and precision, as well as the low cost of instrumentation for this analytical method. ASV is based on previous electrolytical accumulation of the compound to be determined on the working electrode, followed by voltammetric dissolution (oxidation) of the reduced substance formed. In addition, some anions or organic compounds can be accumulated on a mercury electrode to form an insoluble compound with the mercury ions obtained by dissolution of the mercury electrode at positive potentials. In this type of cathodic stripping voltammetry (CSV), the reduction process of the mercury compound on the electrode surface is studied. The most important step, leading to a substantial increase in the sensitivity in both types of methods is electrolytic accumulation of the species on the working electrode.

The high sensitivity of adsorptive stripping method is obviously their greatest advantage. On the other hand, a serious drawback is interference from other surface-active substances that may be present in the solution. In this case, competitive adsorption usually occurs and leads to a decrease in the measured current or, at very high surface-active substance (s.a.s.) concentrations, to significant suppression of the signal. Interfering effects depend on the nature of both the analyzed and interfering substances and on their concentration ratio in the determination.

Evidently, the interfering effect of s.a.s. can be minimized by employing short accumulation times; however, this approach is not suitable in the determination of trace amounts of analyte. It is then necessary to employ suitable separation of interfering compounds, e.g. the application of LC or gel chromatography and extraction procedures [242-244].

**1.4.2 X–Ray fluorescence analysis**

For the analysis of liquid samples by X-ray fluorescence (XRF) spectrometry, the liquid in a specially designed liquid sample holder is irradiated directly with X-rays. However, for direct analysis of liquid samples, the abundance of effective amounts of analytes in the irradiated volume is insufficient for the determination of trace metals in natural water. Therefore, for the quantitation of trace metals in aqueous samples by XRF,
it is necessary to preconcentrate the analytes through accepted sample pretreatments [245,246].

In this case, group concentration, and more rarely individual concentration, is used and the concentrate should preferably be a solid which can be analyzed directly. Otherwise, several operations must be successively performed. Thus for example, extracts are often decomposed and the trace elements sorbed on cellulose powder or silica gel carrying functional groups. It is more convenient to do the extraction with low-melting reagents at increased temperature. In this case, the solidified concentrate may at once be pressed into a tablet and analyzed.

1.4.3 Spectrophotometric determination

Combinations of concentration, especially extractive, with spectrophotometry in the visible and ultraviolet regions are widely known. Individual concentration steps or successive separations of several elements are usually used. The matrix is very rarely separated in this case. Most frequently, the reagent used for concentration also gives a colored complex with the element to be determined. However, two reagents may also be used: first, the most selective reagent is used for the separation and then, for the determination, a reagent which may not be selective but is the most suitable form for the photometry is employed. The second reagent may also be added after back-extraction or mineralization of the extract [248-250].

1.4.4 Neutron-activation analysis

There are two distinct ways of trace concentration in this method: before irradiation and after it. Separation of the matrix before irradiation is necessary if it is strongly activated and the radioisotopes formed have long half-lives. In the concentration, trace components which are strongly activated (but are not to be determined) may also be separated. However, concentration before irradiation nullifies one of the main advantages of activation analysis—that a blank correction is unnecessary. This advantage is still valid, however, in the case of concentration after irradiation. In analytical practice, both variants are used but the second is used more frequently [251-253].

1.4.5 Electrothermal Atomic Absorption Spectrometry

Nowadays, electrothermal atomic absorption spectrometry (ETAAS) is most powerful and popular analytical tools for the determination of low concentration of metal ions present in environmental samples and biological materials due to their high
selectivity and sensitivity for analyte determination. Nevertheless, they are potentially prone to spectroscopic and/or non-spectroscopic interferences. Various schemes have been suggested to alleviate the interfering effects and facilitate reliable analysis such as protocols ranging from instrument modifications (e.g. background correction) to experimental designs (e.g. standard addition or internal standardization). However, instead of implementing such approaches, there is a much simpler and effective solution to the problem, namely to subject the sample to appropriate pretreatments before it is presented to the detector. Preconcentration addresses these serious problems for the determination of metal ions. Concentration of the desired trace elements can extend the detection limits, remove interfering constituents, and improve the precision and accuracy of the analytical results [254-256].

1.4.6 Inductively coupled plasma optical emission spectrometry (ICP-OES)

Inductively coupled plasma optical emission spectrometry is an analytical technique often employed to determine metal ions in various types of samples [257]. But, their sensitivity and selectivity are usually insufficient for direct determination of these contaminants at a very low concentration level in complex matrix environmental samples. Moreover, several types of spectral interference have been reported in the determination of metal ions by ICP-OES. Thus, preconcentration and separation procedures have been devised to allow trace amounts of metal ions to be determined in complex matrices using ICP-OES [258,259].

1.4.7 Inductively coupled plasma mass spectrometry (ICP-MS)

Although inductively coupled plasma-mass spectrometry (ICP-MS) capable of the rapid simultaneous analysis of multiple elements over a large range of concentrations from a single aliquot of sample, the high salinity concentrations of open ocean samples can cause substantial salt precipitation and build-up, unpredictable suppression or enhancement effects as well as mask the analyte signal through the formation of isobaric and polyatomic interferences due to the presence of high salt content in the samples. In particular, the matrix elements in the sample can combine with carbon in the atmosphere and/or argon in the plasma and result in the formation of polyatomic species which may interfere with the determination of numerous analytes including transition metals and rare earth elements. In addition, when the sample contains a very high concentration of dissolved salts, e.g. seawater; clogging of the sample introduction system or of the injector tube of the torch may occur. Therefore, direct sample injection is impractical,
requiring sample pre-treatment to remove the high salinity matrix by either dilution of the sample or using analyte extraction/separation techniques. Despite the sensitivity of ICP-MS, open ocean trace metal concentrations are often at or below the detection limit, prohibiting the further dilution of samples. It is therefore advisable to concentrate and separate the trace metals from the seawater matrix prior to ICP-MS analysis [260,261].

