



Preparation and Characterization of Chelating resins to Develop Column Preconcentration Method with Flame Atomic Absorption Spectrometric Determination of Trace Elements

By

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Under the supervision of Dr. Aminul Islam

Submitted in fulfillment of the requirement of the degree of

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Abstract

The present thesis comprises of six chapters. The first chapter is of **Introduction**. It details with metal ion toxicity. This is followed by a discussion on the basic principle of SPE, advantages over other preconcentration methods and their automation with sophisticated analytical techniques in the determination of trace analyte ions. It gives an account of the different types of chelating sorbent employed as metal extractors and discusses the mechanism involved in the complexation of the metal ions with the active sites on to the sorbent including nanosorbents. This chapter also gives a brief account on the use of flame atomic absorption spectrometric determination of trace metal ions after preconcentration using SPE. It concludes with a brief discussion on statistical treatment of analytical data.

The chapter two is about **Preparation & Characterization of Chelating Resins**. It accounts the details of the synthesis and preparation of different types of chelating sorbents discussed throughout the thesis. It gives an account of the step by step preparation along with the schemes. The chapter also briefs the vendor information and significant reference to this work of the various instrumental techniques used in the characterization of the prepared sorbents.

The third chapter is of **Optimization & Validation of Analytical Methods**. This chapter takes into account the different general experimental procedures followed during the work. Various details that have been covered in this chapter include vendor information, sample pretreatment and details of methods employed for sample digestion. This chapter concludes with a brief account on the procedures followed during method validation.

The rest of the chapters comprise of a brief introduction focusing the metal ion analyzed in the corresponding chapter. This is followed by the detailed results and

discussion for each of the following works. All these synthesized or prepared chelating sorbents were characterized, and then systematically explored for the separation/preconcentration and determination of metal ions in real environmental, biological and food samples by flame atomic absorption spectrophotometer. The chapters also take into account the analyses of Standard Reference Materials and recovery experiments of the spiked real samples for the validation of the developed method in order to evaluate accuracy, precision, limit of detection and limit of quantification.

The fourth chapter is about **Poly(GMA-MMA-EGDMA) terpolymer functionalized with EDA**. Here, a procedure is detailed for the trace determination of Cd(II) and Zn(II) by terpolymeric resin as a solid phase extractant coupled to Flame Atomic Absorption Spectrophotometer (FAAS). Terpolymeric resin was synthesized by chemical functionalization of Glycidylmethacrylate-Methylmethacrylate-Ethyleneglycoldimethacrylate (GMA-MMA-EGDMA) resin beads with ethylenediamine chelating ligand. The modified resin was characterized using FT-IR spectroscopy, Scanning electron microscopy (SEM) along with Energy Dispersive X-ray Analysis (EDAX), Elemental analysis (CHN), Thermogravimetric analysis (TGA)/Differential thermal analysis (DTA), surface area and pore size analysis. The optimum sorption was at pH 8 ± 0.1 . The resin shows maximum sorption capacity of 53.96 mg g⁻¹ for Cd(II) and 24.19 mg g⁻¹ for Zn(II). Under the optimized conditions, many competitive anions and cations studied did not interfere at all in the determination of cadmium and zinc. In isotherm studies Langmuir, Dubinin-Radushkevich, Scatchard and Temkin models were found to be the best fitted. Analytical figures of merit were evaluated by accuracy (SRMs and recovery experiment), precision (RSD <5%), and detection limit. The applicability was demonstrated by determination of cadmium and zinc in rice, bread and fruit juice samples.

The fifth chapter details the research on **Immobilization of GO sheets to GMA-DVB copolymer beads via DETA spacer arm unit.**

In this chapter, Graphene oxide (GO) based glycidylmethacrylate chelating resin was synthesized by immobilization of GO sheets on to glycidylmethacrylate-divinylbenzene resin beads via ethylenediamine (EDA) and diethylenetriamine (DETA) as spacer arms and used as novel solid phase extractant for the development of column preconcentration method. The synthesized resin was characterized by elemental analysis, infrared spectroscopy, surface area and pore size analysis, scanning electron microscopy, transmission electron microscopy, energy dispersive spectroscopy, surface area and pore size analysis, thermogravimetry and solid state ^{13}C NMR. The resin was used for the first time as an effective sorbent for the preconcentration of cadmium and nickel in electroplating waste water, river water, tap water and fruit juice samples. The analytical variables like pH, flow rate for sorption/desorption, and eluate selection were systematically investigated and optimized. Under optimized conditions, the resin was observed to show a good sorption capacity of 17.02, 38.78 mg g^{-1} for Ni(II) and Cd(II), respectively with a regeneration cycle of 36. The prepared resin gives >96% recovery of Ni(II) and Cd(II) even in presence of a large number of competitive ions. The average column to column and day to day coefficient of variation of the method was found to be 2.96, 3.14% for Cd(II) and 3.46, 2.54% for Ni(II), respectively, which supports the good precision of the developed method. The reliability, reproducibility and linearity were evaluated by analysis of SRM, recovery experiments, and a good linear coefficient of variation (<5). The applicability of the resin for the systematic preconcentration of Ni(II) and Cd(II), is substantiated by the analysis of electroplating waste water and river water samples.

The sixth chapter is about **Immobilization of Graphene oxide sheets onto Amberlite Xad-16 by azo (-N=N-) linking.** A novel Graphene oxide immobilized Amberlite Xad-16 resin has been explored for its efficacy and applicability in solid phase extraction/ preconcentration coupled to atomic

absorption spectrometric determination Pb, Cd and Zn ions. The prepared resin was characterized by infrared spectroscopy, Raman spectroscopy, scanning electron microscopy, transmission electron microscopy, energy dispersive X-ray analysis, thermogravimetry, and powder X-ray diffraction analysis. The maximum static sorption capacity of prepared resin was 186.5, 140.5 and 39.24 mg g⁻¹ for Pb, Cd and Zn ions, respectively. Its ability to extract trace amount of lead, cadmium and zinc was exhibited by low preconcentration limits of 2.0, 5.88 and 5.88 µg L⁻¹ and high preconcentration factor 500, 170 and 170 for Pb, Cd and Zn ions, respectively. The experimental parameters affecting the solid phase extraction of cadmium including sample pH, sorption time, flow rate for sorption and desorption, volume and concentration of eluent, sample volume and concomitant ions were investigated. The accuracy of the method was verified by determining the Pb, Cd and Zn content in the Standard Reference Material (SRM) Vehicle exhaust particulates NIES 8 and by recovery experiments in spiked real samples. The proposed method was successfully used to preconcentrate Pb, Cd and Zn ions from electroplating waste water, river water local city tap water with >95% recovery.



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In the Memory of My Father
Dedicated to My Mother

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Certificate

This is to certify that the thesis entitled “**Preparation and characterization of chelating resins to develop column preconcentration method with flame atomic absorption spectrometric determination of trace elements**” being submitted by **Mr. Suneel Kumar**, for the award of **Doctor of Philosophy in Chemistry**, is a bonafide research work carried out by him under my supervision. The thesis, to my knowledge, fulfills the requirements for submission for the award of the degree of Doctor of Philosophy in Chemistry.

Dr. Aminul Islam

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At the outset, I wish to pay my most humble obeisance to the almighty God for giving me the wisdom and inspiration to take up this task. It has been Thy guiding hand that has brought this task to a successful completion.

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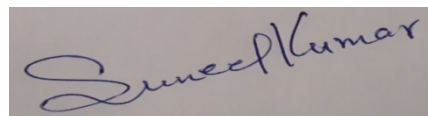
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LIST OF PUBLICATIONS

1. Aminul Islam and **Suneel Kumar**, Glycidylmethacrylate based resin functionalized with graphene oxide for column preconcentration and trace determination of Cd(II) and Ni(II) in environmental and food samples. **RSC Advances** 6 (2016) 77629
2. Aminul Islam, **Suneel Kumar**, Noushi Zaidi, Hilal Ahmad, SPE coupled to AAS trace determination of Cd(II) and Zn(II) in food samples using amine functionalized GMA-MMA-EGDMA terpolymer: isotherm and kinetic studies. **Food Chemistry** 213 (2016) 775-783.
3. Aminul Islam, Hilal Ahmad, Noushi Zaidi, **Suneel Kumar**, Copper selective self-sorting polymeric resin with mixed mode functionality for column preconcentration and atomic absorption spectrometric determination. **RSC Advances** 6 (2016) 5590 - 5598.
4. Aminul Islam, Hilal Ahmad, Noushi Zaidi, **Suneel Kumar**, A graphene oxide decorated with triethylenetetramine modified magnetite for separation of chromium species prior to their sequential speciation and determination via FAAS. **Microchimica Acta** 183 (2016) 289-296.
5. Aminul Islam, Noushi Zaidi., Hilal Ahmad, **Suneel Kumar**, Efficacy of dihydroxy-mercaptopurine functionalized polymeric resin for the trace determination of Cd by SPE coupled flame atomic absorption spectrometry- **RSC Advances** 5 (2015) 46662 -4667.

6. Aminul Islam, Noushi Zaidi., Hilal Ahmad, **Suneel Kumar**, Amine functionalized mesoporous polymer as potential sorbent for nickel preconcentration from electroplating wastewater. **Environmental Science and Pollution Research** 22 (2015) 7716 -7725.

7. Aminul Islam, Hilal Ahmad, Noushi Zaidi, **Suneel Kumar**, Graphene Oxide Sheets Immobilized Polystyrene for Column Preconcentration and Sensitive Determination of Lead by Flame Atomic Absorption Spectrometry. **ACS Applied Material Interfaces** 6 (2014) 13257-13265.

LIST OF PAPERS UNDER COMMUNICATION

1. Aminul Islam, **Suneel Kumar**, Hilal Ahmad, Noushi Zaidi, Graphene oxide supported on Amberlite resin for the development of analytical method based on systematic approach of column preconcentration/sensitive FAAS determination of toxic metal ions in environmental samples, **Analytica Chimica. Acta (Elsevier)**, **Communicated.**

2. Aminul Islam, Noushi Zaidi, Hilal Ahmad and **Suneel Kumar**, Functionalized MWCNTs for dispersive solid phase extraction and FAAS determination of Cd and Pb in environmental samples, **International Journal of Environmental Science and Technology (Springer)**, **Communicated.**

Dedication
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Chapter 1

Introduction



1.1 Scope and Objective of the Proposed Work

Since, the water and food samples are contaminated by toxic metal ions by their uncontrolled release into the environment through various activities. Therefore, it is important to examine and monitor its effect on human health and aquatic life. However, these metal ions are present at very low concentration and associated with other matrix ions in different samples. Thus, their direct determination is very challenging and required separation and preconcentration step. We illustrate the combination of solid phase extraction (SPE) with low sensitive analytical methods for accurate measurements of chemical species at extremely low concentrations. By considering their occurrence, the characteristics of toxic metal ions in the environment as well as the evolution of the directives which stipulate the limit values in water samples, the general objective of this thesis is the preparation of new chelating resins and to develop a new SPE method to separate and preconcentrate lead, cadmium, nickel, and zinc in real environmental samples prior to their accurate, precise and rapid determination using flame atomic absorption spectrometer (FAAS).

More specifically the following objectives were proposed as shown in figure 1.

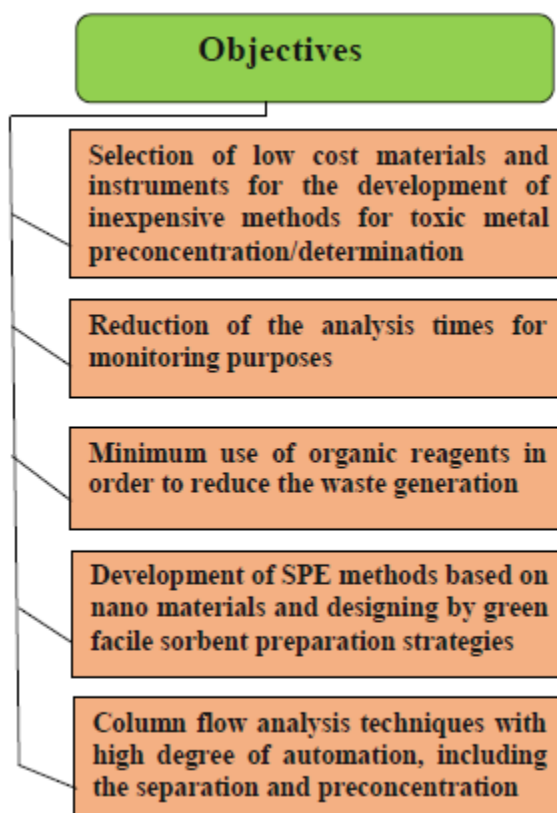


Figure 1.1: Objectives of the work

1.2 Heavy metals exposure in the environment

The basic and most important need of human and all living organism is water which comes from various water bodies such as rivers, lakes, wells, and natural hydrologies. Water is not only a need but also a prime source of survival in biosphere. Unfortunately, these systems are contaminated with various pollutants, as a result of sewage waste, marine dumping, industrial waste, underground storage leakages, atmospheric deposition and eutrophication due to uncontrolled expansion of industrializations and urbanization [1]. The pollutant contains various toxic metals such as lead, cadmium, nickel, arsenic, chromium, copper, mercury and zinc [2]. Human health is directly affected by these metals as they enters into the body through various pathways like inhalation, skin exposure or commonly via consumption of contaminated drinking water and food stuffs [3]. Metals in the environment may be present in the different states as solid, liquid and 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 [4-6]. The toxicity of these ions comes from its non-biodegradable nature and accumulation behavior in the human body for a longer time period; this would also affect the aquatic habitats. The metals having density greater than that of water is known as heavy metals and the ultimate sink for these metals are earth crust, soils and sediments. Among the various toxic metals, Arsenic, chromium, cadmium, lead, and mercury are extensively used in the industrial manufacturing. Few Heavy metals are listed below with their toxicological effect.

Lead

Lead is emerged as a highly toxic cumulative poison in humans as it is listed in B2 human carcinogen group by the US Environmental Protection Agency (USEPA) [7]. It brought into the all parts of the environment includes air, soil and water by its extensive use at large scale, such as construction material for equipment used in sulphuric acid manufacture, petrol refining, halogenation, sulfonation, extraction and condensation, in storage batteries, alloys, solder, ceramics and plastics. It is also used in the manufacture of pigments, tetraethyl lead and other lead compounds,

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. Among the children, the most significant source of lead is dust contaminated by lead-based paint used for window and floor renovation [8]

Health effect: Lead may cause fatigue, irritability, memory problems, reduction in sensory and motor reaction times, decision making impairment, and lapses in concentration. High exposure of lead can lead to irreversible brain damage, seizure, coma, and death. Children are much more easily affected by lead exposure than adults. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders [9]. About 50% of lead is absorbed with inhalation of dusts, 10-15% absorbed orally, out of which 90% is distributed to bones [10]. The maximum allowable limit of lead is $10 \mu\text{g L}^{-1}$ in drinking water as recommended by World Health Organization [11].

Cadmium

Cadmium is classified as one of the human carcinogenic, since it has exerts toxic effect on the skeletal, kidney and respiratory systems. It is generally present in the environment at low levels. However, human activity has greatly increased those levels. Cadmium is mostly used in production of metal like zinc, lead, or copper. It also used in batteries, pigments, stabilizers for plastics and photovoltaic devices. The major source of cadmium exposure is cigarette smoking in smokers while for non-smoking population it enters through food stuffs.

Health effect: Chronic exposure of cadmium induces genomic instability through complex and multifactorial mechanisms, including proteinuria, a decrease in glomerular filtration rate, and an increase in the frequency of kidney-stone formation that causes certain forms of cancer [12]. 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 [13,14].

Nickel

Nickel is the 24th most abundant element in earth's crust and used in the production of stainless steels, non-ferrous alloys, and super alloys [15]. It is also used in electroplating, as catalysts, nickel-cadmium batteries, coins, welding products, and in certain pigments and electronic products [16]. It released in to the environment through various processes like windblown dust, volcanoes, combustion of fuel oil, municipal incineration, and industries involved in nickel refining. Humans are exposed to it through breathing of air or smoking of tobacco containing nickel, eating contaminated food, as well as drinking of water.

Health effect: 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 can have asthma attacks following exposure to nickel. Lung effects, including chronic bronchitis causes reduced lung function, and has been observed in workers who breathed large amounts of nickel. Long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation. High levels of Nickel in the diet may be associated with an increased risk of thyroid problems, cancer, and heart diseases [17,18]

Zinc

Zinc is an essential trace element and has much great importance for humans, plants and animals. It plays a vital role in several biochemical processes and its compounds shows bactericidal activity. Zinc is widely used in industry to make paint, rubber, dye, wood preservatives, and ointments [19]. 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; extraction of gold by the cyanide process, purifying fats for soaps.

Health effect: Deficiency of zinc causes various diseases like ulcerative colitis, Crohn's disease, diarrhoea, and short bowel syndrome Anorexia, lethargy, diarrhoea, growth restriction (delayed bone maturation), impaired immune function and

susceptibility to infection. The overdoses of zinc can results abdominal pain, nausea, vomiting and diarrhoea [20-23].

Arsenic

In the priority list of hazardous substances issued by ATSDR 2015, arsenic got first rank [24]. The order of toxicity of arsenic in human is as, arsines> inorganic arsenites > organic (trivalent compounds arsenoxides) > inorganic arsenates>organic pentavalent compounds > elemental arsenic [25]. Reduced trivalent As(III) and oxidized pentavalent As(V) are two form of inorganic arsenic and can be absorbed and accumulated in tissues, and body fluids, particularly in keratin-rich tissues like hair, nail, and skin [26,27].

Health effect: The acute exposure of arsenic gives rise to vomiting, diarrhoea, weakness, prostration, and weight loss, cutaneous manifestations, hyperpigmentation, conjunctivitis, photophobia, pharyngitis, or irritating cough, asthma while chronic exposure results Bowen's disease, palmar keratosis, skin cancer, squamous cell carcinoma, acute myeloid leukemia [27, 28].

Mercury

Mercury is another toxic heavy metal which combines with several other elements to form inorganic and organic mercury compounds. 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 [29]. The primary sources of inorganic mercury (metallic mercury and inorganic mercury compounds) are mining ore deposits, burning coal and waste, and from manufacturing plants [30]. It enters in the water or in 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 vapour is the most important route of uptake of elemental mercury [31]. An organic mercury compound, namely methyl mercury, is produced mainly by small organisms in water and soil.

Health Effect: Mercury poisoning can result in several diseases, including Hunter-Russell syndrome, and Minamata disease. Mercury vapours are considered as highly toxic because it consists of free, neutral atoms. When the vapours inhaled, the atoms

diffuse from lungs to bloodstreams and cross the blood brain barrier and to reach in brain [30]. Acute inhalation of mercury (Hg^0) results noncardiogenic pulmonary edema leading to death. Inorganic mercury ingestion causes gastroenteritis, nephritic syndrome, or acute renal failure and cardiovascular collapse. High exposure of mercury during pregnancy causes derangement of fetal neuronal migration which gives severe mental retardation [28].

Chromium

Chromium is released into ecosystem from its wide usage in chrome tanning, electroplating, printing industries, oxidative dyeing and cooling water towers [32,33]. In an aqueous environment, chromium usually exists in trivalent Cr(III) and highly mobile toxic hexavalent Cr(VI) forms. USEPA and International Agency for Research on Cancer (IARC) [34, 35] declared Cr(VI) as carcinogenic since, it can causes cancer, mutations and skin dermatitis.

Health effect: Although Cr(III) is essential element for biological mechanisms but its high dose in the cell can leads to DNA damage. Human exposure to Cr (VI) compounds is associated with a higher potential risk of chromium-induced respiratory system cancer [36]. Chromium can diffuse in the forms of CrO_4^{2-} and HCrO_4^- through the cell membrane, and subsequently oxidize the biological molecules [37]. 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.

Drinking water guidelines for heavy metals: USEPA [38], World Health Organization (WHO) [39] and Canadian organization [40] recommended a regulatory limit of heavy metals in drinking water out of which some of them are listed in table 1.1.

Table 1.1 Recommended limits of heavy metal ions in drinking water

Heavy Metal	USEPA MAC^a (µg/L)	WHO MAC^a (µg/L)	Canadian MAC^a (µg/L)
Pb	15	10	10
Cd	5	3	5
Cr	100	50	50
As	10	10	10
Hg (inorganic)	2	6	1

^aMAC= maximum allowable concentration

1.3 Preconcentration

Quantitative determination of heavy metal in various samples including wastewater, water bodies, food and biological samples is important in order to control their assessment and effect on ecosystem. These metals present at a very low concentration in different systems associated with some coexisting ions. Hence, their direct determination is not possible even though, there are large numbers of sophisticated instrumentations available for metal ion determination [41-43]. So, it requires a preconcentration step for the selective separation by removing coexisting ions before subjected to determination without interferences. Preconcentration is an operation as a result of which the ratio of the concentrations or the amount of trace analytes increases, lowering the detection limit and simultaneously eliminates matrix interferences [44]. Preconcentration is not only concentrating the analytes but it also enhances the sensitivity and accuracy of the results, facilitates calibration and offers high degree of selectivity towards analytes. The coupling of preconcentration method to that of detection techniques improves the quality and reliability of the final results [45]. SPE is well established preconcentration technique helpful in sample-preparation step for both highly sensitive techniques like, ICP-MS [46-49] and ET-AAS [50-52] often suffering from strong interferences, and the less sensitive techniques such as FAAS [53-56], often exhibiting a limit of quantification (LOQ) that is insufficient for metal ion determination in many real samples. The use of chelating sorbents in the preconcentration of analyte in a flow mode process shortens the time of the overall procedure where the analyte separation takes place under non-

equilibrium conditions. Depending on the purpose, trace elements can be enriched selectively or in combinations and either separation of the matrix or separation of the trace components can be used. For the analysis of high purity metals, matrix removal is used. If the matrix contains several elements forming complex compounds (geological and biological materials), it is better to isolate 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. Of the analytical techniques for preconcentration and separation, SPE has been preferred over other traditional procedures, such as liquid-liquid extraction (LLE) [57,58], cloud point extraction (CPE) [59,60], and electrochemical methods mainly because it is more efficient and much less time-consuming also avoids large use of organic solvents. Many other methods are used for analyte preconcentration can be classified according to their mode of separations and the number and nature of the phases involved in the separation process are as follows:

- ✓ Membrane filtration [61]
- ✓ Co-precipitation [62,63]
- ✓ Electrochemical Techniques [64]
- ✓ Crystallization [65]
- ✓ Ion Flotation [66]
- ✓ Stir-bar sorptive extraction (SBSE) [67]
- ✓ Solidified floating organic drop microextraction (SFODME) [46]
- ✓ Solid-phase extraction (SPE) [68,69]

1.4 Solid Phase Extraction (SPE)

Analytical chemists are continuously searching and giving priority towards sample preparation methods that are faster, easier, safer, eco-friendly and less expensive, to provide accurate and precise data. SPE is a sample preparation technique for the separation of analyte ions from a mixture of impurities according to their physical and chemical properties. Now a day's use of chelating resin in SPE turned out to be active research area in the field of separation science. The basic approach for SPE requires a packed sorbent which could be a column, a cartridge, a tube or a disk that retains the analyte after passing the aqueous sample through it. The separation ability of SPE 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. After passing of the sample solution, the desired analytes were adsorbed or retain by the sorbent and finally eluted by using some stripping agents and their concentration can be determined by sophisticated analytical instruments.

Basic principles

SPE is similar in principle as that of liquid-liquid extraction (LLE), since it involving the partitioning of solute between two phases. The only difference is that, LLE involves the partitioning of two immiscible liquid phases while in SPE partitioning of liquid phase (sample matrix) and a solid phase (sorbent) takes place. SPE enables the concentration and separation of analytes from different solutions by sorption on a solid sorbent. The sorption of analytes from aqueous solutions by sorbent is carried out by various mechanism, like adsorption, chelation, ion exchange and other chemical reactions on or in the sorbent. In highly non-polar solid sorbent, the trace elements are usually adsorbed on solid phase through van der Waals forces or hydrophobic interaction. The strong or weak cationic or anionic functional groups in ion exchange sorbent can exchange with the associate counter analyte ion. Chelating sorbents having functional groups that contains donar atoms like N, O and S which form chelates with metal ions particularly in metal ion extraction. The entire solution that contains analytes has been passed through the sorbent and the sorbed analytes are then subsequently recovered by using an appropriate eluting agent.