1.4.8 Inductively coupled plasma atomic emission spectrometry (ICP-AES)

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is widely recognized as a multi-element technique for the determination of elemental species, though direct determinations in environmental and biological samples at trace level is difficult, because the aspiration of solutions with high salt concentrations in the plasma can cause problems such as blockage of the nebulizer, considerable background emission, and transport and chemical interferences with a consequent drop in sensitivity and precision. This limitation can be overcome by using enrichment methods in that metals ions of interest from solutions are selectively separated and preconcentrated into smaller volumes to achieve better detection by ICP-AES [262,263].

1.4.9 Atomic absorption spectrometry

Flame atomic absorption is a very common technique for detecting metals and metalloids in environmental samples [264]. It is a well established technique for the quantification of nearly 70 elements in a variety of sample types with sensitivity at the ppm level or less. Aqueous samples can be determined with no sample preparation; solid samples must be dissolved or digested. This sample preparation for solids can be time consuming. Although atomic absorption spectroscopy dates to the nineteenth century, the modern form was largely developed during the 1955s by a team of Australian chemists. They were led by Alan Walsh and worked at the CSIRO (Commonwealth Science and Industry Research Organization) Division of Chemical Physics in Melbourne, Australia. The time since 1955 can be divided into seven years period. The first was an induction period (1955-1961) when atomic absorption received attention from only a very few people. This was followed by a growth period (1962-1969) when most of what we see today was developed, and then by a period of relative stability (1969-1976) when atomic absorption contributed greatly to other fields. We are now in a period of great change, which started in about 1876, due to the impact of computer technology on individual laboratory instruments [265]. Flame atomic absorption spectrometry is among the most widely used methods for the determination of the heavy metals at trace levels. This
technique presents desirable characteristics such as operational facilities, good selectivity and low cost. However, in the presence of very high excess of diverse ions compared with the level of analyte, some limitations, mainly those related to the sensitivity are observed. In trace analysis, therefore, a preconcentration and/or separation of trace elements from the matrix are frequently necessary to improve the detection limit and selectivity of their determination [39,266]. Particularly, solid phase extraction has been demonstrated in various procedures to be a very effective preconcentration technique in combination with atomic absorption spectrometry. The main advantage of this technique is the possibility of using a relatively simple detection system with flame atomization instead of a flameless technique, which require more expensive equipment and are usually much more sensitive to interferences from macro components of various natural matrices [39].

Principles

The technique (Figure 1.4) makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It relies therefore heavily on Beer-Lambert law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals for a short amount of time by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy (or wavelength) is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity.

As the quantity of energy (the power) put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, from Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured.

In order to analyze a sample for its atomic constituents, it has to be atomized. The sample should then be illuminated by light. The light transmitted is finally measured by a detector. In order to reduce the effect of emission from the atomizer (e.g. the black body radiation) or the environment, a spectrometer is normally used between the atomizer and the detector.

Types of Atomizer

The technique typically makes use of a flame to atomize the sample [267], but other atomizers such as a graphite furnace [268] or plasmas, primarily inductively coupled plasma, are also used [269].
When a flame is used it is laterally long (usually 10 cm) and not deep. The height of the flame above the burner head can be controlled by adjusting the flow of the fuel mixture. A beam of light passes through this flame at its longest axis (the lateral axis) and hits a detector.

**Analysis of liquids**

A liquid sample is normally turned into an atomic gas in three steps:

1. Desolvation (Drying) – the liquid solvent is evaporated, and the dry sample remains.
2. Vaporization (Ashing) – the solid sample vaporises to a gas.
3. Atomization – the compounds making up the sample are broken into free atoms.

**Radiation Sources**

The radiation source chosen has a spectral width narrower than that of the atomic transitions.

**Hollow cathode lamps**

Hollow cathode lamps are the most common radiation source in atomic absorption spectroscopy. Inside the lamp, filled with argon or neon gas, is a cylindrical metal cathode containing the metal for excitation, and an anode. When a high voltage is applied across the anode and cathode, gas particles are ionized. As voltage is increased, gaseous ions acquire enough energy to eject metal atoms from the cathode. Some of these atoms
are in excited states and emit light with the frequency characteristic to the metal [270]. Many modern hollow cathode lamps are selective for several metals.

**Diode lasers**

Atomic absorption spectroscopy can also be performed by lasers, primarily diode lasers because of their good properties for laser absorption spectrometry [271]. The technique is then either referred to as diode laser atomic absorption spectrometry (DLAAS or DLAS) [272], or since wavelength modulation most often is employed, wavelength modulation absorption spectrometry.

**Interferences**

Various factors which may interfere with the determination of metal ions are as follows:

- *Chemical interferences* may also be eliminated by separating the metal from the interfering material. Although complexing agents are employed primarily to increase the sensitivity of the analysis, they may also be used to eliminate or reduce interferences.

- The presence of high dissolved solids in the sample may result in interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked. Preferably, samples containing high solids should be extracted.

- *Ionization interferences* occur when the flame temperature is sufficiently high to generate the removal of an electron from a neutral atom, giving a positively charged ion. This type of interference can generally be controlled by the addition, to both standard and sample solutions, of a large excess (1,000 mg/L) of an easily ionized element such as K, Na, Li or Cs.

- *Spectral interference* can occur when an absorbing wavelength of an element present in the sample but not being determined falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal.

- Interference can also occur when resonant energy from another element in a multielement lamp, or from a metal impurity in the lamp cathode, falls within the band-pass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.
The interference, known as *background absorption*, arises from the presence in the flame of gaseous molecules, molecular fragments and some time smoke. In addition background effects can be caused by light scatter.