SPE Procedure

SPE technique involves four steps operation as shown in figure 1.2.

STEP 1: Conditioning

The solid sorbent should be preconditioned using an appropriate solvent, followed by the same solvent as the sample solvent. It removes the impurities initially contained in the sorbent and enables the wetting of the packing material and the solvation of the functional groups. It also removes the air present in the column and fills the void volume with solvent.

For the effective sorption and good recoveries care must be taken, solid sorbent do not allows drying between conditioning and treatment step.

STEP 2: Sample loading

The second step is the percolation or loading of the sample containing analytes through the solid sorbent packed in the column. The volume of the sample varied according to the system used. The sample loading can be applied to the column by

gravity, pumping through peristaltic pump, aspirated vacuum or by an automated system.

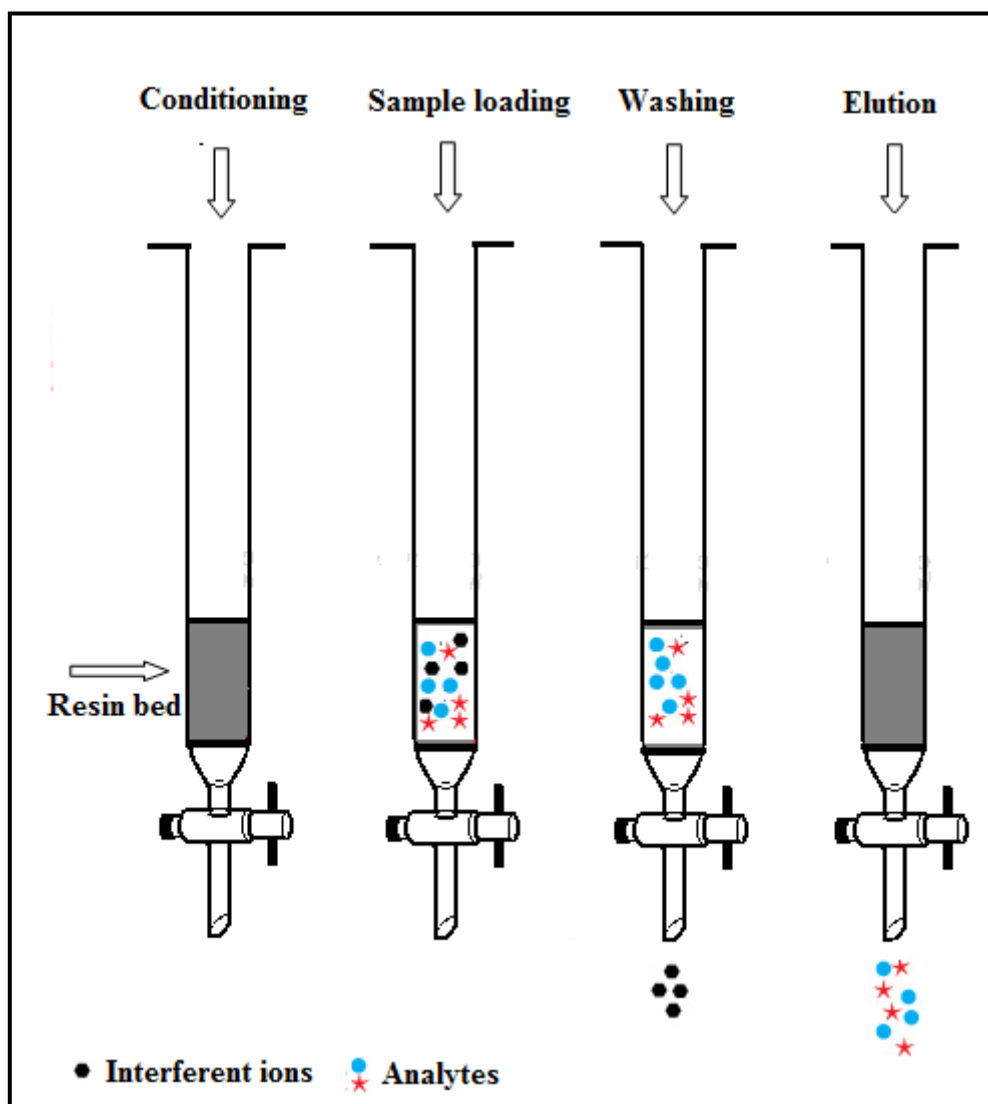


Figure 1.2: SPE operation steps

STEP 3: Washing

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. 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: Elution

The final step is the elution of the analytes of interest by an eluting solvent, without removing retained matrix components. The volume of the eluting solvent should be varying in such a manner so that quantitative recovery of the analyte is achieved subsequent low dilution.

Advantages and scope of SPE

SPE offers a number of important advantages compared to classical liquid-liquid extraction (LLE), these are as follows:

- ✓ Simple methodology
- ✓ Flexibility and stability
- ✓ Fast phase separation with absence of emulsion
- ✓ High enrichment factor with reproducibility
- ✓ Low cost because of lower consumption of reagents
- ✓ Field sampling
- ✓ Safety with respect to hazardous samples
- ✓ Ease of automation and more scope for coupling to different detection techniques

Moreover, SPE can eliminate the use of consumption of organic solvents from the sample preparation process since, the analyte can be directly extracted from the liquid sample onto the solid sorbent, and principally when analytical measurements are carried out on the solid support. SPE is a popular preconcentration technique for its ability to work when coupled with different sophisticated analytical detection techniques such as inductively coupled plasma optical emission spectrometry (ICP-OES), and ICP-MS, and FAAS: off-line and on-line. For the analysis of complex samples offline SPE are preferred due to its greater flexibility and ability to analyze the same extract using various techniques. However, compared to off-line procedures, adaptation of the SPE technique to on-line flow conditions enables us to obtain the higher sample throughput, thanks to the whole procedure being realized automatically under non-equilibrium conditions, lower sample consumption, and lower sample contamination resulting from working in a closed system [53]. In addition, the limits of detection (LODs) and enrichment factors (EF) of the offline method are far better than those of on-line.

1.5 Types of SPE sorbents

SPE sorbents have been classified into various categories according to their nature of solid support or matrix as listed in figure 1.3.

Polymeric resins/materials

Number of sorbents, such as polystyrene and glycidylmethacrylate resins, silica and silica gel, cellulose, polyurethane foam, molecular imprinted polymers (MIPs) and other supports, has been employed as SPE sorbents. Nascent polystyrene divinylbenzene resins such as Amberlite XAD-1180 [70], XAD-4 [71], and XAD-16 [72] are used directly for enrichment of inorganic species as their halide or thiocyanate complex. Polystyrene resins involves van der Waals forces interaction mechanism; 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. The properties of macroporous hydrophobic resins of the Amberlite XAD series (polystyrene divinylbenzene resins) such as porosity, uniform pore size distribution, high surface area and chemical stability towards acids, bases, and oxidizing agents and ease in regeneration makes it superior support for the preparation of chelating resin over other matrices. Silica-based sorbents have drawbacks like, instability at extreme pH values, low recovery for polar compounds, presence of residual groups; they are not wettable by water alone and always need a conditioning step with a wetting solvent, such as methanol. Despite the high selectivity in MIPs, they suffers with the limitations like as number of steps, long time requirement, low binding capacity and less site accessibility.

Nanostructured materials as SPE sorbents

In recent years, nanomaterials are extensively used as SPE sorbents in the various field of analytical chemistry for the development of new methodologies, since their specific physical and chemical properties associated with their nano-size structure also provide numerous advantages in this field. The nano-sized structures may be in the form of particles, pores, wires or tubes have great application in sample preparation procedures for sample clean-up and preconcentration of the analytes [73-75]. The physicochemical properties of these nanomaterials can be controlled through structural design, incorporation of suitable metallic or non-metallic component or by modification of their surfaces. The physical, material and chemical properties of

nanomaterials are directly related to their intrinsic composition, apparent size and extrinsic surface structure. Majority of nanomaterials were classified as metallic nanomaterials (Au Nps, Ag NPs, Fe₃O₄, TiO₂, Al₂O₃, ZrO₂, MnO, CeO₂ etc.), silica nanomaterials (Si NPs) and carbon based nanomaterials (CNTs, MWCNTs) [75-77]. Nanoparticles were used for the separation and determination of analytes [77], or even detection after chemical modification by a desired reagent. Nanoparticles are immobilized onto solid support, mainly with magnetic properties. Iron oxide nanoparticles such as magnetite (Fe₃O₄) or its oxidized form maghemite (γ-Fe₂O₃) are the most popular because of its strong magnetic character and can be easily isolated from sample solution by applying external magnetic field without additional filtration process [78,79]. The silanol groups on the silica coating provide many possibilities for surface functionalization of metallic nanoparticles [80].

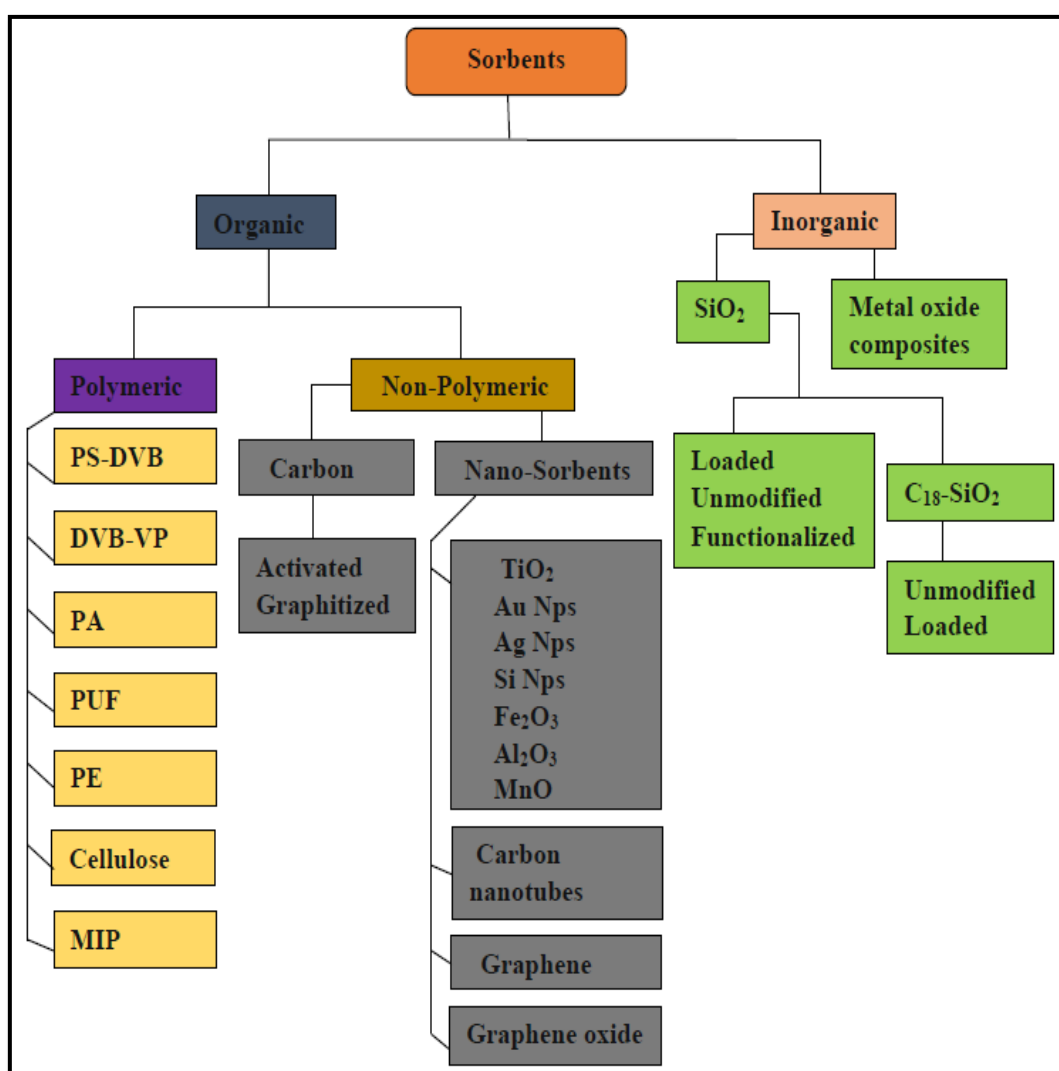


Figure 1.3: Different types of SPE sorbents

Carbonaceous nanomaterials as SPE sorbent

Carbon nanotubes

Since the discovery of carbon nanotubes (CNTs) in 1991 [81], the development of SPE sorbent using CNTs has been turned out to be the active research area in analytical sciences. The properties like as large surface area, especially on the outside, and interstitial spaces within nanotubes bundles and also their chemical, mechanical and thermal stability makes CNTs as suitable sorbent for SPE. In addition, the manipulable surface chemistry of CNTs offer both chemical or physical functionalization by introducing simple organic groups, biomolecules, polymers, bacteria, and others; in order to optimize specificity of the approach or improve subsurface mobility for sorption of target analytes even at trace levels. Multiwalled carbon nanotubes (MWCNT) have been more frequently used as SPE for analyte extraction rather than single walled carbon nanotubes (SWCNTs) in analyte extraction although they are smaller in diameter and have high surface area. This may be due to the fact that SWCNTs are more acid resistant than of MWCNTs and required more aggressive conditions for the acidic oxidation in order to introduce functional groups like hydroxyl, carboxyl and carbonyl, in latter it is favourable [82].

Graphene and Graphene oxide

In the last few years, researchers from the entire globe have been focusing to graphene and graphene oxide as superior SPE sorbent for the sample preparation [83]. Since, it shows adsorptive properties apart from mechanical, optical and thermal properties. Graphene oxide contains large quantities of oxygen atoms on its surface as hydroxyl, epoxy, carbonyl groups [84], therefore, possesses much more hydrophilicity and these oxygen atoms have lone pair electrons and by the sharing of electrons they can efficiently bind metal ion to form a metal complex and in addition with the large specific surface, it shows high sorption capacity towards metal ions [85]. For selective separation or speciation of metal ions graphene or graphene oxide can be further chemically modified and studied in both batches as well as in column procedures [86,87]. The irreversible aggregation of graphene oxide can be restricted by its immobilization onto a solid support for column operation. Graphene/graphene oxide are used for preconcentration of organic compounds and trace metal ions from water,

food, biological and environmental samples using chromatography and spectroscopy techniques [88-91] by SPE, SPME and magnetic SPE methods.

1.6 Commonly analytical techniques coupling with SPE

Some frequently used analytical techniques in combinations with preconcentration may be described as follows:

Adsorptive stripping Voltammetry

Anodic Stripping Voltammetry (ASV) is popular in trace analysis, mainly of heavy metal ions, because of the low limit of determination-ranging to sub ppb concentrations, its accuracy and precision, as well as the low cost of instrumentation. 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 cathodic stripping voltammetry (CSV), the reduction process of the mercury compound on the electrode surface is studied. The electrolytic accumulation of the species on the working electrode is leading to a substantial increase in the sensitivity in both types of methods.

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 [92-94].

X-Ray fluorescence analysis

X-ray fluorescence (XRF) spectrometry is used for the analysis of liquid samples, the liquid in a specially designed liquid sample holder is irradiated directly with X-rays. However, the abundance of effective amounts of analytes in the irradiated volume is insufficient for the determination of trace metals in natural water for direct analysis of liquid samples. Therefore, for the quantitation of trace metals in aqueous samples by XRF, it is necessary to preconcentrate the analytes through accepted sample pretreatments [95-97]. 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.

Spectrophotometric determination

In the visible and ultraviolet regions combinations of concentration, especially extractive, with spectrophotometry are more commonly used.

Individual concentration steps or successive separations of several elements are usually preferred. The matrix is very rarely separated in this case. Most frequently, the reagent used for concentration also gives a colored complex with the analyte. 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 [98-100].

Neutron-activation analysis

In this method two distinct ways of trace concentration are involved: 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 [101,102].

Electrothermal Atomic Absorption Spectrometry

Electrothermal atomic absorption spectrometry (ETAAS) is most powerful and popular analytical tool due to their high selectivity and sensitivity for the determination of low concentration of metal ions, present in environmental samples and biological materials. 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 [50-52].

Inductively coupled plasma optical emission spectrometry (ICP-OES)

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique often employed to determine metal ions in various types of samples [103]. However, 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. Hence, preconcentration and separation procedures have been devised to allow trace amounts of metal ions to be determined in complex matrices using ICP-OES [104].

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 [46-48].

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 [105,106].

Flame Atomic Absorption Spectrometry (FAAS)

Flame atomic absorption is well established and most widely used technique for the elemental determination in various types of samples with good sensitivity at the level of ppm or less. FAAS is applicable where the analyte is in solution or readily solubilised. The priority of FAAS among various other sophisticated techniques for metal detection is because of its desirable characteristics such as operational facilities, good selectivity, and low cost. However, in the presence of a very high excess of

diverse ions compared with the level of analyte, some limitations, mainly those related to the sensitivity are observed. Under trace analysis, it needs a preconcentration and /or separation step of trace elements which is responsible for the improvement of detection limits and selectivity of their determination. For effective preconcentration, solid phase extraction has been coupled with FAAS. The prime advantage of this technique is the possibility of using a relatively simple detection system with flame atomization instead of a flameless technique, which requires more expensive equipment and is usually much more sensitive to interferences from macro components of various natural matrices [41].

Principles

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample and it relies therefore heavily on Beer-Lambert law. The sample that contains analyte is aspirated into a flame and converted to atomic vapours. Now flame only has atoms of the analyte. Amongst these atoms few of them are promoted to excited state but most of them are in ground state. The ground state atoms absorb energy of particular wavelength. The absorption of resonant radiation by ground state atoms of the analyte is used as the analytical signal. 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. A block diagram of Atomic absorption spectrometer has been shown in figure 1.4 indicating the necessary equipments.

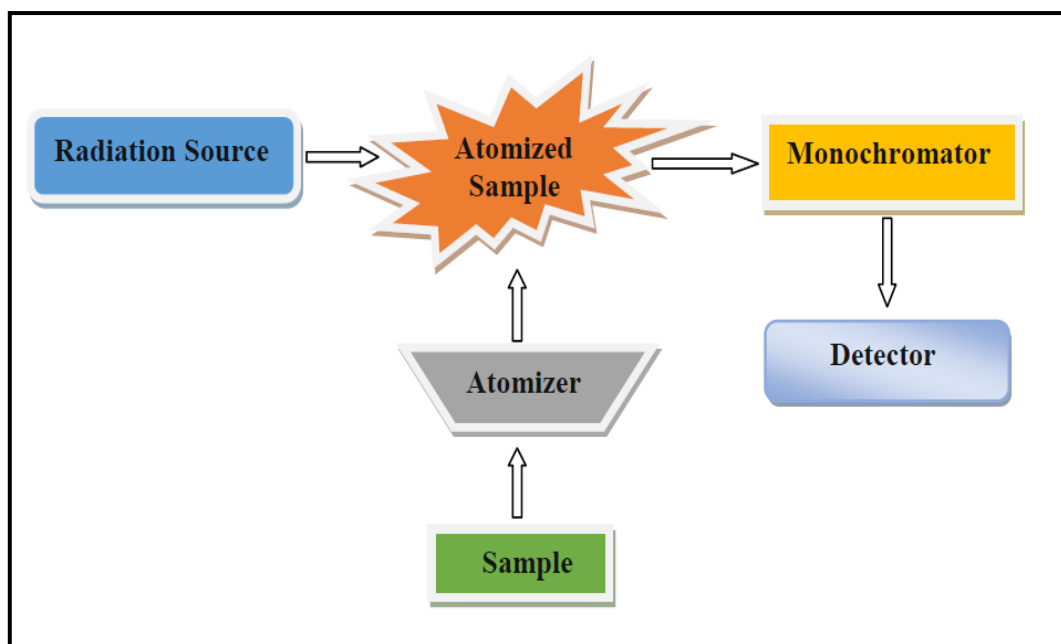


Figure 1.4: Atomic absorption spectrometer block diagram

Atomizer

In this technique flame used as atomizer for the sample [107], but other atomizers such as a graphite furnace [108] or plasmas, primarily inductively coupled plasma, can also be used [109].

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. During atomization several process are involved as described in block diagram (Figure 1.5).

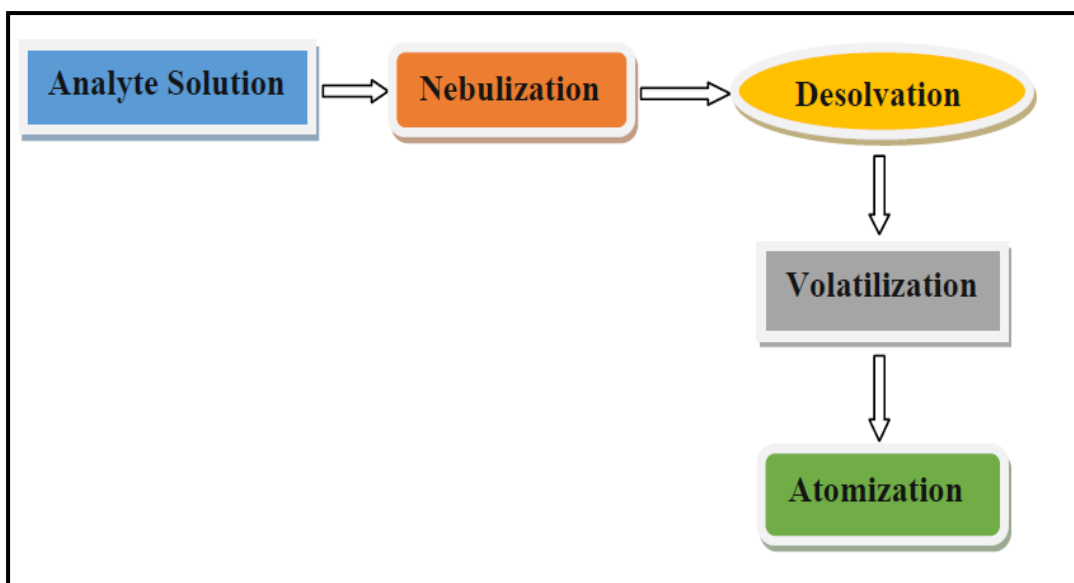


Figure 1.5: Different process during analysis of sample

Analysis of liquids

Nebulization: A solution of the analyte is nebulized by a flow of gaseous oxidant, mixed with a gaseous fuel, and carried into a flame.

Desolvation - the liquid solvent is evaporated, and the dry sample remains.

Volatilization (Ashing) - the solid sample vaporizes to a gas.

Atomization- the compounds making up the sample are broken into free atoms

Radiation Sources

In Atomic absorption, a sharp line source is selected, since the width of the absorption line is very narrow.

Hollow cathode lamps (HCLs)

HCLs are the most common radiation source in atomic absorption spectroscopy. Inside HCL, 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 returned to lower energy states and emit light with the frequency characteristic to the metal [107,110]. The HCL emitted lines are passed through the flame and absorbed by the test analyte. Many modern HCLs are selective for several metals.

Diode lasers

Diode lasers can also be used in atomic absorption spectroscopy because of their good properties for laser absorption spectrometry [107]. The technique is then either referred to as diode laser atomic absorption spectrometry (DLAAS or DLAS) [111], or since wavelength modulation most often is employed, wavelength modulation absorption spectrometry.

Interferences

Spectral interferences

Spectral interferences in FAAS are less frequently observed compare to plasma generated techniques (ICP-OES). Spectral interferences are arises mainly due to following causes:

- ✓ When the resonance line emitted by the HCL overlap with absorption line of the other element.
- ✓ The presence of combustion products that exhibits broad band absorption or scatter radiation that reduces the power of the transmitted radiation and results positive analytical error.
- ✓ Source of absorption or scattering originates in the sample matrix which reduces the power of the transmitted beam not the incident beam and lead to a positive error in absorbance and thus concentration results.
- ✓ Sample contains organic species or organic solvents used in order to dissolve the sample. Carbonaceous particles that are left by the incomplete combustion of organic matrices are responsible for the scattering of light.

Chemical Interference

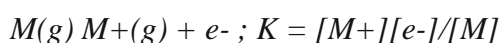
Chemical interference may encounter when the sample containing components are not decomposed in flame. Usually, these components form refractory oxides when aspirated into the flame and fail to convert into free atoms. This problem can be overcome by the following methods, one is by adding an excess of another element, which will also form a thermally stable compound with the interferent. In the case of calcium, lanthanum is added to tie up the phosphate and free the calcium to be atomised making the calcium absorbance independent of the amount of phosphate. In second method, problem can be overcome by increasing the amount of energy means using hotter flame. The nitrous oxide-acetylene flame is considerably hotter than air-acetylene and can often be used to minimise chemical interferences for the elements

generally determined with air-acetylene. In case of calcium, interference by phosphate is not observed in nitrous oxide-acetylene flame, eliminating the need for the addition of lanthanum.

Ionisation Interference

Ionization interference is often observed in very hot flame where ground state gaseous atoms may be further ionized. If additional energy is applied, the ground state atom can be thermally raised to the excited state; or if thermal energy is sufficient, the electron may be totally removed from the atom, creating an ion. As these electronic rearrangements deplete the number of ground state atoms available for light absorption, atomic absorption at the resonance wavelength is reduced. When an excess of energy destroys the ground state atom, ionisation interference exists.

For any element, the gas phase ionization equation is



Ionisation interference can be overcome by adding excess of an element which is more easily ionised, creating a large number of the electrons in the flame and suppressing the ionisation of an analyte. Sodium or potassium is commonly used as an ionisation suppressant.

Physical interferences:

Physical interferences originate when the variation in the physical properties of the analytical sample such as viscosity, surface tension, vapour pressure and temperature were observed. The change in these properties will have effect on sample intake and atomization efficiency. Physical interferences can be controlled by dilution of sample and using internal standard.

Methods for reduction of interferences

- ✓ Ensure if possible that standard and sample solutions are of similar bulk composition to eliminate matrix effects.
- ✓ Change 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

The atomic line split in to two sidebands when magnetic field is used. 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 HCL 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 [112].

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.

1.7 Statistical Treatment of Data

Analytical chemistry besides providing the methods and tools needed for insight into our material world [113], seeks to improve the reliability of existing techniques to meet the demands for better chemical measurements which arise constantly in our society [114]. 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 [115]. 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 ($\sum x_i$) 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.

$$\bar{x} = \frac{\sum x_i}{N}$$

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.

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N - 1}}$$

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 μ (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 (μ) expected to lie, within a given degree of probability. The likelihood that the true value lays within the range is called confidence level or probability usually expressed as a percent.

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

where t is the student's t value which depends on the desired confidence level and this equation holds when σ , 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.

$$r = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{\sqrt{[n\sum x_i^2 - (\sum x_i)^2][n\sum y_i^2 - (\sum y_i)^2]}}$$

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

$$b = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{n\sum x_i^2 - (\sum x_i)^2}$$

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 $a = \bar{y} - b\bar{x}$ 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 [110, 115-116].

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): $\gamma = m/s_s$; where s_s = standard deviation of measurement and m is the slope of the calibration curve [117].

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 [118]:

$C_L = \bar{x} + k \cdot s_{bl}$; where \bar{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 [119].

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 \bar{x} ; and errors in the slope and the intercept of the regression line is acceptable as a measure of s = 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 [119]. Hence, the detection limit (C_L) may also be expressed as: $CL = 3s_{bl}/b$; where b is the slope of the calibration line.

References

- [1] Howard Frumkin, Environmental Health: From Global to Local (Public Health/Environmental Health) 2nd ad. (2010).
- [2] Fenglian Fu, Qi Wang, Removal of heavy metal ions from wastewaters: A review, Journal of Environmental Management 92 (2011) 407-418.
- [3] U. Förstner, G. T. W. Wittmann, Metal Pollution in the Aquatic Environment, 2nd ad. (1983).
- [4] E.S. Deevey, Mineral Cycles. In: The Biosphere. A Scientific American Book, p. 83 (1970).
- [5] G.D. Guthrie Jr., Biological effects of inhaled minerals, Am. Mineral 77 (1992) 225.
- [6] W. S. Fyfe, Toward 2050: the past is not the key to the future- challenges for the science of geochemistry, Environ Geol. 33 (1998) 92.
- [7] Wagner HP, Determination of lead in beer using Zeeman background corrected graphite furnace atomic absorption spectrometry, J. Am. Soc. Brew. Chem. 53 (1995) 141–144
- [8] S. L. Dixon, J. M. Gaitens, D. E. Jacobs, W. Strauss, J. Nagaraja, T. Pivetz, J. W. Wilson, P. J. Ashley, Exposure of U.S. Children to Residential Dust Lead, 1999–2004: II. The Contribution of Lead-Contaminated Dust to Children's Blood Lead Levels, Environ Health Perspect. 2009 117(3): 468-474.
- [9] D C Rice, Behavioral effects of lead: commonalities between experimental and epidemiologic data, Environ Health Perspect 104 (1996) 337–351.
- [10] J. M. Schwartz, B. S. Simon, D. B. Roche, K. Stewart, Characterization of toxico kinetics and toxico dynamics with linear systems theory: application to lead associated cognitive decline, Environ. Health Perspect. 109 (2001) 361.
- [11] WHO, Lead in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. World Health Organization, Geneva, 2003 (WHO/SDE/WSH/03.04/9).
- [12] M. Khairy, Sherif A. El-Safty, M. A. Shenashen, Environmental remediation and monitoring of cadmium, TrAc, Trends Anal. Chem. 62 (2014) 56.
- [13] International agency for research on cancer (IARC), in: Proceedings of the meeting of the IARC working group on beryllium, cadmium, mercury and exposures in the glass manufacturing industry, Scand. J. Work. Environ. Health. 19 (1993) 360.

- [14] M. Satoh, H. Koyama, T. Kaji, H. Kito, C. Tohyama, Perspectives on cadmium toxicity research, *Tohoku J Exp Med.* 196 (2002) 23-32.
- [15] Nickel in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality (2005).
- [16] IARC Nickel and nickel compounds. In: Chromium, nickel and welding. Lyon, International Agency for Research on Cancer, pp. 257–445 (1990) (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 49).
- [17] M. Stoeppler, Analysis of nickel in biological materials and natural waters, in: J.O. Nriagu (Ed.), John Wiley, New York(1980).
- [18] E. Nieboer, D.M. Templeton, Nickel biogeochemistry, *Sci. Total Environ.* 148 (1994) 109.
- [19] L.G. Cockerham, B.S. Shane, Basic Environmental Toxicology, CRC Press, Boca Raton, Ann Arbor, London(1994).
- [20] MA Brown, JV Thom, GL Orth, P Cova, J. Juarez, Food poisoning involving zinc contamination. *Arch. Environ. Health* 8 (1964) 657–660.
- [21] Zinc - Fact Sheet for Health Professionals; Office of Dietary Supplements, National Institutes of Health, June 2013.
- [22] Risk Assessment - Zinc; Expert Group on Vitamins and Minerals (2003).
- [23] Acrodermatitisenteropathica; Genetic and Rare Diseases Information Center, US Department of Health & Human Services, Sept 2011
- [24] ATSDR, Division of Toxicology and Human Health Sciences, Priority List of Hazardous Substances (2015).
- [25] H. B. Bradl, Heavy metals in the environment, Vol. 6, 2005, Elsevier, UK.
- [26] K. Ueki, T. Kondo, Y. H. Tseng, C.R. Kahn, Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse, *Proc. Natl. Acad. Sci. U.S.A.* 101 (2004)10422-10427.
- [27] Sarmishtha Chatterjee, Shuvasree Sarkar, and Shelley Bhattacharya, Toxic Metals and Autophagy, *Chem. Res. Toxicol* 27 (2014) 1887-1900.
- [28] H.Hu, In Harrison's Principles of Internal Medicine,D. L. Kasper, E. Braunwald, A. S. Fauci, S. L. Hauser, D. L. Longo, J. E. Jameson, Eds. McGraw-Hill: New York, Chapter 376(2005) pp 2577–2580.

- [29] D. Jay, Glutathione inhibits SOD activity of Hg, Arch. Inst. Cardiol. Mex. 68 (1998) 457.
- [30] C. Baird, M. Cann, Environmental chemistry (4th ed.). New York: Freeman, Ch 15 (2008).
- [31] WHO, Environmental Health Criteria 101: Methyl Mercury. World Health Organization, Geneva (1990).
- [32] N. Unceta, F. Seby, J. Malherbe, Donard OFX, Chromium speciation in solid matrices and regulation: a review. Anal Bioanal Chem 397 (2010)1097–1111.
- [33] A. A. Hasin, S. J. Gurman, L. M. Murphy, A. Perry, T. J. Smith, P. H. E. Gardiner, Remediation of chromium(VI) by a methane-oxidizing bacterium. Environ Sci Technol 44 (2010)400–405.
- [34] USEPA, Toxicological Review of Hexavalent Chromium, Washington, DC, (1998)
- [35] S. Langard, M. Costa, Handbook on the Toxicology of Metals, CA, USA, (2007)
- [36] B. Weiss, Vulnerability of children and the developing brain to neurotoxic hazards, Environ. Health Perspect. 108 (2000) 375.
- [37] C. J. Lin, S. L. Wang, P. M. Huang, Y. M. Tzou, J. C. Liu, C. Chen, J. H. Chen, C. Lin, Chromate reduction by zero-valent Al metal as catalyzed by polyoxometalate, Water Res. 43 (2009) 5015.
- [38] 2012 Edition of the Drinking Water Standards and Health Advisories, Office of Water U.S. Environmental Protection Agency Washington, DC Spring 2012.
- [39] Guidelines for Drinking-water Quality 4th edn, WHO, 2011.
- [40] Health Canada (2014), Guidelines for Canadian Drinking Water Quality- Summary Table, Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.
- [41] K. Pyrzynska, M. Trojanowicz, Functionalized cellulose sorbents for preconcentration of trace metals in environmental analysis, Crit. Rev. Anal. Chem. 29 (1999) 313.
- [42] C. Karadas, D. Kara, A. Fisher, Determination of rare earth elements in seawater by inductively coupled plasma mass spectrometry with off-line column preconcentration using 2,6-diacetylpyridine functionalized Amberlite XAD-4, Anal. Chim. Acta 689 (2011) 184.

- [43] S. Qian, Z. Huang, J. Fu, J. Kuang, C. Hu, Preconcentration of ultra-trace arsenic with nanometre-sized TiO_2 colloid and determination by AFS with slurry sampling, *Anal. Methods* 2 (2010) 1140.
- [44] E. Jackwerth, A. Mizuike, Y. A. Zolotov, H. Berndt, R. Hohn, N. M. Kuzmin, separation and preconcentration of trace substances preconcentration for inorganic Trace analysis pure appl. Chem. 51 (1979) 1195.
- [45] L. A. Escudero, L. D. Martinez, J. A. Salonia, J. A. Gasquez, Determination of Zn(II) in natural waters by ICP-OES with on-line preconcentration using a simple solid phase extraction system, *Microchem. J.* 95 (2010) 164.
- [46] Shizhong Chen, Jianfen Li, Dengbo Lu, Yan Zhang, Dual extraction based on solid phase extraction and solidified floating organic drop microextraction for speciation of arsenic and its distribution in tea leaves and tea infusion by electrothermal vaporization ICP-MS, *Food Chemistry* 211 (2016) 741–747.
- [47] Shaowei Su, Beibei Chen, Man He, Bin Hu, Zuowei Xiao, Determination of Trace/Ultratrace Rare earth elements in environmental samples by ICP-MS after magnetic solid phase extraction with $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{polyaniline-graphene oxide}$ composite, *Talanta* 119 (2014) 458–466.
- [48] J. E. Osullivan, R. J. Watson, E. C. V. Butler, An ICP-MS procedure to determine Cd, Co, Cu, Ni, Pb and Zn in oceanic waters using in-line flow injection with solid-phase extraction for preconcentration, *Talanta* 115 (2013) 999.
- [49] C. G. Vogiatzis, G. A. Zachariadis, Tandem mass spectrometry in metallomics and the involving role of ICP-MS detection: A review, *Anal. Chim. Acta* 819 (2014) 1.
- [50] B. E. S. Costa, N. M. M. Coelho, L. M. Coelho, Determination of arsenic species in rice samples using CPE and ETAAS, *Food Chemistry* 178 (2015) 89.
- [51] J. Wang, E. H. Hansen, Trends and perspectives of flow injection/sequential injection on-line sample-pretreatment schemes coupled to ETAAS, *Trac Trends Anal. Chem.* 24 (2005) 1.
- [52] M. Savioa, B. Parodi, L. D. Martinez, P. Smichowski, R. A. Gil, On-line solid phase extraction of Ni and Pb using carbon nanotubes and modified carbon nanotubes coupled to ETAAS, *Talanta* 85 (2011) 245.

- [53] A. Tobiasz, S. Walas, Solid-phase-extraction procedures for atomic spectrometry determination of copper, *Trac Trends Anal. Chem.* 62 (2014) 106.
- [54] R. Gurkan, N. Altunay, Determination of total Sn in some canned beverages by FAAS after separation and preconcentration, *Food Chemistry* 177 (2015) 102.
- [55] Zahra DehghaniFirouzabadi, Ali Mohammad Haji Shabani, Shayessteh Dadfarnia, Mohammad Hassan Ehrampoush, Preconcentration and speciation of thallium by ferrofluid based dispersive solid phase extraction and flame atomic absorption spectrometry, *Microchemical Journal* 130 (2017) 428-435.
- [56] Xue Qi, Shuang Gao, Guosheng Ding, An-Na Tanga, Synthesis of surface Cr (VI)-imprinted magnetic nanoparticles for selective dispersive solid-phase extraction and determination of Cr (VI) in water Samples, *Talanta* 162 (2017) 345–353.
- [57] S. Mitra, Sample preparation techniques in analytical chemistry, in: *Chemical analysis: A series of monographs on analytical chemistry and its applications*, J. D. Winefordner (Ed), vol. 162, John Wiley & Sons, Inc., Hoboken, New Jersey (2003).
- [58] Keng-Chang Hsu, Cheng-Fa Lee, Yu-Ying Chao, Chih-Chang Hung, Po-Chih Chen, Chien-Hu Chiang and Yeou-Lih Huang, Ultrasound-assisted hollow fiber/ionic liquid-based liquid phase microextraction using an ionic liquid solvent for preconcentration of cobalt and nickel ions in urine samples prior to FAAS determination, *J. Anal. At. Spectrom.*, 31(2016) 2338.
- [59] Reyhaneh Rahnema, Marzieh Najafi, The use of rapidly synergistic cloud point extraction for the separation and preconcentration of trace amounts of Ni (II) ions from food and water samples coupling with flame atomic absorption spectrometry determination, *Environ Monit Assess* (2016) 188: 150.
- [60] Muhammad Bilal, Tasneem G. Kazi, Hassan I. Afridi, Mohammad Balal Arain, Jameel A. Baig, Mustafa Khan, Naeemullah Khan, Application of conventional and modified cloud point extraction for simultaneous enrichment of cadmium, lead and copper in lake water and fish muscles, *Journal of Industrial and Engineering Chemistry* 40 (2016) 137–144.
- [61] M. Soylak, Y. E. Unsal, N. Kizil, A. Aydin, Utilization of membrane filtration for preconcentration and determination of Cu(II) and Pb(II) in food,

water and geological samples by atomic absorption Spectrometry, Food Chem. Toxicol. 48 (2010) 517.

- [62] Mustafa Soylak, Ayse Aydin, NebiyeKizil, Multi-Element Preconcentration/Separation of Some Metal Ions in Environmental Samples by Using Co-precipitation, Journal of AOAC International, 99 (2016) 273-278.
- [63] Volkan Numan Bulut, Hamit Demirci, Duygu Ozdes, Ali Gundogdu, OlcayBekircan, Mustafa Soylak, Celal Duran, A Novel Carrier Element-free Co-precipitation Method for Separation/Preconcentration of Lead and Cadmium Ions from Environmental Matrices, Environmental Progress & Sustainable Energy 2016, DOI 10.1002/ep
- [64] A. J. Bard, Electroanalytical chemistry, vol. 16, Marcel Dekker, New York (1989).
- [65] H. E. Usdowski, Fraktionierung der spurenelementebei der kristallisation, Springer-Verlag, Berlin (1975).
- [66] N. Hagadone, H. Zeitlin, The separation of vanadium from sea water by adsorption colloid flotation, Anal. Chim. Acta 86 (1976) 289.
- [67] J. I. Cacho, N. C. Campillo, P. Vinas, M. Hernandez-Cordoba, Stir bar sorptive extraction polar coatings for the determination of chlorophenols and chloroanisoles in wines using gas chromatography and mass spectrometry, Talanta 118 (2014) 30.
- [68] M. J. M. Wells, Essential guides to method development in solid-phase extraction, in: I. D. Wilson, E. R. Adlard, M. Cooke, and C. F. Poole (Eds), Encyclopedia of separation science, vol. 10, Academic Press, London, p 4636 (2000).
- [69] J. S. Fritz, Analytical solid-phase extraction, Wiley-VCH, New York, p.264 (1999).
- [70] N. Rajesh, S. Manikandan, Spectrophotometric determination of lead after preconcentration of its diphenylthiocarbazone complex on an Amberlite XAD-1180 column, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 70 (2008) 754.
- [71] C. Li, M. Xu, X. Sun, S. Han, X. Wu, Y. Liu, J. Huang, S. Deng, Chemical modification of Amberlite XAD-4 by carbonyl groups for phenol adsorption from wastewater, Chemical Engineering Journal 229 (2013) 20.

- [72] E. V. Oral, I. Dolak, H. Temel, B. Ziyadanogullari, Preconcentration and determination of copper and cadmium ions with 1,6-bis(2-carboxy aldehyde phenoxy)butane functionalized Amberlite Xad-16 by flame atomic absorption spectrometry, *J. Hazard. Mater.* 186 (2011) 724.
- [73] X. Jiang, K. Huang, D. Deng, H. Xia, X. Hou, C. Zheng, Nanomaterials in analytical atomic Spectrometry, *Trac Trends Anal. Chem.* 39 (2012) 38.
- [74] C. Chang, X. Wang, Y. Bai, H. Liu, Applications of nanomaterials in enantioseparation and related techniques, *Trac Trends Anal. Chem.* 39 (2012) 195.
- [75] K. Scida, P. W. Stege, G. Haby, G. A. Messina, C. D. Garcia, Recent applications of carbon-based nanomaterials in analytical chemistry: Critical review *Anal. Chim. Acta* 691 (2011) 6.
- [76] Yingying Wen, Ling Chen, Jinhua Li, Dongyan Liu, Lingxin Chen, Recent advances in solid-phase sorbents for sample preparation prior to chromatographic analysis, *Trends in Analytical Chemistry* 59 (2014) 26–41.
- [77] Mazaher Ahmadi, Hatem Elmongy, Tayyeb Madrakian, Mohamed Abdel-Rehim Nanomaterials as sorbents for sample preparation in bioanalysis: A review, *Analytica Chimica Acta* (2016) 1-21. doi.org/10.1016/j.aca.2016.11.062
- [78] S. F. Xu, H. Z. Lu, X. W. Zheng, L. X. Chen, Stimuli-responsive molecularly imprinted polymers: versatile functional materials, *J. Mater. Chem. C* 1 (2013) 4406.
- [79] S. F. Xu, J. H. Li, X. L. Song, J. S. Liu, H. Z. Lu, L. X. Chen, Photonic and magnetic dual responsive molecularly imprinted polymers: preparation, recognition characteristics and properties as a novel sorbent for caffeine in complicated samples, *Anal. Methods* 5 (2013) 124.
- [80] K. Pyrzynska, Use of nanomaterials in sample Preparation, *Trac Trends Anal. Chem.* 43 (2013) 100.
- [81] S. Iijima, Helical microtubules of graphitic carbon, *Nature* 354 (1991) 56–58.
- [82] J. Płotka-Wasyłka, N. Szczepanska, M. Guardia, J. Namiesnik, Modern trends in solid phase extraction: New sorbent media, *Trends in Analytical Chemistry* 77 (2016) 23–43.

- [83] R. Sitko, B. Zawisza, E. Malicka, Graphene as a new sorbent in analytical chemistry, *Trends Anal. Chem.* 51 (2013) 33–43.
- [84] D.R. Dreyer, S. Park, C.W. Bielawski, R.S. Ruoff, The chemistry of graphene oxide, *Chem. Soc. Rev.* 39 (2010) 228–240.
- [85] Rafal Sitko, Edyta Turek, Beata Zawisza, Ewa Malicka, Ewa Talik, Jan Heimann, Anna Gagor, Barbara Feista and Roman Wrzalik, Adsorption of divalent metal ions from aqueous solutions using graphene oxide, *Dalton Trans.*, 42 (2013) 5682–5689.
- [86] S. Sayar, K. Mehrani, F. Hoseinzadeh, A. Mehrani, O.Sadeghi, Comparison of the performance of different modified graphene oxide nanosheets for the extraction of Pb(II) and Cd(II) from natural samples, *Microchim Acta* 181 (2014)313–320.
- [87] C. J.Madadrang, H. Y.Kim, G.Gao, N.Wang, J. Zhu, H. Feng, M. Gorrington, M. L. Kasner, S. Hou, Adsorption Behavior of EDTA-Graphene Oxide for Pb (II) Removal. *ACS Appl. Mater. Interfaces*, 4 (2012) 1186–1193.
- [88] Q. Liu, J. B. Shi, L.X. Zeng, T. Wang, Y. Q. Cai, G. B. Jiang, Evaluation of graphene as an advantageous adsorbent for solid-phase extraction with chlorophenols as model analytes, *J. Chromatogr. A* 1218 (2011) 197.
- [89] L. L. Xu, J. J. Feng, J. B. Li, X. Liu, S. X. Jiang, Graphene oxide bonded fused-silica fiber for solid-phase microextraction-gas chromatography of polycyclic aromatic hydrocarbons in water, *J. Sep. Sci.* 35 (2012) 93.
- [90] K. J. Huang, S. Yu, J. Li, Z. W. Wu, C. Y. Wei, Extraction of neurotransmitters from rat brain using graphene as a solid-phase sorbent, and their fluorescent detection by HPLC, *Microchim. Acta* 176 (2012) 327.
- [91] V. Chandra, J. Park, Y. Chun, J. W. Lee, I. Hwang, K. S Kim, Water-Dispersible Magnetite-Reduced Graphene Oxide Composites for Arsenic Removal, *ACS Nano* 4 (2010) 3979.
- [92] R. Kalvoda, M. Kopanica, Adsorptive Stripping Voltammetry In Trace Analysis, *Pure & Appl. Chem.* 61 (1989) 97.
- [93] M.A. Taher, E. Rezaeipour, D. Afzali, Anodic stripping voltammetric determination of bismuth after solid-phase extraction using amberlite XAD-2 resin modified with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol, *Talanta* 63 (2004) 797.