*Procedures for reduction of interferences*

- Ensure if possible that standard and sample solutions are of similar bulk composition to eliminate matrix effects.
- Alteration of flame composition or of flame temperature can be used to reduce the likelihood of stable compound formation within the flame.
- Selection of an alternative resonance line will overcome spectral interferences from other atom or molecules and from molecular fragments.
- Separation, for example by solvent extraction or an ion exchange process, may occasionally be necessary to remove an interfering element.

*Background Correction methods*

The narrow bandwidth of hollow cathode lamps makes spectral overlap rare. That is, it is unlikely that an absorption line from one element will overlap with another. Molecular emission is much broader, so it is more likely that some molecular absorption band will overlap with an atomic line. This can result in artificially high absorption and an improperly high calculation for the concentration in the solution. Three methods are typically used to correct this, namely

*Zeeman correction*- A magnetic field is used to split the atomic line into two sidebands. These sidebands are close enough to the original wavelength to still overlap with molecular bands, but are far enough not to overlap with the atomic bands. The absorption in the presence and absence of a magnetic field can be compared, the difference being the atomic absorption of interest.

*Smith-Hieftje correction*- The hollow cathode lamp is pulsed with high current, causing a larger atom population and self-absorption during the pulses. This self-absorption causes a broadening of the line and a reduction of the line intensity at the original wavelength [273].

*Deuterium lamp correction*- In this case, a separate source (a deuterium lamp) with broad emission is used to measure the background emission. The use of a separate lamp makes this method the least accurate, but its relative simplicity (and the fact that it is the oldest of the three) makes it the most commonly used method.
1.5 Statistical Treatment of Data

Analytical chemistry besides providing the methods and tools needed for insight into our material world [274], seeks to improve the reliability of existing techniques to meet the demands for better chemical measurements which arise constantly in our society [275]. Statistical analysis is necessary to understand the significance of collected data and to set limitations on each step of the analysis. The design of experiments is determined from proper understanding of what the data will represent. It is impossible to perform chemical analysis that is totally free from errors, or uncertainties. Every measurement is influenced by many uncertainties, which combine to produce a scatter of results. It is seldom easy to estimate the reliability of experimental data. However, the probable magnitude of the error in a measurement can often be evaluated statistically. Limits within which the true value of a measured quantity lies at a given level of probability can then be defined.

Chemical analyses are affected by at least two types of errors namely systematic and random based on their source. Systematic errors have definitive value, assignable cause and unidirectional of nature. Systematic errors can be reduced to a negligible level if an analyst pays careful attention to the details of the analytical procedure including methods, periodic calibration of the instruments and self discipline. Random errors are the accumulated effect of the individual indeterminate uncertainties. It is caused by the uncontrollable variables that are an inevitable part of physical and chemical measurement. It causes the data to scatter more or less symmetrically around the mean in a random manner which are assumed to be distributed according to the normal error law (Gaussian curve). They are revealed by small differences in replicate measurements of a single quantity and affect the precision of the results. They are more difficult for an analyst to eliminate, but they can be minimized by increasing the number of replicate measurements. The random error in the result of an analysis can be evaluated by the method of statistics [276].

The most common applications of statistics to analytical chemistry include:

- To establish confidence limits for the mean of a set of replicate data.
- To determine the number of replicate measurements required to decrease the confidence limit for a mean to a given probability level.
- To determine at a given probability whether an experimental mean is different from the accepted value for the quantity being measured (t test or test for bias in an analytical method).
➢ To determine at a given probability level whether two experimental means (different methods) are different.

➢ To determine at a given probability level whether the precision of two sets of measurements differs (F test).

➢ To decide whether a questionable data is probably the result of a gross error and should be discarded in calculating a mean (Q test).

➢ To define an estimate detection limit of a method.

**The commonly used terms in the statistical analysis**

**Mean-** The mean ( \( \bar{x} \) ) is obtained by dividing the sum of the replicate measurements (\( \Sigma x_i \)) by the number of observations (\( N \)) performed in a set. The mean is considered to be the best estimate of the true value, which can only be obtained if an infinite number of measurements are performed.

\[
\bar{x} = \frac{\Sigma x_i}{N}
\]

**Accuracy-** The term accuracy as used in analytical chemical literature is a measure of the degree to which a mean ( \( \bar{x} \) ) obtained from a series of experimental measurements, agrees with the value, which is accepted as the true or correct value for the quantity. It is expressed by the error; either absolute or relative error. There are two types of errors according to their nature and source- systematic errors and random errors. Systematic error causes the mean of a set of data to differ (unidirectional) from the accepted value. They have definitive value, assignable cause and affect the accuracy of results. Random error causes data to be scattered more or less symmetrically around a mean value. It mainly affects the precision of measurement.

**Precision-** Precision describes the agreement among the replicate measurements and is generally expressed as standard deviation, coefficient of variation (relative standard deviation) and variance.

**Standard deviation-** Standard deviation(s) measures how closely the data are clustered about the mean. When measurements are repeated, the scatter of the results will be around the expected value of the results, if no bias exists.

\[
s = \sqrt{\frac{\Sigma (x_i - \bar{x})^2}{N-1}}
\]

In analytical chemistry, this scatter is more often than not of such a nature that it can be described as a normal distribution. The mean gives the centre of the distribution. Standard deviation measures the width of the distribution. Therefore, an experimental technique
that produces a small standard deviation is more reliable (precise) than one that produces a large standard deviation provided that they are equally accurate. The square of the standard deviation, \( s^2 \), is known as variance.

**Coefficient of variation**- Precision is more often expressed as the coefficient of variation which is the standard deviation divided by the average and multiplied by 100. Since the average and standard deviation have same dimension, the coefficient of variation is dimensionless; it is only a relative measure of precision. Therefore, it is sometimes also known as relative standard deviation (RSD).

\[
RSD = \frac{s}{\bar{x}} \times 100
\]

**Confidence limit and confidence interval**- Calculation of \( s \) (standard deviation) for a set of data provides an indication of the precision inherent in a particular method of analysis. But unless there is a large number of data, it does not by itself give any information about how close the experimentally determined mean (\( \bar{x} \)) might be to the true mean value \( \mu \) (population mean). Statistical theory, though, allows us to estimate the limits around an experimentally determined mean (\( \bar{x} \)) within which the population mean or true value (\( \mu \)) expected to lie, within a given degree of probability. The likelihood that the true value lies within the range is called confidence level or probability usually expressed as a recent.

\[
\text{Confidence limit for } \mu = \bar{x} \pm \frac{ts}{\sqrt{N}}
\]

where \( t \) is the student’s \( t \) value which depends on the desired confidence level and this equation holds when \( \sigma \), population standard deviation is not known.

**Correlation coefficient**- The correlation coefficient is a measure of the linear relationship between two variables. In order to establish the linear relationship between variables \( x_i \) and \( y_i \) the Pearson’s correlation coefficient \( r \) is used. The \( r \) may have values between +1 and –1. When the \( r \) is +1, there is a perfect correlation, i.e. an increase in one variable is associated with an increase in the other. When the \( r \) is –1, there is perfect negative correlation, i.e., one variable increases where as the other decreases. When \( r \) is zero, one variable has no linear relationship to the other. The correlation coefficient \( r \) is calculated by using the above equation.
Regression equation- When two variables exhibit a linear relationship, we may be interested in quantifying the relationship so that a value of one variable may be estimated from the other. A common example is the construction of calibration curve, which relates the concentration of analyte to absorbance or any measurable property. The least square curve fitting technique is the most commonly accepted mathematical procedure known for this purpose. The equation derived by this technique produces a line whose position is such that the sum of the squares of the vertical distances of each data point to the line is a minimum if the line is to be used to predict y from x values, or the sum of the squares of the horizontal distances is a minimum if x is to be predicted from y. If \( y = bx + a \) represents the equation for a straight line, where \( y \) is dependent variable and \( x \) is independent variable, then slope ‘b’ and intercept ‘a’ are derived from the following equations [276-278].

\[
b = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{n\sum x^2 - (\sum x_i)^2}
\]

\[
a = \bar{y} - b\bar{x}
\]

Sensitivity- It is measure of the ability of an instrument or method to discriminate between small differences in analyte concentration. There are two factors which limit the sensitivity:

- the slope of the calibration curve.
- reproducibility or precision of the measuring device.

Of two methods that have equal precision, the one that has the steeper calibration curve will be the more sensitive. A corollary to this statement is that if two methods have calibration curves with equal slopes, the one that exhibits the better precision will be the more sensitive. The quantitative definition of sensitivity that is accepted by the International Union of Pure and Applied Chemists (IUPAC) is calibration sensitivity, which is the slope of the calibration curve at the concentration of interest. Most calibration curves that are used in analytical chemistry are linear and may be represented by the equation: \( S = mc + S_{bl} \), where \( S \) is the measured signal, \( c \) is the concentration of the analyte, \( S_{bl} \) is the instrumental signal of the blank, and \( m \) is the slope of the straight line. The quantity \( S_{bl} \) should be the intercept of the straight line. With such curves, the calibration sensitivity is independent of the concentration \( c \) and is equal to \( m \). The calibration sensitivity as a figure of merit suffers from its failure to take into account the precision of individual measurements.
To include precision in a meaningful mathematical statement of sensitivity, **analytical sensitivity** is proposed (γ): \( \gamma = \frac{m}{s_s} \); where \( s_s \) = standard deviation of measurement and \( m \) is the slope of the calibration curve [270,279].

**Detection limits**- It is defined as the minimum concentration or mass of analyte that can be detected at a known confidence level. This limit depends upon the ratio of the magnitude of the analytical signal to the size of the statistical fluctuations in the blank signal. The limit of detection (C_L) according to the definition of International Union of Pure and Applied Chemists can be expressed as [280]: \( C_L = x + k.s_{bl} \); where \( x \) = mean of blank signal, \( s_{bl} \) = standard deviation of the blank measures and \( k \) = numerical constant. A value of \( k=3 \) was strongly recommended by IUPAC. The Analytical Methods Committee of the Royal Society of Chemistry sought to clarify the IUPAC definition [281].

The estimation of the detection limit is best understood by considering a calibration graph. Using the linear regression method, it is possible to obtain the intercept and slope of the best-fit line. The value of the intercept can be used as \( x \); and errors in the slope and the intercept of the regression line is acceptable as a measure of \( s_{bl} \). Hence, the detection limit (C_L) may also be expressed as: \( C_L = 3s_{bl}/b \); where \( b \) is the slope of the calibration line [270,277,282,283].

### 1.6 Present work and its scope

SPE has found its most effective use as a **sample clean-up** and **concentration** method prior to further analysis. Solid phase extraction, in combination with atomic absorption spectrometry, has been developed as the technique for preconcentration prior to determination of trace metal ions in varying complex matrices, which includes both biological and environmental samples.