- [94] D. Afzali, A. Mostafavi, M.A. Taher, E. Rezaei-pour, M.K. Mahani, Natural Analcime Zeolite Modified with 5-Br-PADAP for the Preconcentration and Anodic Stripping Voltametric Determination of Trace Amount of Cadmium, *Anal. Sci.* 21 (2005) 383.
- [95] R. Sitko, P. Janik, B. Zawisza, E. Talik, E. Margui, I. Queralt, Green approach for ultratrace determination of divalent metal ions and arsenic species using total-reflection X-ray fluorescence spectrometry and mercapto-modified graphene oxide nanosheets as a novel adsorbent, *Anal. Chem.* 87 (2015) 3535-3542
- [96] W. Abe, S. Isaka, Y. Koike, K. Nakano, K. Fujita, T. Nakamura, X-ray fluorescence analysis of trace metals in environmental water using preconcentration with an iminodiacetate extraction disk, *X-Ray Spectrom.* 35 (2006) 184.
- [97] H. Barros, Lué-Merú M. Parra, L. Bennun, E.D. Greaves, Determination of arsenic in water samples by Total Reflection X-Ray Fluorescence using preconcentration with alumina, *Spectrochim. Acta Part B* 65 (2010) 489.
- [98] C. Sivani, G.R. Naidu, J. Narasimhulu, D. Rekha, J.D. Kumar, P. Chiranjeevi, Determination of Co(II) in water and soil samples using spectrophotometry coupled with preconcentration on 4-amino methyl pyridine anchored silica gel column, *J Hazard Mater.* 146 (2007) 137.
- [99] M. Soylak, Y.E. Unsal, M. Tuzen, Spectrophotometric determination of trace levels of allura red in water samples after separation and preconcentration, *Food Chem. Toxicol.* 49 (2011) 1183.
- [100] P. Liang, J. Yang Cloud point extraction preconcentration and spectrophotometric determination of copper in food and water samples using amino acid as the complexing agent, *J Food Compos Anal.* 23 (2010) 95.
- [101] R.R. Rao, A. Chatt, Preconcentration neutron activation analysis of trace elements in seawater by coprecipitation with 1-(2-thiazolylazo)-2-naphthol, pyrrolidinedithio carbamate and N-nitroso-phenylhydroxylamine, *J. Radioanal. Nucl. Chem.* 168 (1993) 439.
- [102] M. Nomura, M. Sato, H. Amakawa, Y. Oura, M. Ebihara, Precise determination of Sc in natural waters by neutron activation analysis coupled with preconcentration of Sc, *Anal. Chim. Acta* 553 (2005) 58.

- [103] A. Montaser, D.W. Golightly, Inductively Coupled Plasmas in Analytical Atomic Spectrometry, second ed., VCH, New York, 1992.
- [104] R.E.S. Froes, W.B. Neto, R.L.P. Naveira, N.C. Silva, C.C. Nascentes, J.B.B. da Silva, Exploratory analysis and inductively coupled plasma optical emission spectrometry (ICP-OES) applied in the determination of metals in soft drinks, *Microchem. J.* 92 (2009) 68.
- [105] S. Baytaka, F. Zereenb, Z. Arslanb, Preconcentration of trace elements from water samples on a minicolumn of yeast (*Yamadazyma spartinae*) immobilized TiO₂ nanoparticles for determination by ICP-AES, *Talanta* 84 (2011) 319.
- [106] B. Debelius, J.M. Forja, A.D. Valls, L.M. Lubian, Toxicity and bioaccumulation of copper and lead in five marine microalgae, *Ecotoxicol. Environ. Saf.* 72 (2009) 1503.
- [107] C. T. J. Alkemade, T. Hollander, W. Snelleman, P. J. T. Zeegers, *Metal Vapours in Flames*, Pergamon Press, Oxford (1982).
- [108] B.V. Lvov, Forty years of electrothermal atomic absorption spectrometry. Advances and problems in theory, *Spectrochim. Acta B* 52 (1997) 1239.
- [109] J.A.C. Broekaert, *Analytical Atomic Spectrometry with Flames and Plasmas*, 3rd edn, Wiley-VCH, Weinheim, Germany (1998).
- [110] Gary D. Christian, *Analytical chemistry*, 6th ed. John Wiley and Sons (2003).
- [111] A. Zybin, J. Koch, H. D. Witzemann, J. Franzke, K. Niemax, Diode laser atomic absorption spectrometry, *Spectrochim. Acta B* 60 (2005) 1.
- [112] S. B. Smith, Jr G.M. Hieftje, A New Background-correction Method for Atomic Absorption Spectrometry, *Appl. Spectrosc.* 37 (1983) 419.
- [113] K. Cammann, *Analytical chemistry-today's definition and interpretation*, *Fresenius J. Anal. Chem.* 343 (1992) 812.
- [114] G. M. Hieftje, The two sides of analytical chemistry, *Anal. Chem.* 57 (1985) 256A.

- [115] D.A. Skoog, D.M. West, F.J. Holler, Fundamentals of analytical chemistry, 5th edn, Harcourt Asia Pte Ltd., Singapore (2001).
- [116] D. C Harris, Quantitative chemical analysis, 4th edn, W.H. Freeman and company, New York (1995).
- [117] J. Mandal, R. D. Stiehler, Sensitivity-A Criterion for the Composition of Methods of Test, J. Res. Natl. Bur. Std. 53 (1954) 155.
- [118] Anon, Nomenclature, symbols, units and their usage in spectrochemical analysis II, Spectrochim. Acta 33B (1978) 242.
- [119] Analytical Method Committee, Recommendation for the definition, estimation and use of the detection limit, Analyst 112 (1987) 199.

Chapter 2

Preparation & Characterization of Chelating Resins



The present research work done for the fulfillment of the requirement of doctoral thesis submission comprises of the preparation of three different types of solid phase chelating resins. After preparation, resins were characterized and systematically explored to develop a method for the separation, preconcentration and determination of metal ions by flame atomic absorption spectrophotometry. The thesis compiles the chapters individually taking into account a brief introduction followed by the results and discussions, method validation and conclusion for each work. In this chapter all the three modes of preparation for each work and instruments used for their characterization are detailed.

2.1 Preparation of chelating resins

2.1.1 Source and pretreatment of solid support and chelating ligands

Glycidylmethacrylate (GMA), methylmethacrylate (MMA), ethyleneglycoldimethacrylate (EGDMA) were purchased from Fluka (Buchs, Switzerland). Ethylenediamine (EDA) and diethylenetriamine (DETA) were purchased from Merck (Mumbai, India). 2,2-azobisisobutyronitrile (AIBN) and polyvinyl alcohol (PVA) were procured from Otto Chemie (Mumbai, Maharashtra, India). Non-ionic polystyrene-divinylbenzene co-polymer, Amberlite XAD-16 (AXAD-16) (particle size 20-60, pore size 100 Å, pore volume 1.82 mL g⁻¹, and surface area 900 m² g⁻¹) was procured from Sigma-Aldrich (Steinem, Germany). The AXAD-16 resin was pretreated by using ethanol-hydrochloric acid-water (2:1:1) solution for overnight and subsequently washed thoroughly with distilled water until pH of the supernatant water became neutral so that it becomes free from any impurities.

2.1.2 Synthesis of poly-glycidylmethacrylate-methylmethacrylate-ethyleneglycoldimethacrylate-terpolymeric resin

GMA based terpolymeric resin has been synthesized by a free radical polymerization. In this reaction GMA, MMA and EGDMA (1:1:1) were mixed with 0.2 g of AIBN and stirred for about 1 h. The reaction mixture was then transferred to a beaker containing 20 mL of toluene and stirred for another half an hour followed by its slowly transfer to a flask containing 5% of PVA under continuous stirring and nitrogen purging. The reaction temperature was maintained at 70 °C for 2 h and then at 80°C for 1 h so that the system is below the boiling point of components while still

enabling decomposition of AIBN at an enough rate. The obtained Poly (GMA-EGDMA-MMA) beads were filtered and successively washed with distilled water and methanol in order to remove unreacted components. The resin beads were then oven dried at 50 °C for 24 h. The dried beads were immersed in minimum amount of distilled water for an hour to swell completely. The prepared resin was abbreviated as PGEM.

Functionalization of terpolymeric resin with ethylenediamine

PGEM resin beads were swelled in distilled water before functionalization with EDA ligand. PGEM swelled beads were refluxed with EDA (2 ml diluted with 20 ml of water) for 10 h with continuous stirring. The obtained EDA functionalized Poly(GMA–EGDMA–MMA) resin beads (PGEME) were thoroughly washed with excess TDW in order to remove the unreacted ethylenediamine and oven dried at 60 °C for 24 h. The synthesis scheme of the PGEME terpolymeric resin was shown in figure 2.1.

The characterization of PGEME terpolymeric resin was done by Fourier transform infrared (FT-IR) spectral studies, thermogravimetric analysis/differential thermal analysis (TGA/DTA), scanning electron microscopy (SEM) and Brunauer-Emmett-Teller (BET) surface area analysis. The prepared terpolymeric resin was used as solid phase extractant for the determination of Cd(II) and Zn(II) in different food samples with flame atomic absorption spectroscopy. The application of the prepared resin was detailed in **Chapter 4**.

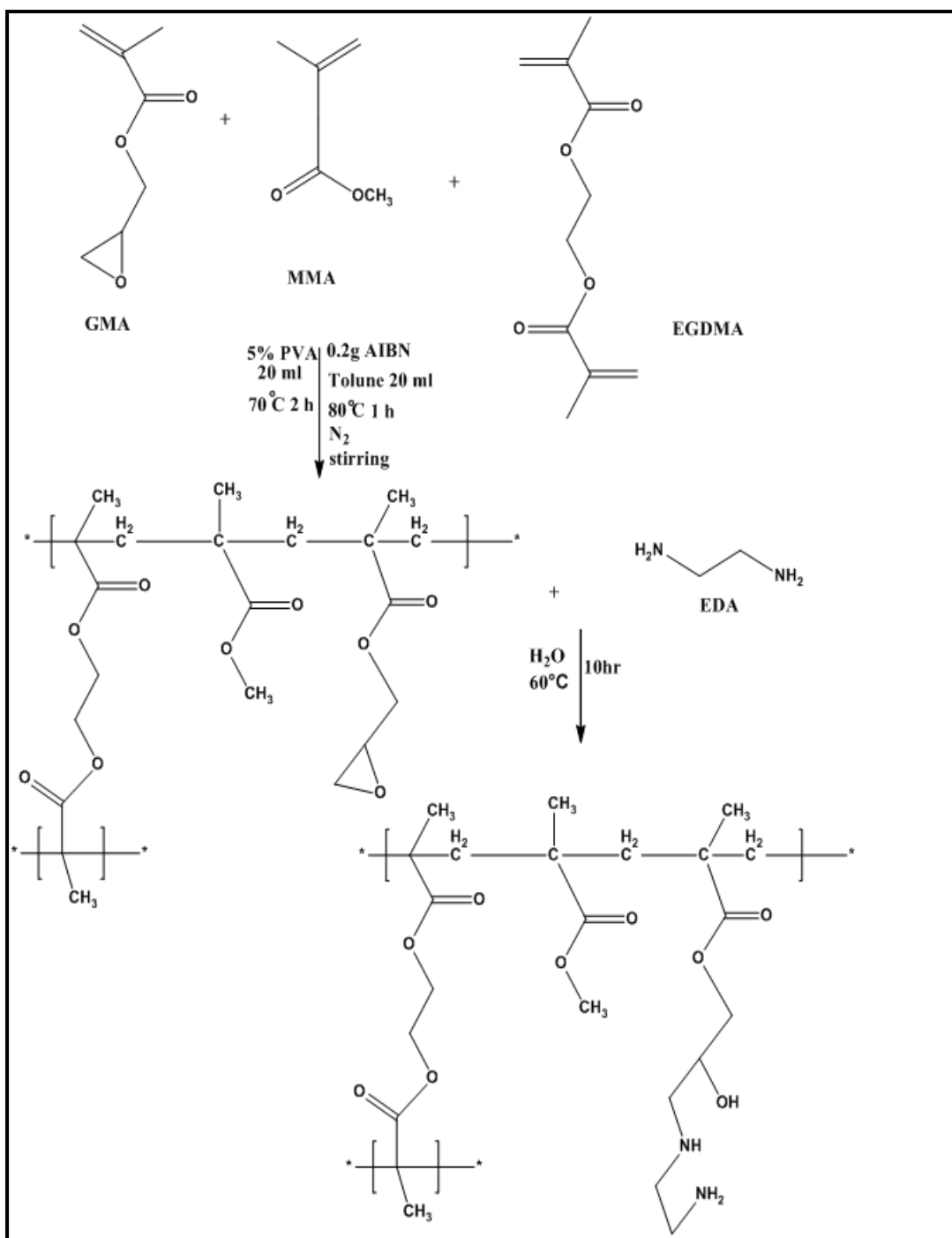


Figure 2.1: Synthesis of poly(GMA-MMA-EGDMA-EDA) terpolymeric resin

2.1.3 Graphene oxide immobilization onto glycidylmethacrylate based chelating resin

Graphene oxide (GO), contains numerous functional group such as epoxy (-COC), hydroxyl (-OH), carbonyl (-CO) and carboxyl (-COOH), was synthesized by oxidation of graphite [1]. GO is highly hydrophilic in nature due to presence of various oxygen rich functional groups responsible for metal ion complexation and can

be used as potential solid phase extraction sorbent for the preconcentration of metal ions [2-5].

Synthesis of graphene oxide

GO was synthesized by hummers method using natural graphite as starting material [1]. 200.0 mL of H₂SO₄ (95%) was added into a flask and cooled in an ice bath. After this, 5.0 g of graphite powder and 2.5 g of NaNO₃ were added under vigorous stirring to avoid agglomeration. After dispersion of graphite powder, 15 g of KMnO₄ was added slowly over about 2 h under stirring at temperature below 10°C. The ice bath was then removed and the mixture was stirred at room temperature (30°C) for 5 days. The mixture gradually became thick and the colour turned light brown as the reaction progressed. 350.0 mL of water was slowly added to the paste with continuous stirring and the temperature was kept below 98°C. The diluted suspension was stirred at 98°C for a day and then temperature reduced to 60°C. Excess MnO₄⁻ was eliminated by addition of 50.0 mL of 30% H₂O₂. And, in final step resulting mixture was filtered and washed with 2M HCl to remove metal ions followed by distilled water washing till the neutral pH. The mixture was then dried at 80°C. The obtained graphite oxide was exfoliated to GO sheets by bath ultrasonication (500 W, 40 kHz) for 2 h and was subjected to centrifugation at 3000 rpm to remove any unexfoliated graphite oxide.

Immobilization of GO onto GMA-DVB resin

GO was immobilized onto glycidylmethacrylate-divinylbenzene (GMA-DVB) copolymer by epoxy ring opening reaction by using different amines spacer arms unit. In the first step GMA-DVB copolymer beads were synthesized as reported in one of our publication [6]. GMA-DVB resin beads were divided into two portions, each of 5 g and swelled in distilled water for 2h. After swelling, first 5 g of resin beads were mixed with 3 ml of EDA in 20 ml of TDW and the remaining 5 g resin was treated with DETA (2 ml in 25 ml TDW). The resultant mixtures individually undergo on refluxing for 10 h, after refluxing the products were thoroughly washed with distilled water and methanol to remove the unreacted EDA, DETA and then oven dried. The resulting products abbreviated as p-EDA, p-DETA, respectively.

In the second step, GO was immobilized onto the p-EDA resin, taking 3 g of resin beads in a 250 ml of round bottom flask containing 25 ml of DMF, 5 ml of GO solution (2 mg ml⁻¹) and a coupling agent N, N'-dicyclohexylcarbodiimide (DCC) [7].

The solution mixture refluxed for 12 h with continuous stirring at a temperature of 60 °C. Similarly, 2.5 g of p-DETA resin was refluxed with a mixture of 4 ml of GO solution (2 mg ml⁻¹) and DCC in 25 ml DMF at 60 °C for 12 h. The proposed schemes of the resins were shown in figure 2.2 and 2.3 for p-EDAGO and p-DETAGO, respectively.

The prepared resins were characterized by solid state C¹³ NMR spectroscopy, scanning electron microscopy (SEM) along with energy dispersive x-ray spectroscopy (EDS), transmission electron microscopy (TEM). Column based preconcentration method was developed by using this solid phase extractant and used for the determination of Cd and Ni in various environmental and food samples which was discussed in detail in **Chapter 5**.

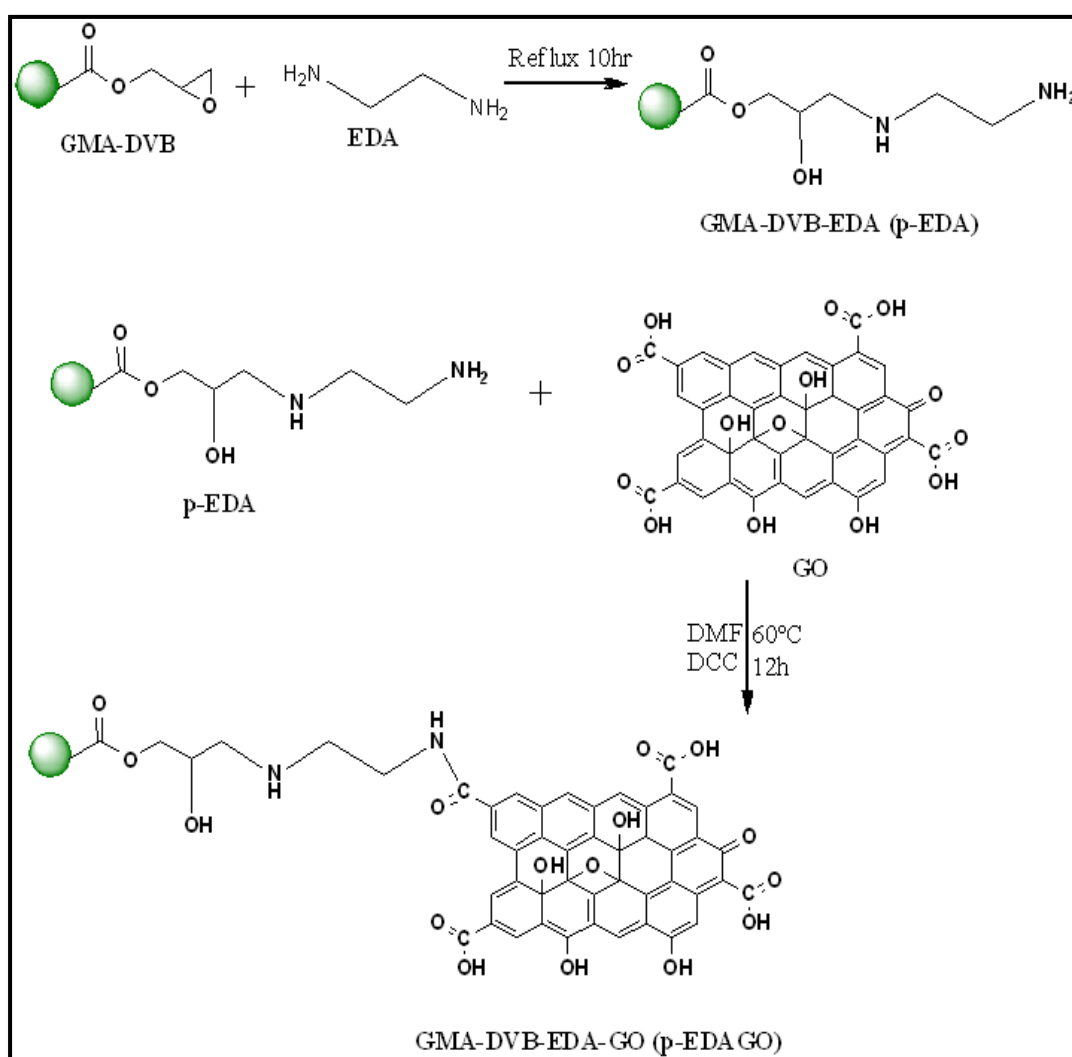


Figure 2.2: Synthesis of GO immobilized GMA-DVB-EDA resin

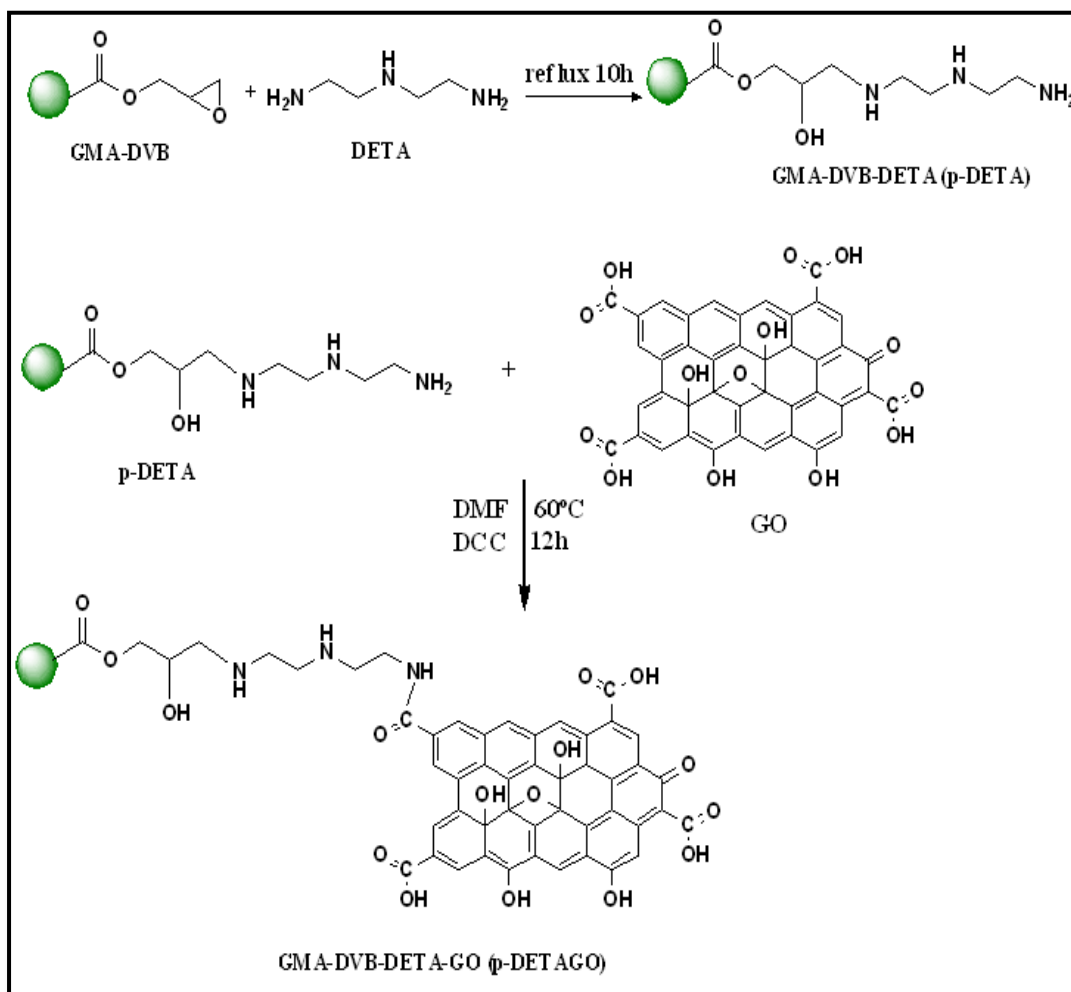


Figure 2.3: Synthesis of GO immobilized GMA-DVB-DETA resin

2.1.4 Synthesis of graphene oxide immobilized Amberlite XAD-16 chelating resin

Amberlite XAD chelating resins are best suitable solid support for the immobilization or linkage of different chelating ligands on to it by various methods. The superiority of the support is due to its high surface area and its porosity which provides more accessibility for incorporation of chelating ligands results high sorption capacity.

Nitration of XAD-16 resin

For nitration of XAD-16, 5g of resin was taken in a round bottom flask followed by the addition of HNO_3 and H_2SO_4 (2:3) under ice cold then temperature increase up to 70° and maintain for 2 hr, the resulting product was poured into ice water. After

cooling resin beads were filtered and washed with distilled water. This product was yellow colour resin beads.

Amination of XAD-16 resin

Nitrated resin was subjected amination by taking resin beads along with hydrochloric acid and ethanol and reflux for 12 hr at 90 °C in the presense of tin chloride (SnCl_2). After completion of the reaction the product washed with a mixture of hydrochloric acid and ethanol followed by sodium hydroxide and distilled water, respectively.