Amberlite XAD-4 and 16 resins have been successfully used as the polymeric supports in previous works (Table 1.2 and 1.3). Hence, we have used the same because of its good surface area (725 \( \text{m}^2 \ \text{g}^{-1} \) and 800 \( \text{m}^2 \ \text{g}^{-1} \)) and high porosity. The functional groups used satisfactorily for this work are salicylic acid, \( \sigma \)-hydroxybenzamide, salicylanilide and p-aminobenzene sulfonic acid on account of their smaller size and good number of chelating sites. This functionalities would render the modified resin more hydrophilic so as to facilitate faster equilibrium (of solute between solid and aqueous phases) whereby enhancing the extraction ability.
The modification of polystyrene-divinyl benzene adsorbent resin has been accomplished through azotization reaction, by conventional reaction techniques, by incorporating neutral organic hydrophilic group through –N=N- spacer. It is important to note that these functional groups are neutral, that is they bear no positive or negative charge but, however, they possess the characteristic features of a chelating agent. This is important in order to allow for effective contaminant removal of the inorganics since either anionic or cationic charged resins may often pick up undesirable materials that are present such as other matrices. However, neutral functionalized or modified resins such as those described here in act differently than charged resins and take up the inorganics by adsorbtion/chelation rather than ion exchange.

Chelating resin and the aqueous solution containing the heavy metals along with other matrices may be contacted using both batch and column methods which would result in intimate contact between the resin and the solution. The columns used for extractions were packed with 100 to 500 mg of resin. Varying volume of aqueous solutions of a sample, containing metals in the concentration of the order of parts per million, is passed through the column at the optimum flow rate whereby the solute gets retained onto the resin.

Investigations of the optimum experimental conditions sorption and elution have been carried out by considering the following influential parameters.

**Synthesis and Characterization**

The introduction of the organic groups onto the polymeric material (XAD-series resin) can make the stable chelating compounds for the uptake of trace metal ions. The aim of chemical modification with different organic reagent, which has mainly N and O donor atoms, is to make the material have excellent coordination properties with trace metal ions and to obtain a novel sorbent with high loading of metal ions. The FT-IR analysis is a very useful technique in identifying the immobilization process by comparing the precursor and modified resin. The thermogravimetric analysis (TGA) curve of the chelating resin shows mass loss in two steps. In first step, it may be due to the loss of sorbed water of the resin and second step, due to the loss of functional moieties of the chelating resin. It was also use to performed, to study the water-regaining capacity of the resin matrix in order to evaluate its hydrophilic character. The CHN analysis was performed during each stage of chemical modification to study the extent of ligand functionalization to the polymeric matrix.
The modified resin soaked in acidic (HCl/HNO₃, 1-5 mol L⁻¹) and basic medium (NaOH, 1-4 mol L⁻¹) for 6 h, filtered and then washed with triply distilled water until free from acid or alkali. The resin shows no loss in sorption capacity. Hence, the resin exhibited high chemical stability. The water regain capacity and hydrogen ion capacity have also been studied. This value reflects the high hydrophilicity of the resin which is satisfactory for column operation. In case of AXAD-16-SALD, the overall hydrogen ion capacity amounts to 6.08 mmol g⁻¹ of resin, which may be contributed both by the hydroxylic and amide groups present within the molecule. Theoretically, if 2.65 mmol of the reagent constituted per gram of the resin, the hydrogen ion capacity, due to the hydroxylic group should have been 2.65 mmol g⁻¹. The durability and reusability nature of the chelating resin was tested with metal ions solutions by batch equilibration method. Thereafter, the sorption and desorption of metal ions were repeated on the same resin. The capacity of the resin found to be constant up to the several cycles (35-55) showing the multiple use of chelating resin without any loss in its physical and chemical properties.

Sorption studies

Effect of pH of the sample

Sample pH is of prime importance for efficient retention of the trace elements on the sorbent. Its influence strongly depends on the nature of the sorbent used. Careful optimization of this parameter is thus crucial to ensure quantitative retention of the trace elements and in some cases selective retention. In particular with ion exchangers, correct adjustment of sample pH is required to ensure preconcentration. Thus, in the case of cationic-exchangers, low pH usually results in poor extraction due to competition between protons and cationic species for retention on the sorbent. When retention of trace elements is based on chelation (either in the sample or on the solid sorbent), the sample pH is also a very important factor as most chelating ligands are conjugated bases of weak acid groups and accordingly, they have a very strong affinity for hydrogen ions. The pH will determine the values of the conditional stability constants of the metal complexes. By contrast, pH may have no influence with some non-ionizable organic ligands [284]. For inorganic oxides, pH is also of prime importance. In particular, on amphoteric oxides such as TiO₂ or Al₂O₃, cations are adsorbed at elevated pH due to the deprotonation of functional groups, whereas anion retention requires acidic conditions for the protonation of functional groups.
**Adsorption isotherm**

The isotherm parameters of Langmuir and Freundlich for the sorption of metal ions have been studied. The results showed that the regression coefficients $R^2$ obtained from Langmuir model were very close to 1, suggesting the Langmuir model could well interpret the studied adsorption procedure. From the comparison of correlation coefficients, it can be concluded that the data were fitted better by Langmuir equation than by Freundlich equation. Langmuir equation is applicable to homogeneous adsorption, where the adsorption of each adsorbate molecule onto the surface had equal adsorption activation energy. The fact shows that the adsorption of the hybrid sorbent is attributed to monolayer adsorption [285].

**Kinetics of sorption**

In order to determine the uptake rate of metal ions on the synthesized resin and get access to the equilibrium time, studies on sorption kinetics were carried out. The sorbents characterized by good kinetic properties determined by the macroporous structure of the support, large surface area and total accessibility of the functional groups (without steric hindrance). The kinetic studies also showed that the temperature affected the rate constants significantly, that is, saturation was reached at a faster rate at higher temperature. This temperature effect may be a manifestation of the fact that the resin swells more completely at higher temperature, which allows metal ions to diffuse more easily into the interior of the resin, and that the sorption was an endothermic process and hence high temperature facilitates higher sorption. Moreover, both pseudo-first-order equation and pseudo second-order equation were used to express the sorption process of the chelating resin. The results showed that regression coefficients values ($R^2$) of the pseudo-second-order model (>0.99) were better than those of the pseudo-first-order model for the sorbent, suggesting the pseudo-second-order model was more suitable to describe the sorption kinetics of chelating resin [285,286].

**Sample flow-rate**

The sample flow-rate has been optimized to ensure quantitative retention along with minimization of the time required for sample processing. This parameter may have a direct effect on the breakthrough volume, and elevated flow-rates may reduce the breakthrough volume [284]. As a rule, cartridges and columns require lower maximum flow-rates than disks ranging typically from 0.5 to 5 mL min$^{-1}$. This value may be
increased by a factor of 10 using disks. High flow rate has been found for the sorption of metal ions, such high flow rates support the superiority of present chelating resins.

**Elution studies**

**Nature of the eluent**

The nature of the eluent is of prime importance and should optimally meet three criteria: efficiency, selectivity and compatibility, as discussed below. In addition, it may be desirable to recover the analytes in a small volume of eluent to ensure a significant enrichment factor. The eluent may be an organic solvent (when reversed-phase sorbents are used), an acid (usually with ion-exchangers), or a complexing agent. Firstly, the eluting agent has been carefully chosen to ensure efficient recovery of the retained target species and quantitative recovery as far as possible [287]. A further characteristic of the eluent arises with the possibility of introducing selectivity. Using an eluent with a low or moderate eluting power, the less retained analytes can be recovered without eluting the strongly retained compounds. Thus, if the elements of interest are those that remain on the sorbent another elution step with a more eluent will ensure their quantitative recovery. In that way interfering analytes were removed during the first eluting step (also called *washing* step). On the opposite, if the compounds of interest are the less retained on the sorbent their elution with a low or moderate eluting agent ensures their selective recovery, as the interferent compounds will remain on the sorbent due to stronger interactions with the solid support. In some cases, this selectivity may favour speciation. For example, 1 mol L⁻¹ HCOOH removed only Se⁴⁺ from an anion-exchange resin, leaving Se⁶⁺ retained on the sorbent, which was further eluted using 2 mol L⁻¹ HCl [104]. Finally, the eluent should be compatible with the analysis technique. In particular, when using both flame and electrothermal AAS, HNO₃ has been preferred to other acids (namely H₂SO₄, HCl), as nitrate ion is a more acceptable matrix [288].

**Effect of pH of eluent**

As retention of trace elements on solid sorbents is usually pH-dependent, careful choice of the eluent pH may enhance selectivity in the SPE procedure. As an example, once retained on Eriochrome black-T (ERT)-functionalized- silica gel, Mg²⁺ could be eluted first at a pH approximately 4, while increasing the pH to 5–6 was required for eluting Zn²⁺ [237].
**Elution mode**

Most of the time, for practical reasons, sample loading and elution steps are performed in a similar manner. However, to avoid irreversible adsorption and ensure quantitative recoveries, elution in the back flush mode is recommended in some cases. This means that the eluent is pumped through the sorbent in the opposite direction to that of the sample during the preconcentration step. This is especially crucial when carbon-based sorbents have to be used due to possible irreversible sorption of the analytes.

**Flow-rate of eluent**

The flow-rate of the elution was found to be high enough to avoid excessive duration, and low enough to ensure quantitative recovery of the target species. Typical flow-rates are in the range of 0.5 to 5 ml min\(^{-1}\) for cartridges/columns and of 1 to 20 ml min\(^{-1}\) for disks [289]. As a rule, the higher the flow-rate, the larger the eluent volume required for complete elution [290,291].

**Volume of Eluent**

The elution volume may be determined either experimentally or estimated theoretically [292]. The elution volume can usually be reduced by increasing the concentration of the eluent (e.g. acid). However, in this case, problems with subsequent analysis may be encountered (e.g. FAAS). Alternatively, the use of micro-sized disks may allow reduced solvent volume [293]. The elution step should enable sufficient time and elution volume to permit the metallic species to diffuse out of the solid sorbent pores. As a rule, 2 elution cycles are usually recommended as compared to a single step (e.g. two 5 ml elution should be preferred to a single 10 ml elution). Soaking time is also critical and 2 to 5 minute soak is most of the time allowed before each elution.

**Breakthrough profile**

**Sample volume to be percolated**

An important parameter to control in SPE is the breakthrough volume, which is the maximum sample volume that has been percolated through a given mass of sorbent after which analytes start to elute from the sorbent resulting in non-quantitative recoveries (Figure 1.5). The breakthrough volume is strongly correlated to the chromatographic retention of the analyte on the same sorbent and depends on the nature of both the sorbent and the trace element, as well as on the mass of sorbent considered and the analyte concentration in the sample [294]. In addition, it depends on the sorbent containers, as
disks usually offer higher breakthrough volumes than cartridges. This volume may be determined experimentally or estimated using several methods [292]. For that purpose the nature of the sample has to be taken into account, as the possible presence of ligands may dramatically reduce the breakthrough volume [295].

![Concentration vs. Sample Volume](image)

**Figure 1.5** Typical representation of the breakthrough curve (i.e. concentration of the analyte at the outlet of the SPE system vs. sample volume percolated through the system).

$V_B$ is the breakthrough volume, $V_R$ the chromatographic elution volume, $V_C$ the sample volume corresponding to the retention of the maximum amount of analyte and $C_0$ is the initial analyte concentration in the sample.

Preconcentration is a process in which the ratio of the quantity of a desired trace element to that of original matrix is increased but it does not necessarily mean increase in the concentration of the analyte. In order to demonstrate the preconcentration factor, preconcentration limit column procedure has been applied. The closeness of the dynamic capacity to the total sorption capacity reflects the applicability of the column technique for preconcentration.