Diazotization of XAD-16 resin

Aminated resin was taken in a beaker including 1M hydrochloric acid and then at 0°C 2M sodium nitrite (NaNO_2) was slowly added dropwise until the iodide paper turns violet. The washing of the product was done by 1% cold sodium chloride (NaCl) solution.

Immobilization of graphene oxide sheets onto AXAD-16 resin beads

3ml of GO solution (2 mg ml^{-1}) along with 3g of diazotized resin was added into mixture of NaOH and methanol (1:2) and kept at a temperature of 0-2 °C for 48 h and, then washed with TDW in order to remove the alkali and oven dried for 24h. The prepared resin was abbreviated as XAD-GO. The synthesis scheme was shown in figure 2.4.

The prepared resin was explored for the preconcentration of lead (Pb), cadmium (Cd) and Zinc (Zn) in various environmental water samples using column method and detailed in **Chapter 6**.

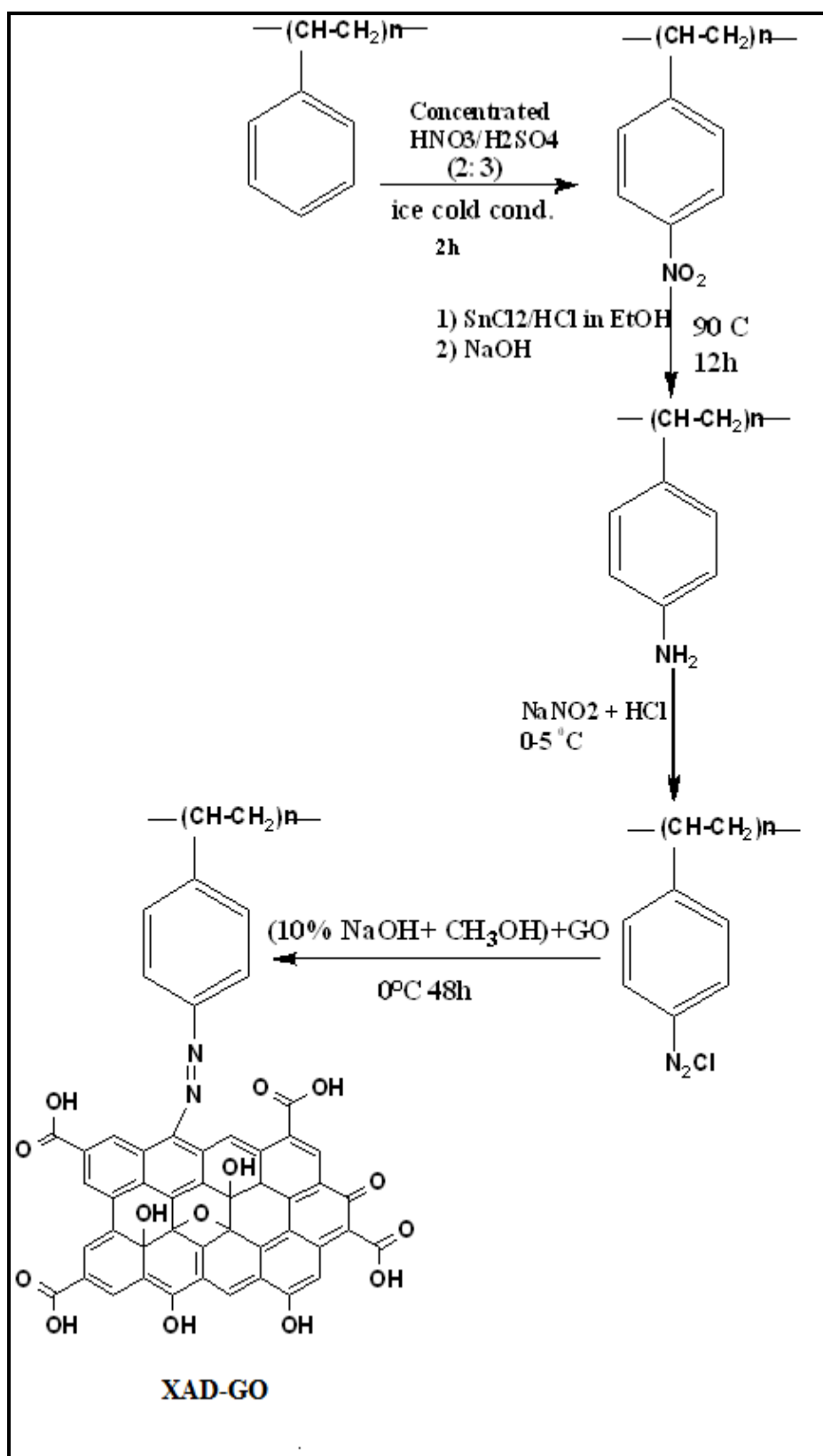


Figure 2.4: Preparation of XAD-GO resin

2.2 Characterization of the chelating resins

2.2.1 Fourier Transform-IR Spectrometer (FTIR) and Far-IR (FIR) Spectrometer

All the infrared (IR) spectra were recorded on Perkin Elmer spectrum two spectrometer (Waltham, MA, USA) using KBr disk method in the range between 500 to 4000 cm^{-1} with a resolution of 2.0 cm^{-1} and the interferograms were recorded by accumulating 32 scans. Far infrared spectra measurements were obtained by using a Perkin Elmer spectrometer in polyethylene pellet under nitrogen atmosphere at room temperature (27°C) in the range between 50 to 500 cm^{-1} . The IR spectra of the prepared and then modified resins (both metal loaded as well as metal free resins) had been taken. The IR spectra of each stage of the preparation of chelating sorbents exhibited bands that support the preparation and then immobilization of the ligands onto the different polymeric support. The IR spectrum of each intermediate favors the proposed synthetic route for the chelating resins.

2.2.2 Thermo gravimetric analysis/differential thermal analysis (TGA/DTA) simultaneous measuring instrument

Thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) was done on Shimadzu TGA/DTA simultaneous measuring instrument, DTG-60/60H (Kyoto, Japan) from the temperature 50 °C to 600 °C at a heating rate of 10 °C/min and under an inert atmosphere (N_2 flow rate of 50 mL/min). The thermal stability of the terpolymeric resin, graphene immobilized glycidylmethacrylate and graphene oxide decorated XAD-16 chelating resins were interpreted by different TGA curve which gives different behaviour of their matrix composition and incorporated ligand. In the TGA curves of all the chelating resin initially weight loss of all the chelating resins may attributed due the decomposition weakly sorbed water.

2.2.3 Elemental analysis (CHNSO)

The elemental composition of the chelating resins was confirmed by the elemental analysis (CHNSO) and was done on Flash EA 1112 Organic Elemental Analyzer (Thermo Fischer Scientific). The extent of the reaction may be interpreted from the composition of the final resin. The total percentage of carbon, nitrogen, hydrogen, Sulfur and oxygen contents can give a good approximation of the quantity of reagent incorporated. Hence, the resins were subjected to CHNSO analysis. CHNSO analysis

was also carried out for the different intermediate products obtained during the synthesis and the results were compared with the theoretical values to get a better interpretation of the composition of the products.

2.2.4 Scanning electron microscopy (SEM) with Energy dispersive X-ray analysis (EDS)

The structural morphologies and micro composition of the resins were checked out by scanning electron microscopy (SEM) images and energy dispersive X-ray analysis (EDS), was performed on Jeol JSM-6510LV (Tokyo, Japan). The presense of donar atoms on the pendant group of the reagent on to the matrix of the resin along with the metal ion complexation was confirmed by EDS analysis.

2.2.5 Transmission electron microscope (TEM)

The structural morphology of the modified chelating resins was confirmed by the Transmission electron microscope (TEM). TEM images were recorded on Jeol JEM-2100 microscope (Peabody, MA) operating at a maximum accelerating voltage of 200 KV.

2.2.6 ^{13}C -NMR spectroscopy

^{13}C NMR was recorded on Bruker Avance III HD (Germany). The characterization of new specific carbon was done by interpreting the ^{13}C NMR spectra wherever it required.

2.2.7 Surface Area Analysis

Surface area analysis measurements were done on an Autosorb-iQ one-station gas sorption analyzer (Quantachrome Instruments, Boynton Beach, FL). For higher sorption of metal ion by the sorbent a good surface area of the solid phase metal ion extractor is an essential requisite. This surface area estimation of the synthesized resins was done by BET analysis.

2.2.8 Powder X-ray diffraction (PXRD) analysis

For the phase identification of graphite and graphene oxide (Chapter 6), powder X-ray diffraction study was carried out. And it was done on a Bruker D8 Advance diffractometer with Ni-filtered Cu-K α radiation.

2.2.9 Raman spectroscopy

The carbon skeleton of the synthesized graphene oxide (Chapter 6) has been characterized by Raman spectroscopic analysis and it was recorded on Raman spectrum on LabRAM HR Evolution microscope Raman system (Horiba Jobin Yvon) equipped with air-cooled diode laser.

References

- [1] W. S. Hummers, R. E. Offeman, Preparation of Graphitic Oxide. *J. Am. Chem. Soc.* 80 (1958) 1339-1339.
- [2] J. Mo, L. Zhou, X. Li, Q. Li, L. Wang, Z. Wang, On-line separation and pre-concentration on a mesoporous silica-grafted graphene oxide adsorbent coupled with solution cathode glow discharge-atomic emission spectrometry for the determination of lead, *Microchem. J.* 130 (2017) 353-359.
- [3] E. Aliyari, M. Alvand, F. Shemirani, Modified surface-active ionic liquid-coated magnetic graphene oxide as a new magnetic solid phase extraction sorbent for preconcentration of trace nickel, *RSC Adv.* 6 (2016) 64193-64202.
- [4] M. Ghazaghi, H. Z. Mousavi, A. M. Rashidi, H. Shirkhanloo, R. Rahighi, Innovative separation and preconcentration technique of coagulating homogenous dispersive micro solid phase extraction exploiting graphene oxide nanosheets, *Analytica Chimica Acta* 902 (2016) 33-42.
- [5] E. Kazemi, S. Dadfarnia, A. M. H. Shabani, Dispersive solid phase microextraction with magnetic graphene oxide as the sorbent for separation and preconcentration of ultra-trace amounts of gold ions, *Talanta* 141 (2015) 273-278.
- [6] A. Islam, N. Zaidi, H. Ahmad, S. Kumar, Amine-functionalized mesoporous polymer as potential sorbent for nickel preconcentration from electroplating wastewater. *Environ Sci Pollut Res* 22 (2015) 7716-7725.
- [7] Q. Liu, J. Shi, J. Sun, T. Wang, L. Zeng, G. Jiang, Graphene and graphene oxide sheets supported on silica as versatile and high- performance adsorbents for solid-phase extraction, *Angew. Chem., Int. Ed.* 50 (2011) 5913-5917.

Chapter 3

Optimization & Validation of Analytical Methods



3.1 Reagents and solutions

3.1.1 Standard solutions of metal ions

All chemicals used were of analytical reagent grade. Stock solutions (1000 mg L^{-1} in 1% HNO_3/HCl) of nitrate salts of Cr(III), Co(II), Ni(II), Cu(II), Fe(III), Pb(II) and chloride salt of Zn(II) and Cd(II), were procured from Merck (Darmstadt, Germany). All the working solutions of the metal ions were prepared on a daily basis at a desired concentration range by dilutions of the stock solutions with triply distilled water (TDW). The metal solutions were standardized by complexometric titration before performing experiment [1-2].

3.1.2 Dilution of nitric acid, hydrochloric acid and sodium hydroxide solution

Nitric acid (15.4 mol L^{-1}) and hydrochloric acid (11.6 mol L^{-1}) of analytical reagent grade were diluted to the required lower concentrations with triple distilled water. The solution of sodium hydroxide was prepared by dissolving an appropriate amount in 1000 mL. It was standardized titrimetrically before use.

3.1.3 Buffer solutions

Buffers covering the pH range 1.2-3.6 were prepared by mixing appropriate amount of glycine (0.1 mol L^{-1}) with hydrochloric acid (0.1 mol L^{-1}) [3]. Buffers for pH 4.0-6.0 were prepared by mixing acetic acid (0.2 mol L^{-1}) and sodium acetate (0.2 mol L^{-1}) solutions in the appropriate ratio [4]. The reported mixing ratio of citric acid solution (0.1 mol L^{-1}) and disodium mono-phosphate solution (0.2 mol L^{-1}) were used to prepare buffer for pH 7.0-7.8. For buffer of pH 8.0-10.0, (0.2 mol L^{-1}) ammonium chloride solution was mixed with (0.2 mol L^{-1}) ammonia solution [5].

3.2 Standard reference materials

Standard reference materials (SRMs) such as NIES, JSS and NBS were received from the National Institute of Environmental Studies (Ibaraki, Japan), the Iron and Steel Institute of Japan (Tokyo, Japan) and National bureau of Standards, U.S. Department of Commerce, (Washington DC, USA), respectively. All the SRMs used throughout the work are enlisted in Table 3.1 along with their compositions.

Table 3.1 Composition of standard reference materials.

SRM	Certified value ($\mu\text{g g}^{-1}$)
Rice flour unpolished NIES	Al:1.5, Cr:0.08, Co:0.007, Se:0.07, Cl:230, Br:0.5, Sr:0.2, As:0.15, Hg:0.005, Sr:0.2, Mn:40.1 \pm 2.0, Zn:23.1 \pm 0.8,
Chromium Steel SCr21 JSS 513-4 ^b	C:1600, Si:2500, Mn:7900, P:120, S:100, Ni:1300, Cr:11500, Mo:100, Cu:740, V:50, Al:250, N:1210
Vehicle exhaust particulates NIES ^a	Al:3300, Sb:6.0, As:2.6, Br:56, Cd:1.1, Ca: 5300, Ce:3.1, Cs:0.24, Cr:25.5, Co: 3.3, Cu: 67, Eu:0.05, La:1.2, Pb: 219.0, Lu:0.02, Mg:1010, Mo: 6.4, Ni:18.5, , Zn: 1040, K: 1150, Na:1920, P:510, Rb:4.6, Sm:0.20, Sc:0.55, Se:1.3,

^aNational Institute of Environmental studies (NIES); ^bIron and Steel Institute of Japan (JSS); ^cNational bureau of Standards (NBS).

3.3 Pretreatment of samples

3.3.1 Collection and pretreatment of water samples

For the preconcentration and determination of metal ions different real water samples were analyzed. The water samples along with the collection steps have been included in table 3.2. All the water samples after collection were immediately filtered through Millipore cellulose membrane filter (0.45 μm pore size), acidified to pH 2 with 0.2 M HNO_3 and stored in pre-cleaned polyethylene bottles. The bottles were cleaned by soaking in an alkaline detergent, 4 mol L^{-1} HCl and finally treated with 2 mol L^{-1} HF and 0.5 mol L^{-1} HNO_3 , and rinsed with triply distilled water between each step.

Table 3.2 Water samples and their collection spots.

Water sample	Collection spot
Tap water 1	From Aligarh Muslim University campus, Aligarh, UP, India
Tap water 2	From the local city Aligarh, UP, India
River water (Ganga river)	From two different cities namely Narora and Kanpur in UP, India
Sewage water (Electroplating industries wastewater)	From an area in the vicinity of local nickel electroplating industry, Aligarh, India

3.3.2 Digestion of food samples

Food samples (rice, fruit juice and bread) were purchased from a local market (Aligarh, India) and digested (rice (1 g) and bread (5 g)) by wet oxidation with concentrated nitric acid, perchloric acid, and 30% H_2O_2 [6]. The fruit juice (200 ml) sample was evaporated to about 5 mL on hot plate and digested by wet oxidation with 10-20 mL each of conc. HNO_3 and HClO_4 and 2 mL of 30% H_2O_2 . The residue obtained after digesting fruit juice sample was dissolved in 5 mL of 0.5 M HNO_3 and finally made up to 50 mL with triply distilled water. Similarly the digested residue of 1g of rice and 5g of bread was dissolved in 2 mL of 0.5 M HNO_3 and finally made up to 50 mL with triply distilled water.

3.3.3 Digestion of multivitamin capsules

Multivitamin capsules (Maxirich) purchased from local drug shop was digested by taking 25 ml of concentrated nitric acid (5 mol L^{-1}) into a beaker containing one capsule and temperature of the mixture was then grown slowly up to 120°C , the resulted residue was then cooled and further dissolve by another 20 ml of nitric acid and dry out on a steam bath until obtained a solid residue, again it was mixed with 10 mL TDW and concentrated nitric acid added drop wise in order to obtained clear solution followed by gentle heating and finally it was make up to 50 mL with TDW [6].

3.3.4 Digestion of standard reference materials (SRMs)

The SRM rice flour NIES10c was digested (4.396 g) as proposed by the international atomic energy agency [6]. The exactly weighed amount of the SRM was agitated with 25 mL of acetone, and then washed three times with distilled water and with 25 mL of acetone. The contact time of the cleaning medium with the sample was 10 minutes. The sample was finally dried for 16 h at 100°C . Then sample was dissolved in 10-20 mL of concentrated nitric acid. After adding 0.5 mL of 30% H_2O_2 , the solution was boiled to dryness. The residue obtained was dissolved in minimum amount of 2% HCl and made up to a 50/100 mL volume in a calibrated flask.

The solution of standard alloy (Chromium steel JSS 513-4) prepared by taking an exactly weighed amount of the SRM (25 mg of JSS 513-4) into a beaker and dissolved in 10-50 mL of aqua-regia. The solution was boiled to near dryness. Finally

the residue was dissolved in minimum volume of 2% HCl and filtered through a Whatman filter paper No.1. The residue was washed with two 5 mL portions of hot 2% HCl. The aqueous layer was evaporated to dryness. The residue was redissolved in 5 mL of 2% HCl and made up to 50 mL with triply distilled water.

Environmental SRM (vehicle exhaust particulates-NIES 8) was digested by dissolving 0.5 g of the sample along with addition of 10 mL of concentrated nitric acid (15.5 mol L^{-1}), 10 mL of concentrated perchloric acid (12.2 mol L^{-1}), and 2 mL of concentrated hydrofluoric acid (22.4 mol L^{-1}) in a 100 mL teflon beaker and evaporated (water bath) to near dryness, redissolved in minimum volume of 2% HCl, filtered and made up to 50 mL volume in a calibrated flask.

3.4 pH meter

Thermo Scientific (Waltham, MA, USA) Orion 2 star model was used for the pH measurement. The pH value displayed corresponds to an error of ± 0.01 .

3.5 Mechanical shaker

A thermostated mechanical shaker NSW-133 (Narang Scientific works, New Delhi, India), equipped with digital display ($\pm 0.2^\circ\text{C}$), with shaking speed variable from 10 to 200 strokes per minute and supplied with gabled cover and lotus clamps for conical flask (9 nos. for 100 mL and 5 nos. for 250 mL), was used for carrying out equilibrium batch studies.

3.6 Flame atomic absorption spectrophotometer (FAAS)

Concentration of various metal ions was determined using a GBC 932+ flame atomic absorption spectrometer (FAAS, Dandenong, Australia) with hollow cathode lamp along deuterium background on an air-acetylene flame. Some metal ions listed in Table 3.3 along with their operating parameters in FAAS.

Table 3.3 FAAS operating parameters set for the determination of elements.

Metal	Wavelength (nm)	Slit width (nm)	Lamp Current (mA)	Flame composition (L min ⁻¹)	
				Air	Acetylene
Co	240.7	0.2	30	9.5	2.3
Ni	232.0	0.2	15	9.5	2.3
Cu	324.8	0.7	25	9.5	2.3
Zn	213.9	0.7	15	9.5	2.3
Cd	228.8	0.5	10	9.5	2.3
Pb	217.0	0.5	8	9.5	2.3

3.7 Column for Dynamic Studies

Dynamic studies were carried out using a short glass column with an inner diameter of 10 mm and a length of 100 mm, provided with sintered porous frits, purchased from J-SIL Scientific industries, Agra, India.

3.8 Recommended procedures for preconcentration and determination of metal ions

3.8.1 Batch ‘static’ method

A fixed amount of the prepared/modified resin was equilibrated with suitable volume of metal solution of appropriate concentration maintained at constant pH for optimized shaking time. The sorbent was filtered and then uptaken metal ions were desorbed by shaking with the appropriate eluent and subsequently analyzed by FAAS. For optimizing the static parameters the batch experiments were done following the univariate approach.

3.8.2 Column ‘dynamic’ method

A known amount of prepared resin beads were swelled in distilled water for 24 h and then transferred into a glass column. Resin bed in the column was further buffered with 5 mL of suitable buffer solution. A solution of metal ions of optimum concentration after adjusting the pH with appropriate buffers was passed through the column at an optimum flow rates. After the sorption operation, recovery experiments were performed; for this purpose the column was washed with distilled water and then appropriate volume of the proper eluent was made to percolate through the bed of

loaded resin whereby the retained metal ions get eluted. The eluent was collected in 5 mL of volume for the subsequent determination by FAAS. For the optimization of various effective parameters the univariate approach was adopted throughout the experiments.

Effect of pH

For optimization of sample pH, excess of metal ion in 50 mL of solution was shaken with suitable amount of resin over a range of pH 1.2-10 for 180 minutes. The pH of metal ion solution was adjusted prior to equilibration with the corresponding buffer system. pH >10 was not studied to avoid metal hydroxide precipitation.

Effect of contact time

To investigate the effect of shaking time on the sorption capacity, a suitable amount of sorbent was stirred with 50 mL of solution containing one of the metal ions for 10, 20, 30, 40, 60, 80, 100, 120 and 180 min (under the optimum conditions).

Kinetics of sorption

The dynamics of the adsorption process in terms of the order and the rate constant can be evaluated using the kinetic sorption data. The kinetic parameters can also be used in the modelling of the sorption operation as explicitly explained by Lagergren pseudo-first-order [7] and pseudo-second-order kinetic models given by Ho and McKay [8].

Pseudo-first-order kinetic model can be explained as:

$$\log(q_e - q_t) = \log q_e - k_1 t / 2.303 \quad (1)$$

the value of q_e (equilibrium adsorption capacity (mg g^{-1})) and rate constant, k were calculated from the slope and intercepts by plotting $\log(q_e - q_t)$ against time (t).

Pseudo second-order kinetic is represented as:

$$t/q_t = 1/k_2 q_e^2 + t/q_e \quad (2)$$

where q_t is adsorption capacity at time t and the values of k and q_e were obtained after drawing t/q_t versus time plot.

Sorption Isotherms

The sorption behavior of the resin (**Chapter 4**) for metal ions was evaluated by analyzing Langmuir, Freundlich, Temkin, Dubinin-Radushkevich (D-R) and Scatchard isotherm. Monolayer sorption behaviour is explained by using Langmuir isotherm model. The linearized form of the model represented by the equation:

$$C_e/Q_e = 1/(Q_m K_b) + C_e/Q_m \quad (3)$$

where C_e , Q_e are the equilibrium concentration of the metal ions (mg L^{-1}) in the solution and corresponding uptake in the resin phase (mg g^{-1}), respectively. Q_m , the equilibrium sorption capacity (mg g^{-1}) and K_b , the sorption equilibrium constant, were calculated from the slope and intercepts of C_e/Q_e versus C_e plot). Separation factor (R_L) is very important parameter in Langmuir isotherm and represented as:

$$R_L = 1/(1 + K_L C_0) \quad (4)$$

C_0 and K_L are initial concentration of metal ions (mg L^{-1}) and Langmuir constant, respectively. Nature of the isotherm model explained on the basis of R_L values e.g. unfavorable ($R_L > 1$), linear ($R_L = 1$), favorable ($0 < R_L < 1$) or irreversible ($R_L = 0$) [9].

Freundlich model equation:

$$\ln Q_e = \ln K_F + 1/n \ln C_e \quad (5)$$

where K_F and $1/n$ are Freundlich constants related to the capacity of the resin and heterogeneity of the system, respectively.