**Interference of sample matrix**

The presence of ligands in the sample matrix may affect trace element retention when stable complexes are formed in the sample with these ligands, as trace elements are less available for further retention. Thus, if metals are present in the sample as strong complexes, they may not dissociate resulting in no retention of the free metal on the sorbent. As an example, reduction in the retention of Cu$^{2+}$ on Amberlite CG50 occurs in the presence of ligands such as glycine [296]. In the case of real samples, the presence of natural organic matter is of great concern as it may form complex with trace elements as observed for Cu$^{2+}$ [296,297]. The most important class of complexing agents that occurs
naturally are the humic substances [298]. Their binding of metals through chelation is one of the most important environmental qualities. Yet, in some cases the presence of ligands may be a valuable tool for adding selectivity to the SPE step. This requires that the added ligands be correctly chosen to complex only the elements that are not of interest so that they are not retained on the sorbent [235]. The presence of ions other than the target ones in the sample may also cause problems during the SPE step. In particular, due to their usually high levels (e.g. Ca\(^{2+}\)), they may hinder the preconcentration step by overloading the sorbent or cause interferences during spectrophotometric analysis. Therefore, their influence has been studied before validating a SPE method. Sometimes the addition of a proper masking agent (such as EDTA, thiourea or ethanolamine for example) may prevent the formation of interferences due to ions present in the sample [287]. Finally, the ionic strength of the sample is another parameter to control for an efficient SPE, as it may influence the retention of trace elements, and thus the value of the breakthrough volume for a given sorbent [299,300]. The chelating resin was found to tolerate high contents of various naturally occurring alkali and alkaline earth metals, anions and complexing agents in the determination of these metal ions.

**Analytical method validation**

Great care has been taken to ensure that accurate results are obtained in the analysis. Every measurement has some imprecision associated with it, which results in random distribution of results. The experiment can be designed to narrow the range of this (confidence limit is set around the mean at some degree of probability), but it cannot be eliminated. Precision of the method is expressed either standard deviation or relative standard deviation. Systematic errors of instrumental and personal type are minimized by periodic calibration of the instruments and volumetric glassware, and self-discipline. However, systematic method errors are difficult to detect and introduces bias in the method. Bias of the analytical method has been estimated by the following procedures:

*Analyzing Standard Reference Materials* (SRM) of biological, environmental and alloy type having complex sample matrix obtained from National Institute of Environmental studies (NIST), Iron and Steel institute of Japan (JSS) and National bureau of Standards (NBS). In order to demonstrate whether the difference of the observed mean of the replicate analysis of SRM and its certified value is due to merely the random error of the measurement or bias in the method, statistical t test is applied.

*Using a Second Independent and Reliable Analytical Method* in parallel with the method being evaluated when standard samples are not available. The independent method should
differ as much as possible from the one under study. This minimizes the possibility that some common factor in the sample has the same effect on both methods. Then a statistical t and f test must be used to determine whether any difference is a result of random errors in the two methods or due to bias in the method under study.

*Performing Recovery Experiment* which requires the spiking of the sample with known amounts of the analyte. The amount determined (recovered) by the method is expressed in terms of percentage recovery which shows accuracy of the developed method. By varying the sample size, the presence of a constant error can be detected.

**Applications**

In order to explore the potential applicability of the chelating resin the developed methods were successfully applied for the determination of metal ions in natural and sewage water, mint leaves, mango pulp, fish, urine, multivitamins tablets, infant milk substitute and hydrogenated oil (ghee).

**1.7 Merits of the present work**

- The main advantage of the present procedure is the simple and fast preparation of the chelating resin and no requirement of organic solvents in the metal elution step.
- The excellent ability for the exclusion of alkali and alkaline earth elements makes it desirable for use in the separation and preconcentration of trace elements because their presence often interfere in the subsequent FAAS determination.
- The use of a column preconcentration technique allows for the assessment of low trace metal concentrations, even by less sensitive determination methods such as FAAS.
- Preconcentration by this material from river water samples do not require any prior digestion of the samples. It can be successfully applied for the analysis of both environmental and biological samples as indicated by the high precision (low relative standard deviation).