Dubinin-Radushkevich (D-R) isotherm was studied to interpret the sorption on a single type of uniform pores. Its linear expression is:

$$\ln Q_e = \ln Q_m - K \epsilon^2 \quad (6)$$

ϵ is the Polanyi potential calculated using equation; $\epsilon = RT \ln(1 + \frac{1}{C_e})$ where R is the universal gas constant ($\text{kJ}/(\text{mol K})$), T is the temperature (K). The mean free energy (E) used to estimate the sorption type can be calculated from constant K (obtained from slope and intercepts of the $\ln Q_e$ versus ϵ^2):

$$E = (-2K)^{-0.5} \quad (7)$$

The nature of binding sites and sorption process was investigated by Scatchard isotherm model, which is represented as

$$Q_e/C_e = Q_m K_b - Q_e K_b \quad (8)$$

where K_b is the Scatchard isotherm constant. The shape of the Scatchard plot describes the type of interactions of metal ions with chelating resin. The presence of a

deviation from linearity on a plot based on Scatchard analysis usually points out the presence of more than one type of binding site, while the linearity of the Scatchard plot indicates that the binding sites are identical and independent.

The adsorbent-adsorbate interaction was related to heat of adsorption of metal ions described by Temkin isotherm model:

$$Q_e = A \ln K_T + A \ln C_e \quad (9)$$

$$A = RT/b \quad (10)$$

where constant b (kJ/mol) is the variation of adsorption energy and K_T is the equilibrium binding constant (L/mg) corresponding to the maximum binding energy. Temkin isotherm constants calculated from slope and intercepts of the plot Q_e versus $\ln C_e$.

Type and volume of eluting agents

For the recovery of metal ions various mineral acids were analyzed. The efficiency of stripping was checked by using different volumes (1-10 mL) and concentrations (0.1-5.0 mol L⁻¹) of the studied mineral acid. The eluent optimized throughout the study was the one which resulted in the maximum percent recovery of the sorbed analyte in minimum eluent volume.

Effect of flow rate for sorption and desorption

The effect of flow rate on the metal ion sorption was studied by varying the sample flow rate 2-10 mL min⁻¹ at selected pH for maximum sorption, and subsequently determined the amount recovered. The eluent flow rate was also optimized by varying it for the chosen eluent and then subsequently determined the analyte recovered.

Resin reusability test

Resin reusability experiment was performed by equilibrating the metal ions several times on a sorbent from a solution having a different concentration and eluting the same by using appropriate eluting agent. The maximum number of loading/elution cycles was set as the reusability after which the sorption capacity tends to decrease.

Effect of matrix components

In real samples cations and anions are inevitably associated with heavy metals, may interfere in the latter's determination through precipitate formation, redox reactions,

or competing complexation reactions. To see the effect of these interfering ions on the determination of analyte ions, various interfering electrolytes and metal ion species at higher concentration along with the target analyte ion (low concentration) were allowed to percolate individually through a column packed with the chelating resin. At the optimized conditions, amount of analyte and interfering ions were changed accordingly up to certain degree of interference.

3.9 Analytical method validation

Calibration

The standard solutions for calibration, were prepared in 50 mL by taking suitable aliquot of metal ions and buffer solutions, and then subjected to the recommended optimized column procedure (for terpolymer: following batch procedure). The aliquot of sample solutions for the calibration run was taken in a manner such that after solid phase extraction the final concentration of the analyte metal ion was much above the corresponding preconcentration limit for the analyte. The calibration curve with the regression equation and correlation coefficient (R^2) was obtained by the method of least-squares. The linearity of the calibration curve was estimated from the R^2 value.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) of the developed method was obtained by performing blank run following the recommended column procedure with the same volume of aqueous solution prepared by adding a suitable buffer (excluding metal ions) and finally eluting the same in 5 mL before subjected to FAAS determination.

The LOD and LOQ values were calculated as $3s/m$ and $10s/m$ of the standard deviation (s) of the blank signal for replicate measurements ($n>10$), respectively, and m is the slope of calibration curve.

Accuracy

The accuracy of the developed method was tested by the analysis of SRMs and performing recovery experiments. The weighed amount of SRMs were digested and then run through the column/batch at the optimized conditions. For the comparison of the observed mean concentration of metal ions with the certified mean values Student's t -test was performed. The recovery experiments were done for the spiked

amount of analyte ions in the real environmental water and food samples using the developed SPE method.

Precision

Using optimum conditions, the precision of the method was evaluated by carrying out five successive sorption and elution cycles of 5 µg of the analyte metal ion studied taken in 100/50 mL following the recommended procedure. The relative standard deviations (RSD) for the observed values were then evaluated.

Preconcentration limit and factor

The lower limit of quantitative preconcentration below which recovery becomes non-quantitative (preconcentration limit) was determined by increasing the volume of metal ion solution and keeping the total amount of loaded metal ion constant at the optimized conditions. The preconcentration factor was calculated as the ratio of the total volume beyond which quantitative recovery fails to that of the volume of eluent.

References

- [1] A. I. Vogel, A textbook of quantitative inorganic analysis, Longman London, 4th edn, p. 319, 1978.
- [2] F. J. Welcher, The analytical uses of ethylenediaminetetraacetic acid, Van Nostrand Company Inc., Princeton, New jersey, New York, 1958.
- [3] G. Gomori, Preparation of buffers for use in enzyme studies, Methods Enzymol 1 (1955) 138.
- [4] H.T.S. Britton, Hydrogen ions: Their determination and importance in pure and industrial chemistry, E.H. Tripp (Ed), vol. 3, Chapman and Hall Ltd, London, 1932.
- [5] J.A. Dean (Ed), Lange's Handbook of chemistry, 15th edn, McGraw Hill, New York, 1999.
- [6] A. Islam, M. A. Laskar, A. Ahmad, Characterization of a novel chelating resin of enhanced hydrophilicity and its analytical utility for preconcentration of trace metal ions, Talanta 81 (2010) 1772.
- [7] S. Lagergren, K. Svenska Vetenskapsakad, Handl 24 (1898) 1-39.
- [8] Y. S Ho, G. McKay, Kinetic models for the sorption of dye from aqueous solution by wood, Process Safety and Environmental Protection 76 (1998) 183-191.
- [9] T. W. Weber, R. K. Chakkravorti, Pore and solid diffusion models for fixed-bed adsorbers, AIChE Journal 20 (1974) 228-238.

Chapter 4

***SPE Coupled to AAS
Trace Determination of
Cd(II) and Zn(II) in Food
Samples using Amine
Functionalized G.M.A-
M.M.A-EGD.M.A
Terpolymer: Isotherm and
Kinetic Studies***

4.1 Introduction

As the global population rises and economies in many regions grow remarkably, industrial production is also predicted to increase. Hence, the level of environmental pollution especially water pollution is increasing considerably. The sources of water pollution are mainly metal extraction, smelting, electroplating, tannery, galvanizing, paper and paint industry [1]. The discharge of toxic waste material into the water bodies results serious effect on human health and environmental ecosystem via their entry in food chain. The primary source of cadmium exposure in non-smokers adults and children is food. In soils and water bodies cadmium is accumulated under certain environmental conditions which results increased risk of future exposure through food [2]. In the priority list of Agency for Toxic Substances and Disease Registry (ATSDR), Cd is at rank 7 and Zn at 75 [3]. Cadmium even at low concentration limit cause harmful effect like kidney damage, renal disorder, high blood pressure, bone fracture, and destruction of red blood cells [4]. Although, Zn comes into the category of essential trace element, at higher concentration it causes nausea vomiting and hematemesis [5]. Thus, removals of heavy metal ions before its discharge into the environment and monitoring of these toxic metals in industrial effluent, biological samples and food stuff are of prime concern. Techniques such as ion exchange [6,7], cloud point extraction (CPE) [8], solvent extraction [9], coprecipitation [10], extraction involving deep eutectic solvent (DES) [11-13], ionic liquids (ILs) [14] and solid phase extraction (SPE) [15-17] were proved to be efficient for the removal, separation and preconcentration prior to determination of toxic metal ions. However, ion exchange lacks selectivity since metal ion uptake involves only ion exchange mechanism and ion exchanger offers less versatility to chemical modification. The limitation of CPE is relatively low partition coefficients of metals. Solvent extraction suffers from the use of large volumes of carcinogenic organic solvent, emulsion formation by the mutual solubility of aqueous and organic phases and analyte loss during multi-step extraction. The low cost and biodegradable DES, allow selective extraction of mainly biological macromolecules. However, the high viscosity and solid state at room temperature restrict their application as extraction solvents. The disadvantages with ILs are due to the high toxicity and high cost of the synthesis of some of their ingredients [11]. SPE has been turned out to be an economical and eco-friendly approach because of

its advantages like ease of separation, regeneration, reproducible quantitative sorption and elution, low consumption of non toxic solvents, high selectivity resulting in efficient removal of interfering constituents thereby, improving the detection limit, precision and accuracy of the analytical method. The effectiveness of the SPE depends on the nature of the sorbent. Previously various solid phase extractants like silica [18-19], chelating resin based on polystyrene [20-22], ion imprinted polymer [23,24] and activated carbon [25] were used. Although the ion imprinting polymers involve high selectivity it has certain limitations such as number of steps, thereby, introducing certain complications, long time requirement, low binding capacity and less site accessibility. The determination of heavy metals in various water samples using sophisticated analytical techniques like ICP-MS, ICP-AES, ETAAS and FAAS suffers from spectral, background and chemical interferences. Among them, FAAS is one of the cheapest and most widely used technique for heavy metal ion determination because of its simplicity, easy handling and less spectral interferences [26].

GMA based copolymeric/terpolymeric chelating resins were of great interest because of its hydrophilic nature that enhances the surface contact with aqueous phase metal ions resulting in better kinetics. The presence of abundant epoxy groups allows the modification with different chelating ligands having donor atoms like N, O, S and P in order to improve complexing behaviour towards metal ions, thereby, increasing the possibility of high sorption capacity and selectivity.

In this chapter, a ethylenediamine (EDA) functionalized terpolymeric resin (PGEME) (Synthesis scheme detailed in **Chapter 2**, Section 2.1.1) has been used as solid phase extractant for the determination of Cd(II) and Zn(II) in rice, bread and fruit juice samples by flame atomic absorption spectrometry (FAAS). In addition, sorption behaviour of the resin with metal ions was also tested by using different sorption isotherm models. Although, the presented route of synthesis was tried by few authors earlier [27-30] the work on metal ions determination were limited [29] in which the removal of only Hg(II) was done without any analytical figures of merits and interference studies. Moreover, no spacer arm or ligand was immobilized onto it. Hence, it is interesting to put forward the further modification of terpolymeric resin for its utilization as solid phase extractant and FAAS determination after developing and validating analytical method in a systematic

way. After optimizing the experimental parameters of SPE, the resin was applied for the separation, determination and removal of Cd(II) and Zn(II) in food samples.

4.2 Experimental

For the development of an analytical method for the determination of Cd(II) and Zn from food samples using PGEME resin several parameters need to optimize and done by various experimental studies. A recommended batch static method was used throughout the experiment for the sorption/ desorption, sorption kinetics, isotherm studies, interference studies, SRMs and recovery experiments of Cd and Zn as described in **Chapter 3** (Section 3.8).

For batch experiments 50.0 mL of metal ion solution in the concentration range of 5.1-1800 and 4.1-1500 $\mu\text{g L}^{-1}$ for Cd and Zn buffered at optimized pH and equilibrated for certain time interval. PGEME resin was characterized by various instruments and techniques with the corresponding recommended procedures stated in **Chapter 2** (Section 2.2).

4.3 RESULTS AND DISCUSSION

4.3.1 Characterization of PGEME resin

In the FT-IR spectra of PGEME resin (Figure. 4.1) the disappearance of peak at 908 and 848 cm^{-1} [31] shows the complete conversion of epoxy groups. Characteristic peaks appeared at 3447 cm^{-1} corresponds to structural OH and NH_2 stretching vibrations [31]. The peak at 2997 and 2954 cm^{-1} are associated with methylene vibrations and 1731 cm^{-1} is due to the carbonyl group of both GMA and MMA [29,32]. The stretching band at 1390 cm^{-1} is assigned for NH stretching vibrations [31]. The appearance of medium intensity peak at 1456 cm^{-1} and 1265 cm^{-1} are corresponds to CN stretching vibrations indicating the presence of NH and NH_2 [31].

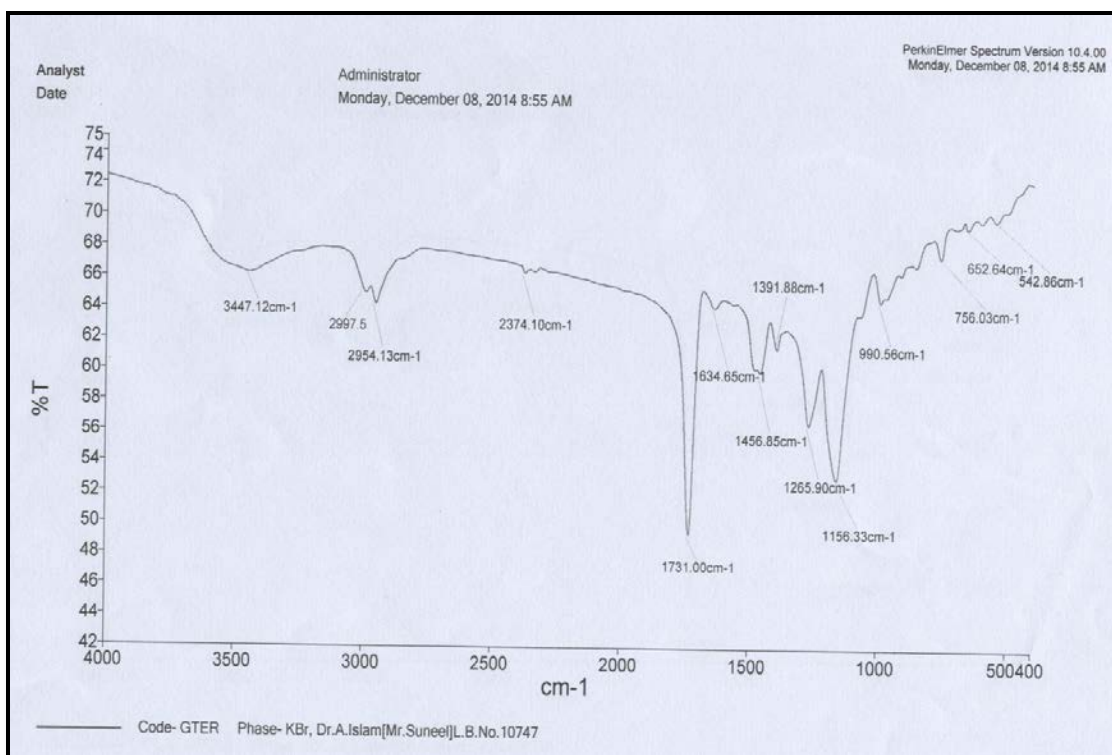


Figure 4.1: FT-IR spectrum of terpolymeric resin

To estimate the amine content 1 g of resin was contacted with 25 mL of 2 M HCl for 24 h. The residual HCl was measured titrimetrically against 0.05M NaOH. The calculated amine content of 1.0 mM g⁻¹ resin account for the immobilization of 0.5 mM of EDA (as indicated in the synthesis scheme that one EDA a unit linked with one epoxy group of GMA) which corresponds to equivalent sorption capacity of 0.48 mM g⁻¹ (Cd(II)) and 0.37 mM g⁻¹ (Zn(II)) of the resin. Elemental analysis of PGEME resulted in C, H and N as 56.5, 7.5 and 1.7%, respectively. This %N data was also used to verify the amount of amine groups immobilized (L₀; mM g⁻¹) onto the resin using the following equation [33]: $L_0 = (\%N / \text{Nitrogen atomic weight}) \times 10$ and was found to be 1.2. The multipoint BET analysis resulting in a surface area of 70.65 m² g⁻¹ which is better than previously reported terpolymer 21.6 m² g⁻¹ [29] 16.2 m² g⁻¹ [27]. The mesoporous nature of the resin is evident from its pore diameter of 1.4 nm, calculated using the BJH (Barrett-Joyner-Halenda) desorption method. EDS spectra along with their corresponding elemental weight% obtained from the SEM (Figure. 4.2) confirms the immobilization of ligand onto the resin matrix as well as its complexation with metal ions. TGA curve (Figure. 4.3) of PGEME resin shows thermal stability up to 250 °C.

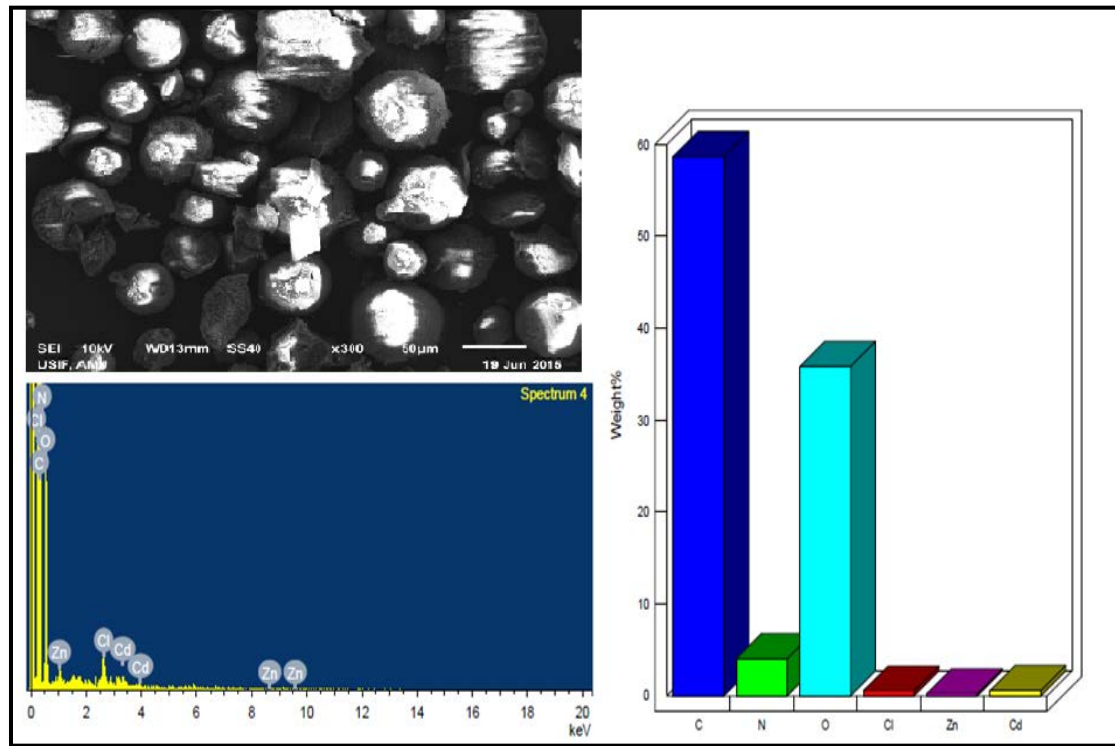


Figure 4.2: SEM analysis along with EDS spectra

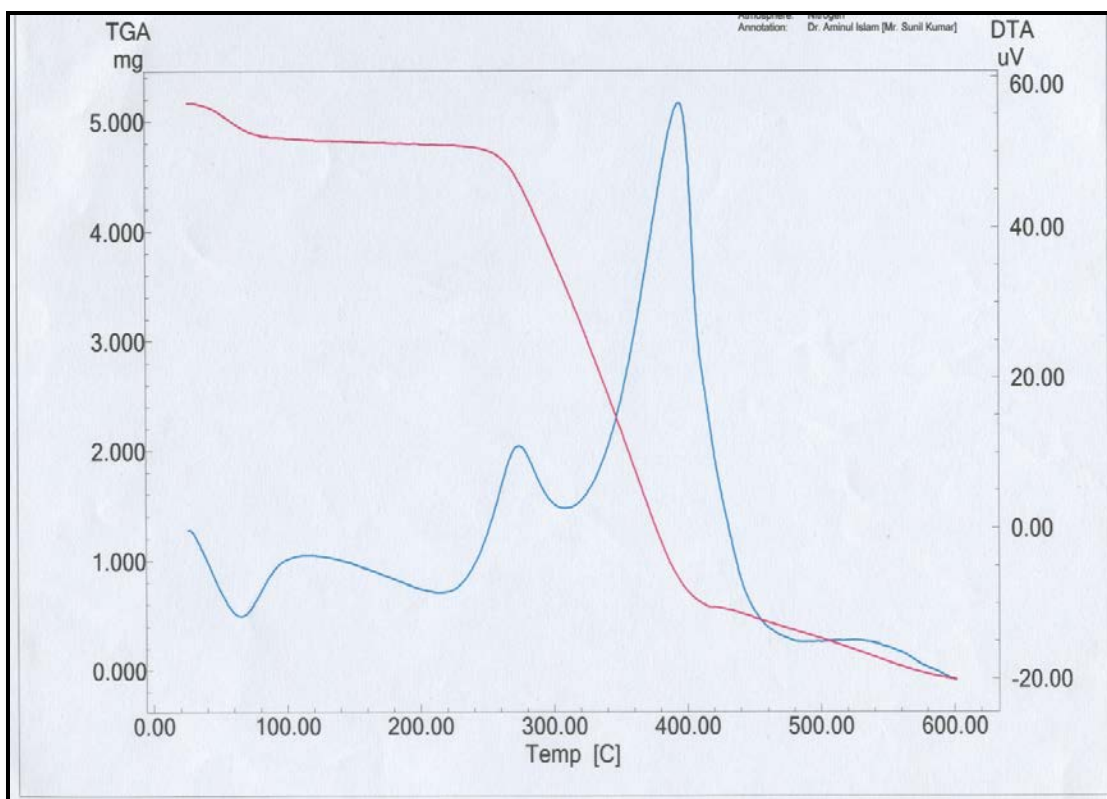


Figure: 4.3 TGA of terpolymeric resin

4.3.2. Optimization of experimental variables

In order to understand the effect of solution pH on the sorption/removal of Zn(II) and Cd(II), PGEME resin was equilibrated for 180 min over the pH range of 4-8. The sorption at higher pH values were not studied due to the probable hydroxide formation. It was observed that the maximum sorption for Cd(II) (53.96 mg g^{-1}) and Zn(II) (24.19 mg g^{-1}) were found at pH 8.0 (Figure. 4.4) which may be ascribed to the increased basicity of amine groups of the ligand at $\text{pH} > 7$ resulting in high affinity for metal chelation. On the contrary, the non functionalized PGEME resin did not sorb any analyte in the studied pH range. Hence, pH 8.0 was considered as the optimum pH for rest of the experiments. Similarly, the effect of sorption time was studied equilibrating for the time interval of 10, 20, 30, 45, 60, 90, 120, and 150 minutes. It was observed that for both the analyte ions, 90 minutes were required to reach the equilibrium (Figure. 4.5) and hence, optimized for further studies. The HCl and HNO_3 have been tested as eluting agents for the recovery of the sorbed analytes varying their volume and concentration. The 5 mL of 2 M HNO_3 was

observed to recover a maximum of 86% of Zn(II) and 100% of Cd(II) while using 5 mL of 2.5 M HCl gave complete desorption (100% recovery) of both the analyte ions. Since elution does not involve the use of any carcinogenic organic solvent, proposed method could be considered as environmentally safe.