The designing and characterization of these four Amberlite XAD-4/16 based chelating resins and the investigation of their metal sorption behavior with their subsequent applications for analysis of various real samples containing varying complex matrices define the scope of the present thesis.
<table>
<thead>
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<th>Mode of preparation</th>
<th>Techniques coupled</th>
<th>Metals</th>
<th>Ref.</th>
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<td>Chemically modified</td>
<td>ICP-AES</td>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;,Ni&lt;sup&gt;2+&lt;/sup&gt;,Co&lt;sup&gt;2+&lt;/sup&gt;,Cd&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>304</td>
</tr>
<tr>
<td>Ammoniumpyrrolidine-dithiocarbamate</td>
<td>Impregnation</td>
<td>ICP-AES</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;,Cu&lt;sup&gt;2+&lt;/sup&gt;,Mn&lt;sup&gt;2+&lt;/sup&gt;,Ni&lt;sup&gt;2+&lt;/sup&gt;,Pb&lt;sup&gt;2+&lt;/sup&gt;,Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>305</td>
</tr>
<tr>
<td>2-Acetylmercapto-phenylidiazooaminobenzene</td>
<td>Chelate complex</td>
<td>FAAS</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;,Co&lt;sup&gt;2+&lt;/sup&gt;,Cu&lt;sup&gt;2+&lt;/sup&gt;,Ni&lt;sup&gt;2+&lt;/sup&gt;,Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>306</td>
</tr>
<tr>
<td>2,6-dihydroxyphenyl-diazoaminobenzene</td>
<td>Chelate complex</td>
<td>FAAS</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;,Co&lt;sup&gt;2+&lt;/sup&gt;,Cu&lt;sup&gt;2+&lt;/sup&gt;,Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>307</td>
</tr>
<tr>
<td>Diethyldithiocarbamates</td>
<td>Chelate complex</td>
<td>FAAS</td>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;, Fe&lt;sup&gt;2+&lt;/sup&gt;, Pb&lt;sup&gt;2+&lt;/sup&gt;, Ni&lt;sup&gt;2+&lt;/sup&gt;,Cd&lt;sup&gt;2+&lt;/sup&gt;,Bi&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>308</td>
</tr>
<tr>
<td>1-Hydrizinophthalazine</td>
<td>Chelate complex</td>
<td>AAS</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;, Co&lt;sup&gt;2+&lt;/sup&gt;,Cu&lt;sup&gt;2+&lt;/sup&gt;,Ni&lt;sup&gt;2+&lt;/sup&gt;,Pb&lt;sup&gt;2+&lt;/sup&gt;,Fe&lt;sup&gt;2+&lt;/sup&gt;,Cr&lt;sup&gt;3+&lt;/sup&gt;Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>309</td>
</tr>
<tr>
<td>1-(2-pyridylazo)-2-naphthol</td>
<td>Impregnation</td>
<td>FI-FAAS</td>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>234</td>
</tr>
<tr>
<td>1-(2-pyridylazo)-2-naphthol</td>
<td>Chelate complex</td>
<td>AAS</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;, Cu&lt;sup&gt;2+&lt;/sup&gt;,Ni&lt;sup&gt;2+&lt;/sup&gt;,Pb&lt;sup&gt;2+&lt;/sup&gt;,Cr&lt;sup&gt;3+&lt;/sup&gt;,Mn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>310</td>
</tr>
<tr>
<td>Salen</td>
<td>Chemically modified</td>
<td>AAS</td>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;,Pb&lt;sup&gt;2+&lt;/sup&gt;,Bi&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>311</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>Chemically modified</td>
<td>Spectrophotometry</td>
<td>U&lt;sup&gt;6+&lt;/sup&gt;</td>
<td>312</td>
</tr>
<tr>
<td>Schiff bases</td>
<td>Chemically modified</td>
<td>FI-FAAS</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;, Co&lt;sup&gt;2+&lt;/sup&gt;,Cu&lt;sup&gt;2+&lt;/sup&gt;,Ni&lt;sup&gt;2+&lt;/sup&gt;,Pb&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>313</td>
</tr>
<tr>
<td>O,O-Diethyldi-thiophosphate</td>
<td>Chelate complex</td>
<td>FI-FAAS</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>314</td>
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<tr>
<td>m-Phenylendi-amine</td>
<td>Chemically modified</td>
<td>ICP-AES</td>
<td>Rh&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>315</td>
</tr>
<tr>
<td>2,3-Diamino-naphthalene</td>
<td>Chemically modified</td>
<td></td>
<td>Se&lt;sup&gt;6+&lt;/sup&gt;</td>
<td>316</td>
</tr>
<tr>
<td>2-Aminothiophenol</td>
<td>Chemically modified</td>
<td>FAAS</td>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;,Ni&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>317</td>
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<tr>
<td>Reagent</td>
<td>Mode of preparation</td>
<td>Techniques coupled</td>
<td>Metals</td>
<td>Ref.</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>---------------------</td>
<td>--------------------</td>
<td>-------------------------------------</td>
<td>------</td>
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<tr>
<td>1,6-bis(2-carboxy aldehydephenoxo)butane</td>
<td>Chemically modified</td>
<td>FAAS</td>
<td>Cu(^{2+}), Cd(^{2+})</td>
<td>318</td>
</tr>
<tr>
<td>2,6-dichlorophenyl-3,3-bis(indolyl)methane Gallic acid</td>
<td>Impregnation</td>
<td>FAAS</td>
<td>Cu(^{2+}), Zn(^{2+}), Mn(^{2+})</td>
<td>319</td>
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<tr>
<td>4-[(2-hydroxyphenyl)imino]methyl]-1,2-benzenediol</td>
<td>Chemically modified</td>
<td>FAAS</td>
<td>Cr(^{3+}), Mn(^{2+}), Fe(^{3+}), Co(^{2+}), Ni(^{2+}), Cu(^{2+}), Zn(^{2+}), Mn(^{2+}), Ni(^{2+}), Pb(^{2+}), Cd(^{2+}), Cu(^{2+}), Fe(^{3+}), Co(^{2+})</td>
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<tr>
<td>Acetylacetone</td>
<td>Chemically modified</td>
<td>AAS</td>
<td>Cr(^{3+}), Cr(^{6+})</td>
<td>321</td>
</tr>
<tr>
<td>3,4-dihydroxy benzoyl methyl phosphonic acid</td>
<td>Chemically modified</td>
<td>FAAS</td>
<td>U(^{6+}), Th(^{4+})</td>
<td>322</td>
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<tr>
<td>Phthalic acid</td>
<td>Chemically modified</td>
<td>AAS</td>
<td>Pb(^{2+})</td>
<td>323</td>
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<tr>
<td>N,N-dibutyl-N- benzoxythiourea (bis-2,3,4-trihydroxybenzyl) ethylene diamine Thiocyanate</td>
<td>Impregnation</td>
<td>Spectrophotometry</td>
<td>U(^{6+})</td>
<td>160</td>
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<tr>
<td>1,5-diphenylcarbazone</td>
<td>Impregnation</td>
<td>FAAS</td>
<td>Ag(^{2+})</td>
<td>324</td>
</tr>
<tr>
<td>Nitrosonaphthol</td>
<td>Chemically modified</td>
<td>AAS</td>
<td>Ni(^{2+}), Cu(^{2+})</td>
<td>165</td>
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