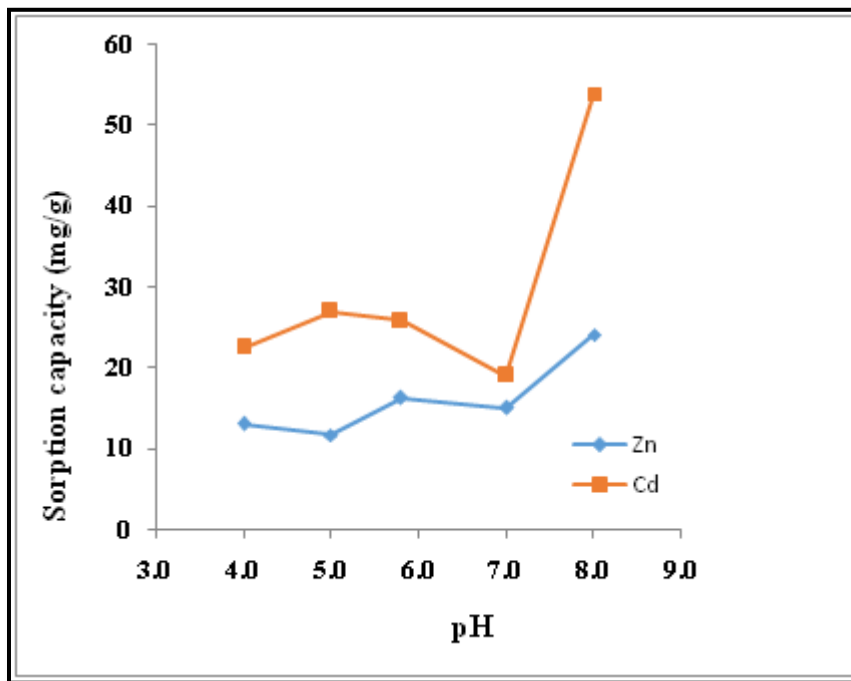


Figure 4.4: Effect of pH on metal ion sorption

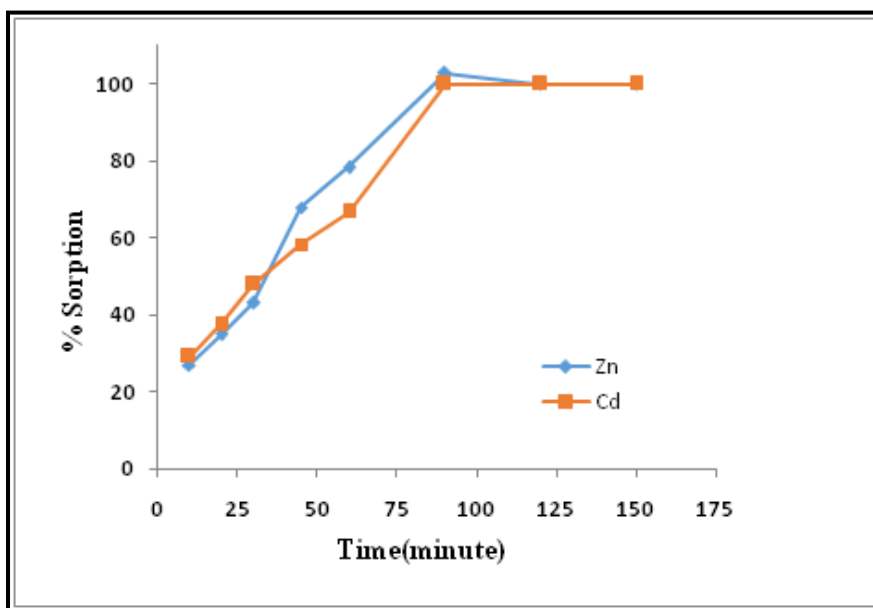


Figure 4.5: Effect of time on % sorption for Cd(II) and Zn(II)

4.4. Kinetic of Sorption

Lagergren pseudo-first-order [34] and pseudo-second-order kinetic models were used for the evaluation of rate constant and order of the sorption reaction (Equation 1 and 2, chapter 2) as shown in figure 4.6 and 4.7, respectively.

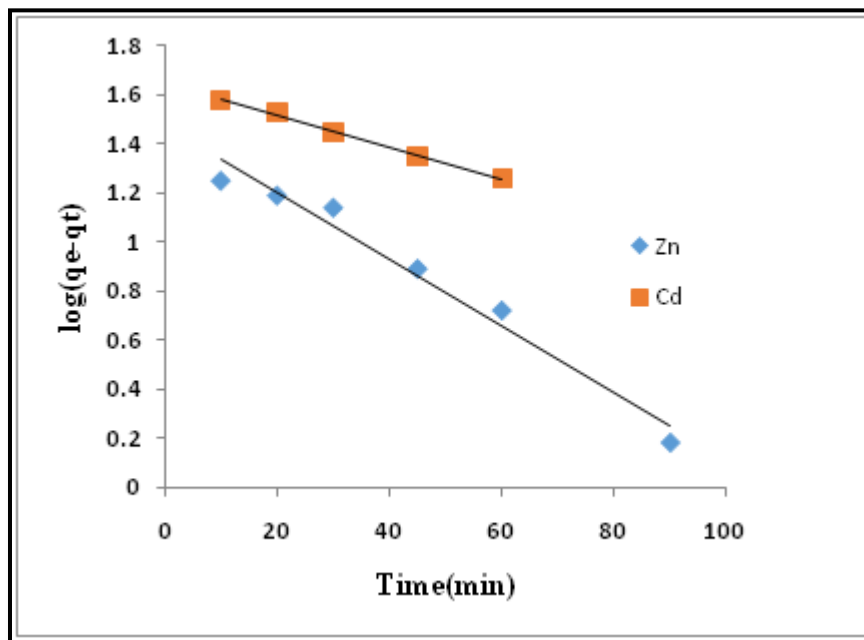


Figure 4.6: Pseudo first order kinetic model for Cd(II) and Zn(II).

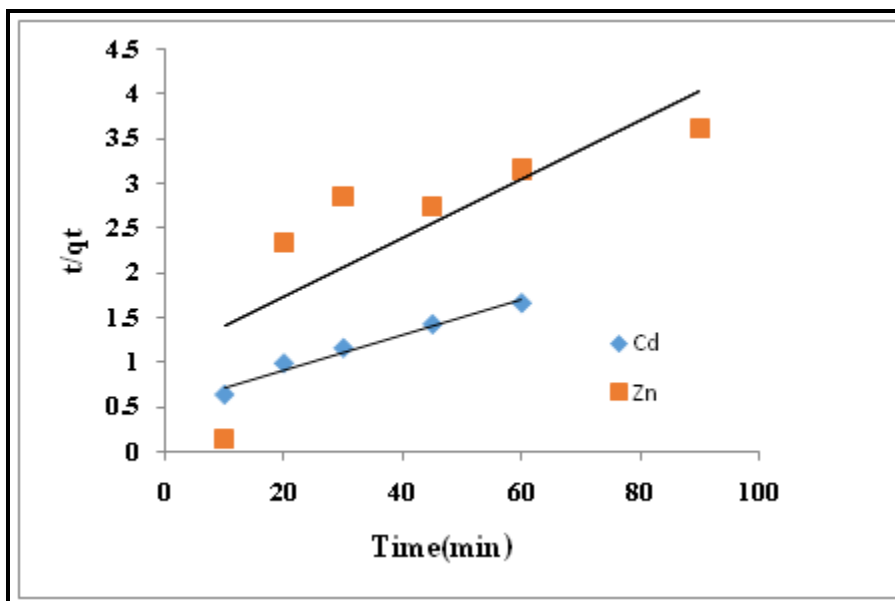


Figure 4.7: Pseudo second order kinetic model for Cd(II) and Zn(II)

From the obtained R^2 values of 0.997 for Cd(II) and 0.971 for Zn (II), it was observed that the pseudo first order kinetic model provided good correlations for the kinetic data of both metal ions rather than pseudo second order kinetics.

4.5 Sorption Isotherms

The sorption behavior of the resin for Cd(II) and Zn(II) were evaluated by analyzing Langmuir, Freundlich, Temkin, Dubinin-Radushkevich (D-R) and Scatchard isotherm as shown in figure (4.8- 4.12) (detailed in chapter 3, Equation 3 to 10) . The concentration for these studies was increased in the range of 45-674 mg L⁻¹ for Cd(II) and 65-392 mg L⁻¹ for Zn(II). Summary of the results was shown in table 4.1.

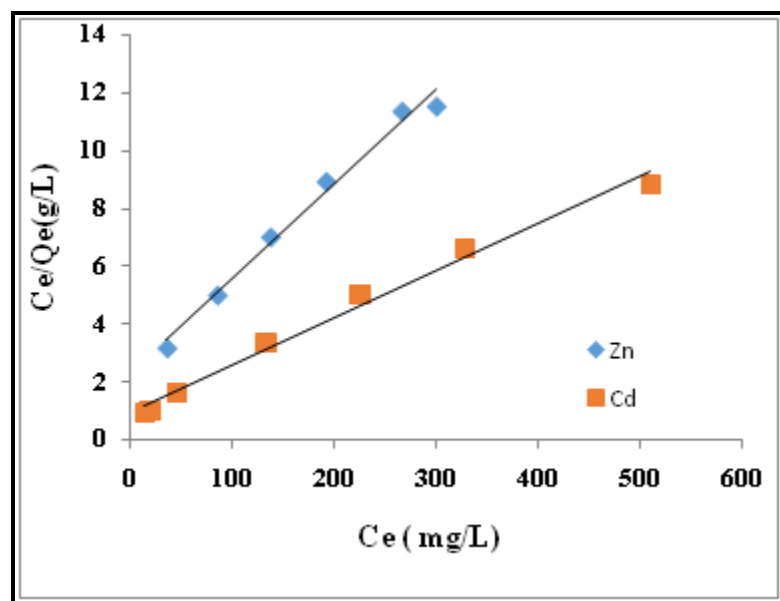


Figure 4.8: Langmuir isotherm model

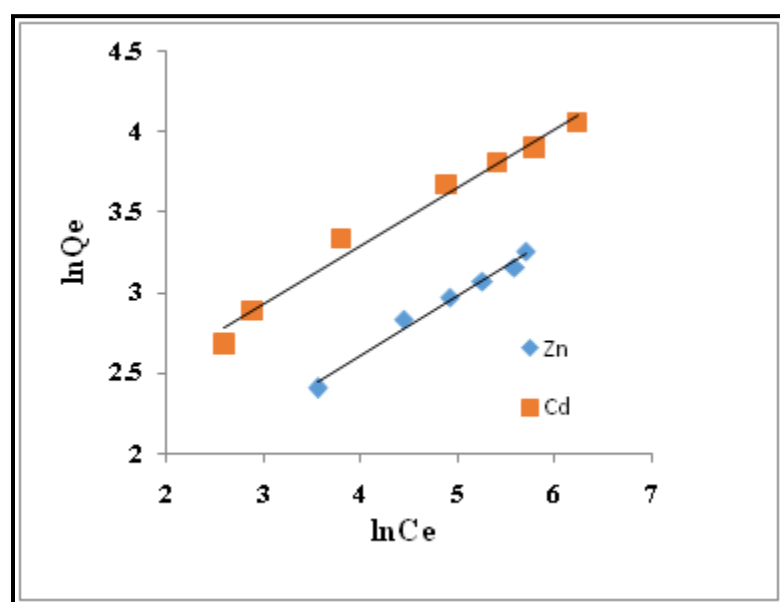


Figure 4.9: Freundlich isotherm model

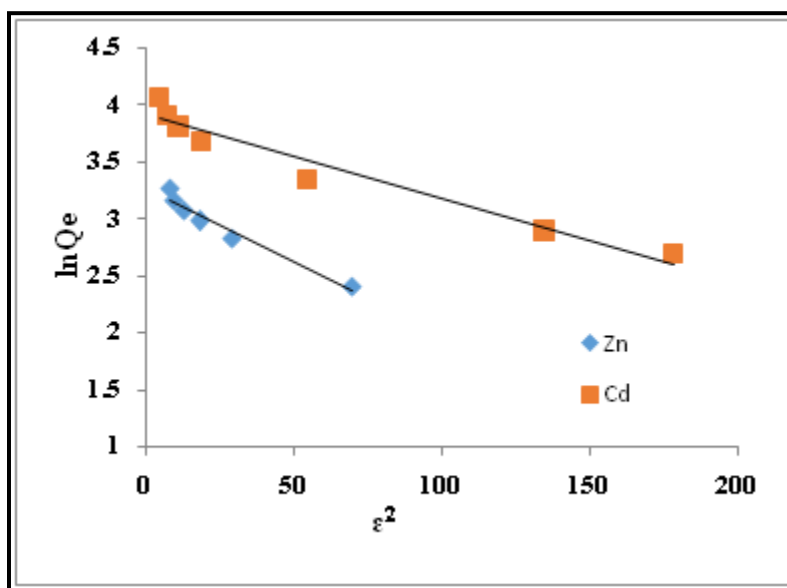


Figure 4.10: Dubinin–Radushkevich (D-R) isotherm model

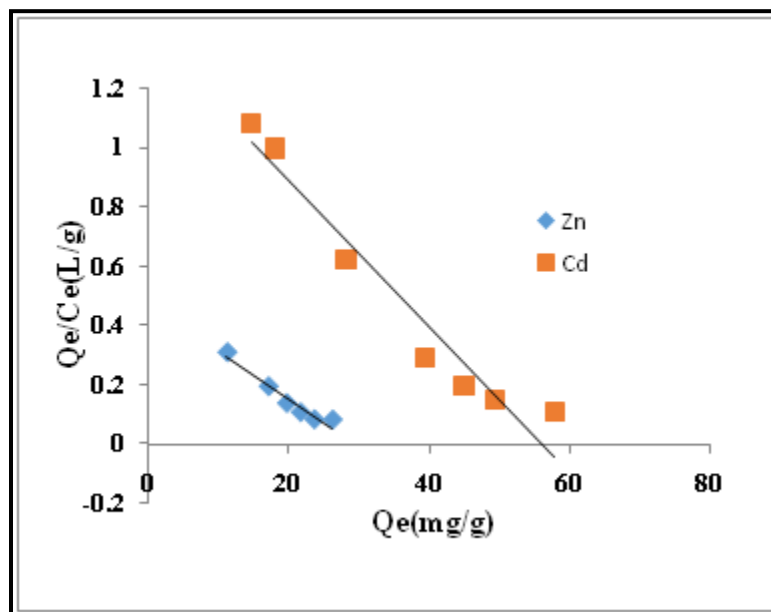


Figure 4.11: Scatchard isotherm model

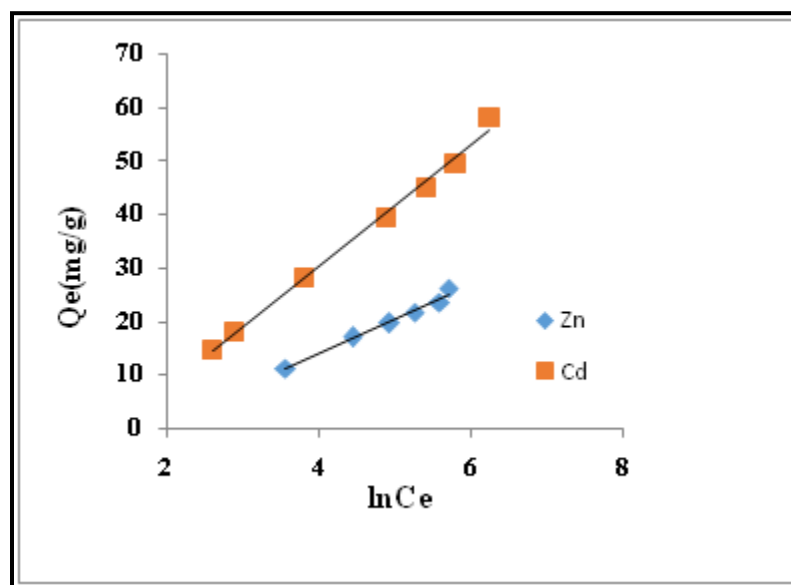


Figure 4.12: Temkin isotherm model

It was observed from results (Table 4.1) that, Langmuir sorption capacity found to be closer to the experimental batch capacity. Hence, Langmuir model considered to be best fit rather than Freundlich model. Moreover, in Langmuir model the nature of the isotherm is also explained by separation factor (R_L). The fact that for all the initial concentration values $0 < R_L < 1$ (Table 4.2), indicates the favorable sorption of Cd(II) and Zn(II). The value of mean free energy (E) in D-R isotherm was found to be in the range of 8-16 kJ mol⁻¹ which gives the idea that chemisorption of Cd(II) and Zn(II) by PGEME resin.

Table 4. 1.

Parameters driven from different isotherm models for Cd(II) and Zn(II) sorption by PGEME resin.

Isotherm models	Parameters	Cd(II)	Zn(II)
	Sorption capacity (mg g ⁻¹)	53.95	24.19
Langmuir isotherm	Q _m (mg g ⁻¹)	62.5	31.25
	k _b (mL mg)	0.015	0.014
	R ²	0.988	0.987
Freundlich isotherm	Q(mg g ⁻¹)	6.27	3.02
	n	2.75	2.67
	R ²	0.981	0.983
Dubinin-Radushkevich	Q(mg g ⁻¹)	49.69	26.18
	K(mol ² kJ ²)	0.007	0.012
	E(kJ mol)	8.45	6.45
	R ²	0.949	0.957
Scatchard isotherm	Q(mg g ⁻¹)	57.33	29.81
	k _b	0.024	0.016
	R ²	0.945	0.948
Temkin	A	11.33	6.538
	K _T (L mg ⁻¹)	3.81	1.88
	R ²	0.994	0.984

Table 4. 2.
 R_L values for Cd(II) and Zn (II) sorption obtained from
Langmuir equation.

Cd(II)		Zn(II)	
C_o (mg L⁻¹)	R_L	C_o(mg L⁻¹)	R_L
45	0.308	65	0.234
56	0.263	131	0.133
112	0.152	196	0.093
224	0.082	261	0.071
337	0.056	326	0.058
450	0.043	392	0.049
674	0.029		

4.6 Interference studies in the SPE coupled FAAS procedure

To investigate the effect of such coexisting ions, a synthetic solution of Cd(II) and Zn(II) (200 $\mu\text{g L}^{-1}$ each) were prepared along with the interferents and the recovery of analyte ions were checked following recommended batch procedure. The results are shown in (Table 4.3). The tolerance level for interfering ions were set as the maximum concentration that caused a variation in the absorbance of eluent concentration more than $\pm 5\%$, than the absorbance of eluent for 200.0 $\mu\text{g L}^{-1}$ of Cd(II) and Zn(II) alone after SPE. The result shows that the tolerance of 3 to 5 times the amount of transition metals, 10^3 to 10^4 times the amount of the alkali, alkaline earth metals and anions which is at par with the other SPE coupled FAAS determination methods for Cd and Zn [35,36] and therefore, indicating fair selectivity for the PGEME resin.

Table 4.3. Effect of interfering ions on the recovery of Cd(II) and Zn(II) (experimental condition pH 8.0, eluent 5mL of 2.5M HCl, 50 mL of sample containing 10µg each of Cd and Zn).

Interfering ions	Added as	Amount added (µg)	Zn % Recovery (RSD) ^a	Cd % Recovery (RSD) ^a
Cu	Cu(NO ₃) ₂	30	98 (1.63)	100 (0.35)
		50	88.9 (2.36)	49.8 (2.83)
Co	Co(NO ₃) ₂	30	100 (3.32)	100 (3.55)
		50	100 (2.18)	47.8 (4.58)
Ni	NiNO ₃	30	99 (2.73)	102 (4.59)
Fe	Fe(NO ₃) ₃	30	100 (3.58)	98 (1.74)
Cr	CrCl ₃	30	101 (1.05)	99 (2.95)
Pb	Pb(NO ₃) ₂	30	97 (1.87)	100 (3.75)
Ca ²⁺	CaCl ₂	20×10 ⁴	96 (1.82)	99 (2.31)
Na ⁺	NaCl	14×10 ⁴	98 (4.15)	99 (0.68)
Mg ²⁺	MgCl ₂	24×10 ³	97 (1.26)	98 (1.09)
NO ₃ ³⁻	NaNO ₃	15×10 ³	96.8 (1.33)	102.3 (2.15)
Cl ⁻	NaCl	23×10 ⁴	98 (4.15)	99 (1.96)
PO ₄ ³⁻	Na ₂ HPO ₄	56×10 ²	100 (2.41)	101 (3.76)

a; N=3

4.7 Analytical Figures of Merits

The mean concentration values for Cd(II) and Zn(II) obtained in SRM, (NIES 10c; Rice flour) by the proposed method were statistically insignificant from the certified values (Table 4.4) since the Student's t-test values for mean concentration of Zn(II) was less than the critical Student's t-value of 4.303 at 95% confidence level for $N = 3$, hence, indicating the absence of systematic method errors. Recovery experiments were carried out after spiking with a known amount (two levels) of Cd(II) and Zn(II) in rice, bread and fruit juice. The mean percentage recoveries were found to be 96-105% with relative standard deviation (RSD) <5% (Table 4.4), indicating the reliability of the present method for the determination of Cd(II) and Zn(II) in food samples of various matrices without significant interference. The calibration curve with the regression equation, correlation coefficient (R^2) and concentration range ($\mu\text{g L}^{-1}$) for Cd(II) and Zn(II) determination were $A=0.196X_{\text{Cd}}+0.151$; $R^2=0.999$; 5.1-1800 and $A=0.213X_{\text{Zn}}+0.029$; $R^2=0.998$; 4.1-1500, respectively. The standard deviation of slope and intercept for Cd(II) and Zn(II) were observed to be 0.2758, 1.62 and 0.071, 1.01, respectively. A procedural blank run was performed applying recommended SPE method with 50 mL of aqueous solution prepared by adding suitable buffer (excluding metal ions) and eluting the same in 5 mL of eluate before subjecting it to FAAS determination. The LOD and LOQ evaluated as $3S_b/m/10$ and $10S_b/m/10$ (where S_b and m are standard deviation of the mean blank absorbance signal and slope of the calibration equation, respectively) were found to be 1.5 and $5.1 \mu\text{g L}^{-1}$ for Cd and 1.2 and $4.1 \mu\text{g L}^{-1}$ for Zn, for 15 replicate blank runs. Analytical figures of merits were not studied for terpolymer resins synthesized earlier [37-39] (some other references are also included in table 4.6).

4.8 Application

The practical applicability of the developed method was examined by determination of both Cd(II) and Zn(II) in bread, fruit juice and multivitamin capsules using optimized batch procedure. The summary of the results was shown in table (Table 4.4). The assay of Zn in multivitamin capsules exhibited close agreement with the reported value while the Cd was not detected. From the experimental results it was found that the concentration of Cd in bread is higher than that of Zn. The results of Cd and Zn content in the rice and

bread samples are comparable with the previous reported literature as evidenced from the data presented in table 4.5. In order to explore the utility of PGEME and demonstrate the reproducibility of its synthetic route, fruit juice sample was analyzed applying recommended procedure using PGEME synthesized in three batches in a replicate manner. Cd and Zn were observed to exhibit mean value of 5.9 and 11.37 $\mu\text{g L}^{-1}$ with corresponding RSD of 3.62 and 4.95%, respectively which is in close agreement with the data presented in table 4.4.

Table 4.4.

Analytical results for FAAS determination of Cd and Zn in SRM, multivitamin and food Samples after SPE.

Samples	Concentration Certified/reported ^d ($\mu\text{g g}^{-1}$) $\pm\text{SD}^g$		Concentration after SPE ^a ($\mu\text{g L}^{-1}$ or $\mu\text{g g}^{-1}$) $\pm\text{SD}^g$	Amount spiked ($\mu\text{g g}^{-1}$ or $\mu\text{g L}^{-1}$)	Amount Found ($\mu\text{g g}^{-1}$ or $\mu\text{g L}^{-1}$) (RSD)	%Recovery
Rice flour NIES-10c ^b	Cd	1.82 \pm 0.06	1.78 \pm 0.08	-	-	-
	Zn	23.1 \pm 0.8	23 \pm 0.57			
Multivitamin in capsules ^d	Cd	NR ^e	ND ^f	-	-	-
	Zn	442.5	441 \pm 2.14			
Rice ^b	Cd	-	-	0	0.073 (4.25)	-
				5	5.04 (0.82)	99.3
				10	10.05 (0.48)	100.3
	Zn	-	-	0	1.03 (5.46)	-
				5	6.13 (3.37)	102.2
				10	11.08 (4.01)	98.87
Bread ^b	Cd	-	-	0	0.089 (4.49)	-
				2	2.066 (0.79)	98.9
				4	4.046 (1.45)	99.02
	Zn			0	0.049 (6.12)	-
				2	2.094 (2.99)	102.3
				4	4.036 (1.43)	97.67
Fruit juice ^c	Cd			0	6.1 (2.96)	-
				40	44.54 (3.26)	96.1
				60	64.63 (2.68)	97.6
	Zn			0	11.26 (4.12)	-
				40	53.53 (3.44)	105.7
				60	71.68 (4.00)	100.7

^a solid phase extraction; ^b $\mu\text{g g}^{-1}$; ^c $\mu\text{g L}^{-1}$; N = 3; ^d multivitamin; ^e NR(Not Reported); ^fND (Not detected); $\pm\text{SD}^g$ standard deviation

Table 4.5. Comparison of Cd and Zn content in rice and bread samples with the previously reported work

Technique	Rice ($\mu\text{g g}^{-1}$)		Bread ($\mu\text{g g}^{-1}$)		Reference
	Cd	Zn	Cd	Zn	
Direct-ETAAS ^a	-	-	0.03	-	[40]
Direct- FAAS ^b	0.029	16.4	-	-	[41]
Direct ICP-MS ^c	0.078	-	-	-	[42]
Direct GF-AAS ^d	0.09	-	-	-	[43]
Direct GF-AAS	-	-	0.039	-	[44]
SPE-FAAS	0.073	1.03	0.089	0.049	This work

^aElectrothermal atomic absorption spectrometer, ^bFlame atomic absorption spectrometer, ^cGraphite furnace atomic absorption spectrometer, ^dInductively coupled plasma mass spectrometer.

4.9 Conclusion

The synthesized PGEME terpolymeric resin offers a greater surface area than the similar reported resins and exhibits reasonable sorption capacity for Cd and Zn, thereby making it a suitable chelating resin. According to our literature knowledge, this is the first attempt to develop an environmentally safe SPE coupled FAAS analytical method for the determination of Cd and Zn in food samples as evidenced from the first five terpolymer reported in Table 4.6. It excludes major concomitant alkali and alkaline earth elements and their counter anions which otherwise often interfere in the subsequent FAAS determination of metals. The calibration curve was found to be linear ($R^2 > 0.998$) in the concentration range of 5.1-1800 and 4.1-1500 $\mu\text{g L}^{-1}$ for Cd and Zn, respectively. The accuracy and precision of the proposed method was evaluated by the analysis of SRM, recovery experiments and RSD of less than 5% in most of the analysis. The resin was successfully applied for the separation, preconcentration and determination of Cd(II) and Zn(II) ions in rice, bread and fruit juice samples. The striking feature about utility of PGEME as solid phase extractant is its reproducibility of synthesis which is indicated by the reproducibility of results in the analysis of real samples. The chemisorption and identical, independent binding site behaviour were evaluated by Dubinin-Radushkevich

isotherm and Scatchard plot analysis. From the fact that equilibrium data fit well to Langmuir adsorption isotherms ($R^2=0.988$) indicating a typical monolayer sorption and adsorbent-adsorbate interaction from Temkin isotherm model, the resin could further explored for the removal of Cd from wastewater.

Table 4.6. Comparison of figures of merits of some solid phase extractants used for metal ion separation and determination.

Materials	Element	pH	SC ^a (mg g ⁻¹)	LOD ^b (µg L ⁻¹)	Method/ Detection	Application	Referenc e
Ethylenediamine functionalized GMA- MMA-EGDMA terpolymer	Cd Zn	8.0	53.96 24.19	1.5 1.2	SPE ^d / FAAS ^e	Food samples	This work
Poly(GMA-MMA- EGDMA) terpolymer	Hg	5.5	124.8	-	SPE/AAS	Waste water	[29]
2-amino-6-nitro- benzothiazole- semicarbazide- formaldehyde terpolymer	Fe, Co, Ni, Cu, Zn, Pb	2.5-6.0	13.16, 5.95, 8.06, 10.99, 8.85,12.35	-	IE ^f	-	[45]
2-amino-6-nitro- benzothiazole- ethylenediamine- formaldehyde terpolymer	Fe, Cu, Ni, Zn, Co, Pb	2.5-6.0	14.49, 9.6, 8.29, 9.26, 7.41, 14.71	-	IE	-	[46]
8-HQ- salicylic acid- formaldehyde terpolymer	Pb, Zn, Cu, Ni, Ba, Co, Mn	3.0-5.5	-	-	IE	-	[47]
Salicylic acid- diaminonaphthalein	Fe, Cu, Ni, Co,	1.5-6.5	-	-	IE	-	[48]

formaldehyde terpolymer	Zn, Cd, Pb						
Diethylenetriamine functionalized GMA-divinyl benzene copolymer	Ni	2.0	20.25	2.29	SPE/FAAS	Environmental water samples	[49]
Amberlite XAD-4-salicylic acid	Cu, Cr, Zn, Cd, Mn, Ni, Co	5.5-8.0	15.57, 8.12, 10.13, 16.29, 6.87, 7.19, 4.12	0.42, 0.57, 0.63, 0.77, 0.94, 0.96, 1.41	SPE/FAAS	Water and food samples	[21]
Amberlite XAD-16-salicylanilide	Cu, Co, Ni, Zn, Cr, Cd, Pb	6.0-9.0	48.94, 431.47, 42.15, 41.31, 31.88, 32.63, 30.77	0.56, 0.64, 0.65, 0.70, 0.75, 0.88, 1.17	SPE/FAAS	Environmental and biological samples	[20]
4-(Phenyl diazenyl) benzene-1,3-diamine	Cu	10	-	0.6	CPE ^g /FAAS	Environmental and biological samples	[8]
Ion imprinting polymer	Cd	6.0	32.9	0.006	MSPE ^h /GFAAS ⁱ	Biological and food samples	[24]
Ion imprinting polymer	Zn	6.0	22.11	1.0	SPE/FAAS	Food and water samples	[23]
Ionic liquid	Cu, Zn, Cd	7.0	-	-	LLE ^j /FAAS	Aqueous solutions	[14]
Silica gel	Pb	8.0	-	0.36	SPE/FAAS	Beer and tea samples	[18]

Deep eutectic solvent	Pb, Cd	-	-	0.008, 0.0002	LPME ^k / ETAAS	Edible oils	[12]
2-hydroxy-5-nonylacetophenone oxime Cyanex 302 Di(2-ethylhexyl) phosphoric acid	Cu, Zn, Cd, Ni	1.90- 2.29	-	-	SX ^l /AAS	Liquor of e-waste	[9]

^a sorption capacity, ^blimit of detection, ^dsolid phase extraction, ^eflame atomic absorption spectrometry, ^fion exchange, ^gcloud point extraction, ^hmicro-solid phase extraction, ⁱgraphite furnace atomic absorption spectrometry, ^jliquid liquid extraction, k liquid phase micro-extraction, ^lsolvent extraction.

References

- [1] C. Baird, M. Cann, Environmental chemistry (4th ed.). New York: Freeman, (Chapter 15) (2008).
- [2] Health risks of heavy metals from long-range transboundary air pollution, WHO/Europe (2007)
- [3] Division of Toxicology and Human Health Sciences, Priority List of Hazardous Substances, ATSDR, (2013).
- [4] H. Hu, In D. L. Kasper, E. Braunwald, A. S. Fauci, S. L. Hauser, D. L. Longo, J. L. Jameson (Eds.), Harrison's principles of internal medicine (pp. 2577–2580), New York: McGraw-Hill, Chapter 376 (2005).
- [5] G. J. Fosmire, Zinc toxicity, The American Journal of Clinical Nutrition, 51 (1990) 225–227.
- [6] N. K. Amin, O. Abdelwahab, E. S. Z. El-Ashtoukhy, Removal of Cu(II) and Ni(II) by ion exchange resin in packed rotating cylinder, Desalination and Water Treatment 55 (2015) 199-209.
- [7] S. A. Nabi, Alimuddin, A. Islam, Synthesis and characterization of anew cation exchanger-zirconium(IV) iodotungstate: Separation and determination of metal ion contents of synthetic mixtures, pharmaceutical preparations and standard reference material, Journal of Hazardous Materials, 172 (2009) 202–207.
- [8] A. Shokrollahi, M. Ghaedi, O. Hossaini, N. Khanjari, M. Soylak, Cloud point extraction and flame atomic absorption spectrometry combination for copper(II) ion in environmental and biological samples, Journal of Hazardous Materials 160 (2008) 435-440.
- [9] M. K. Jha, D. Gupta, P. K. Choubey, V. Kumar, J. Jeong, C. J. Lee, Solvent extraction of copper, zinc, cadmium and nickel from sulphate solution in mixer settler unit (MSU), Separation and Purification Technology, 122(2014) 119–127.
- [10] I. Komjarova, R. Blust, Comparison of liquid–liquid extraction, solid-phase extraction and co-precipitation preconcentration methods for the determination of cadmium, copper, nickel, lead and zinc in seawater, Analytica Chimica Acta, 576 (2006) 221–228.

- [11] Y. Dai, G. J. Witkamp, R. Verpoorte, Y. Hae Choi, Natural deep eutectic solvents as a new extraction media for phenolic metabolites in *Carthamus tinctorius* L, *Analytical Chemistry* 85 (2013) 6272–6278.
- [12] M. Karimi, S. Dadfarnia, A. M. Haji Shabani, F. Tamaddon, D. Azadi, Deep eutectic liquid organic salt as a new solvent for liquid-phase microextraction and its application in ligand less extraction and pre- concentration of lead and cadmium in edible oils, *Talanta* 144 (2015) 648–654.
- [13] A. K. Kumar, B. S. Parikh, M. Pravakar, Natural deep eutectic solvent mediated pretreatment of rice straw: Bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue, *Environmental Science and Pollution Research* 23 (2015) 9265–9275.
- [14] R. Leyma, S. Platzer, F. Jirsa, W. Kandioller, R. Krachler, B. K. Keppler, Novel thiosalicylate-based ionic liquids for heavy metal extractions, *Journal of Hazardous Materials*, 314 (2016) 164–171.
- [15] D. Das, U. Gupta, A. K. Das, Recent developments in solid phase extraction in elemental speciation of environmental samples with special reference to aqueous solutions, *TrAC Trends in Analytical Chemistry* 38 (2012) 163–171.
- [16] A. Islam, A. Ahmad, M. A. Laskar, Preparation, characterization of a novel chelating resin functionalized with o-Hydroxybenzamide and its application for preconcentration of trace metal ions, *Clean-Soil, Air, Water* 40 (2012) 54–65.
- [17] A. Tobiasz, S. Walas, Solid-phase-extraction procedures for atomic spectrometry determination of copper, *Trends in Analytical Chemistry* 62 (2014) 106–122.
- [18] J. Cheng, X. Ma, Y. Wu, Silica gel chemically modified with ionic liquid as novel sorbent for solid-phase extraction and preconcentration of lead from beer and tea drink samples followed by flame atomic absorption spectrometric determination, *Food Analytical Methods* 7 (2014) 1083–1089.
- [19] M. M. Lopez Guerrero, M. T. Siles Cordero, E. Vereda Alonso, A. Garcia de Torres, J. M. Cano Pavon, Synthesis and characterization of a novel mesoporous silica functionalized with [1,5 bis(di-2-pyridyl)methylene thiocarbohydrazide] and its application as enrichment sorbent for determination of antimony by FI– HG–ETAAS, *Talanta*, 129 (2014) 1–8.

- [20] A. Islam, A. Ahmad, M. A. Laskar, A newly developed salicylanilide functionalized Amberlite XAD-16 chelating resin for use in preconcentration and determination of trace metal ions from environmental and biological samples. *Analytical Methods*, 3 (2011) 2041–2048.
- [21] A. Islam, A. Ahmad, M. A. Laskar, Characterization of a chelating resin functionalized via azo spacer and its analytical applicability for the determination of trace metal ions in real matrices, *Journal of Applied Polymer Science*, 123 (2012) 3448–3458.
- [22] A. Islam, N. Zaidi, H. Ahmad, S. Kumar, Efficacy of dihydroxy-mercapto pyrimidine functionalized polymeric resin for the trace determination of Cd by SPE coupled flame atomic absorption spectrometry, *RSC Advances*, 5 (2015) 46662–46671.
- [23] M. Roushani, S. Abbasi, H. Khani, R. Sahraei, Synthesis and application of ion-imprinted polymer nanoparticles for the extraction and preconcentration of zinc ions, *Food Chemistry*, 173 (2015) 266–273.
- [24] B. Zhao, M. He, B. Chen, B. Hu, Novel ion imprinted magnetic mesoporous silica for selective magnetic solid phase extraction of trace Cd followed by graphite furnace atomic absorption spectrometry detection, *Spectrochimica Acta Part B* 107 (2015) 115–124.
- [25] B. Barfi, M. Rajabi, M. Morshedi Zadeh, M. Ghaedi, M. Salavati-Niasari, R. Sahraei, Extraction of ultra-traces of lead, chromium and copper using ruthenium nanoparticles loaded on activated carbon and modified with N, N bis-(*o*-methylsalicylidene)-2,2-dimethylpropane-1,3-diamine, *Microchimica Acta* 182 (2015) 1187–1196.
- [26] K. Pyrzyńska, M. Trojanowicz, Functionalized cellulose sorbents for preconcentration of trace metals in environmental analysis, *Critical Reviews in Analytical Chemistry*, 29 (1999) 313–321.
- [27] G. Bayramoglu, S. Kiralp, M. Yilmaz, L. Toppare, M. Yakup Arica, Covalent immobilization of chloroperoxidase onto magnetic beads: Catalytic properties and stability, *Biochemical Engineering Journal*, 38 (2008) 180–188.

- [28] G. Bayramoglu, Y. Tunali, M. Yakup Arica, Immobilization of b-galactosidase onto magnetic poly(GMA–MMA) beads for hydrolysis of lactose in bed reactor, *Catalysis Communications* 8 (2007) 1094–1101.
- [29] G. Bayramoglu, M. Yakup Arica, Kinetics of mercury ions removal from synthetic aqueous solutions using by novel magnetic p(GMA-MMA-EGDMA) beads, *Journal of Hazardous Materials* 144 (2007) 449-457.
- [30] G. Bayramoglu, M. Yılmaz, A. UlkuSenel, M. Yakup Arica, Preparation of nano fibrous polymer grafted magnetic poly(GMA-MMA)-g-MAA beads for immobilization of trypsin via adsorption, *Biochemical Engineering Journal* 40 (2008) 262–274.
- [31] A. Nastasovi, S. Jovanovic, D. Dordevic, A. Onjia, D. Jakovljevic, T. Novakovic, Metal sorption on macroporous poly(GMA-co-EGDMA) modified with ethylene diamine, *Reactive & Functional Polymers*, 58 (2004) 139–147.
- [32] G. Socrates, *Infrared characteristics group frequencies* (3rd ed.). New York: Wiley Inter Science, (1980).
- [33] S. A. Idris, C.M. Davidson, C. McManamon, M.A. Morris, P. Anderson, Gibson, L.T. Large pore diameter MCM-41 and its application for lead removal from aqueous media, *Journal of Hazardous Materials* 185 (2011) 898-904.
- [34] S. Lagergren, K. Svenska Vetenskapsakad, *Handl* 24 (1898) 1-39.
- [35] T. Dasbasi, S. Sacmaci, A. Ulgen, S. Kartal, A solid phase extraction procedure for the determination of Cd(II) and Pb(II) ions in food and water samples by flame atomic absorption spectrometry, *Food Chemistry* 174(2015) 591–596.
- [36] T. Dasbasi, S. Sacmaci, A. Ulgen, S. Kartal, Determination of some metal ions in various meat and baby food samples by atomic spectrometry, *Food Chemistry* 197(2016) 107–113.
- [37] R. S. Azarudeen, R. Subha, D. Jeyakumar, A. R. Burkanudeen, Batch separation studies for the removal of heavy metal ions using a chelating terpolymer: Synthesis, characterization and isotherm models, *Separation and Purification Technology* 116 (2013) 366–377.

- [38] A. Gupta, R. Jain, D. C. Gupta, Studies on uptake behavior of Hg(II) and Pb(II) by amine modified glycidyl methacrylate-styrene-N, N0-methylenebisacrylamide terpolymer, *Reactive and Functional Polymers*, 93 (2015) 22–29.
- [39] M. M. Jadhao, S. Kumar, L. J. Paliwal, N. S. Bhavé, S. Alam, Resin-V: Synthesis and physicochemical studies of a biphenol-guanidine-formaldehyde terpolymer resin, *Journal of Applied Polymer Science*, 118(2010) 1969–1978.
- [40] R. Tahvonen, J. Kumpulainen, Lead and cadmium contents in Finnish breads, *Food Additives & Contaminants*, 11 (1994) 621–31.
- [41] R. Masironi, S. R. Koirttyohann, J. O. Pierce, Zinc, copper, cadmium and chromium in polished and unpolished rice, *Science of the Total Environment*, 7 (1977) 27–43.
- [42] A. A. Meharg, G. Norton, C. Deacon, P. Williams, E. E. Adomako, A. Price, Y. Zhu, G. Li, F. J. Zhao, S. McGrath, A. Villada, A. Sommella, P. M. C. S. De Silva, H. Brammer, T. Dasgupta, M. R. Islam, Variation in rice cadmium related to human exposure, *Environmental Science & Technology*, 47 (2013) 5613–5618.
- [43] Y. Fang, X. Sun, W. Yang, N. Ma, Z. Xin, J. Fu, X. Liu, M. Liu, A. M. Mariga, X. Zhu, Q. Hu, Concentrations and health risks of lead, cadmium, arsenic, and mercury in rice and edible mushrooms in China, *Food Chemistry* 147 (2014) 147–151.
- [44] E. H. Larsen, N. L. Andersen, A. Møller, A. Petersen, G. K. Mortensen, J. Petersen, Monitoring the content and intake of trace elements from food in Denmark, *Food Additives & Contaminants* 19 (2002) 33–46.
- [45] M. A. Riswan Ahamed, D. Jeyakumar, A. R. Burkanudeen, Removal of cations using ion-binding terpolymer involving 2-amino-6-nitro-benzothiazole and thiosemicarbazide with formaldehyde by batch equilibrium technique, *Journal of Hazardous Materials*, 248–249 (2013) 59–68.
- [46] M. A. Riswan Ahamed, R. Subha, D. Jeyakumar, A. R. Burkanudeen, Separation of metal ions by the influence of a cation-exchange terpolymer involving 2-amino-6-nitrobenzothiazole-ethylenediamine-formaldehyde, *Polymer International*, 64 (2014) 126–137.
- [47] R. S. Azarudeen, M. A. Riswan Ahamed, A. R. Burkanudeen, Chelating terpolymer resin: Synthesis, characterization and its ion-exchange properties, *Desalination* 268(2011) 90–96.

- [43] Dhanraj T. Masram, Narayan S. Bhavé, Kiran P. Kariya, Synthesis of resin IV: Salicylic acid, diaminonaphthalene, and formaldehyde terpolymer and its ion exchange, *Journal of Applied Polymer Science*, 117 (2010) 315–321.
- [49] A. Islam, N. Zaidi, H. Ahmad, S. Kumar, Amine-functionalized mesoporous polymer as potential sorbent for nickel preconcentration from electroplating wastewater, *Environmental Science and Pollution Research*, 22 (2015) 7716–7725.

Chapter 5

***Glycidylmethacrylate
Based Resin
Functionalized with
Graphene oxide for
Column Preconcentration
and Trace Determination
of Cd(II) and Ni(II) in
Environmental and Food
Samples***

5.1 Introduction

Graphene oxide (GO) is a well established two-dimensional single-layered sp^2 -hybridized carbon atoms derived from graphite by chemical oxidation [1]. In solid phase extraction (SPE) and preconcentration of metal ions [2-5] graphene oxide (GO) is used due to its large specific surface area leading to high sorption capacity and the hydrophilic functional groups like epoxy (-COC), hydroxyl (-OH), carbonyl (-CO), and carboxyl (-COOH) present on the edges and basal plane of GO [6,7] form chelates with the metal ion [8] and shows fast metal ion uptake. Direct use of GO in SPE becomes challenging; particularly when the sheet size ranging to the nanoscale, due to unfavorable phase separation and recycling of GO [2]. Recently published data shows the use of GO in the separation and preconcentration of metal ions by dispersive solid phase extraction (DSPE) [9], dispersive magnetic solid phase extraction (DMSPE) [10,11] and cloud point extraction (CPE) [12]. However, these methods have several drawbacks e.g. in dispersive mode filtration of GO leads to its aggregation because of strong van der Waals interactions and π - π stacking between GO sheets which can significantly affect the efficiency, reusability and needs sonication for redispersion of GO. During filtration there is a possibility of escape of GO which is simplified by the use of DMSPE. In CPE, the regeneration of GO is not possible since the analyte along with GO slurry is introduced into atomic spectrometer for analyte determination and gets degraded during atomization [12]. Moreover, in all the three methods, application of large sample volume is restricted for metal ion preconcentration. Direct use of GO in SPE cartridge/column creates back pressure and needs a high pressure peristaltic pump which may result in GO escaping [8]. In order to simplify these problems GO can be immobilized onto a solid support material for column application, providing enough flow rate for large sample volume without a use of high-pressure peristaltic pump. These solids not only play a role of support but also enhance the hydrophilicity due to the presence of functional groups and provide spacer arms. The introduction of spacer arm between the solid supports and GO is a new approach by virtue of which GO behaves in a manner similar to DSPE as well as free monomeric ligand in homogenous system for longer spacer arms and forms complexes with metal ions [13,14].

In this chapter, we have developed a column preconcentration method for determination of Cd(II) and Ni(II) in environmental and food samples using novel GO-based solid phase extractant prepared by the coupling of GO with glycidylmethacrylate beads via diethylenetriamine spacer arm (Synthesis scheme was detailed in **chapter 2**, section 2.1.2). Since, the polymer bound GO sheets restricts to aggregation and leaching from column compared to direct use of GO and can be reused for several sorption elution cycle in SPE.

5.2 Experimental

Prepared p-DETAGO resin has been characterized by solid state ^{13}C NMR, FT-IR, TGA/DTA, SEM along with EDS, TEM analysis by using the recommended instruments as reported in **Chapter 2** (Section 2.2). For the optimization of experimental variables batch dynamic mode had been selected for effect of sorption/desorption and effect of contact time of Ni and Cd as reported in, while the rest of the variables were optimized following column method **Chapter 3** (Section 3.8). Sample solution adjusted to pH 8.0 ± 0.1 of optimum concentration was passed through the column at a flow rate of $2\text{--}6\text{ mL min}^{-1}$. Sorbed Ni(II) and Cd(II) were eluted with 5.0 mL of 2M HCl and the concentration of Ni and Cd was determined subsequently by FAAS.

5.3. Result and discussion

5.3.1 Characterization

The FT-IR spectra of p-DETAGO resin shown in figure 5.1. The O-H stretching vibrations [18,19] of (-COOH and -OH) is appearing at 3373.8 cm^{-1} and peak for sp^2 - CH stretching vibrations appeared at 2943.5 cm^{-1} . The sharp band at 1725.26 cm^{-1} is due the presence of C=O.¹⁹ N-H (bending), C-N and C-O bands are observed at 1560.88 cm^{-1} , 1387.66 cm^{-1} and 1160.59 cm^{-1} , respectively [18,19]. The peak 1271.42 cm^{-1} is due to -CH of the epoxy group. The band 1472.41 cm^{-1} can be attributed to stretching of C=C bonds [19]. The chemical shifts observed in ^{13}C NMR spectra (Figure 5.2) indicates the presence of amide bond (168.37 ppm), $-\text{CH}_2\text{-NHR}$ (45.22 ppm), benzylic $-\text{C-NHR}$ (66.22 ppm, 67.22 ppm), aromatic carbons (106.66 ppm-138.66 ppm), carboxylic carbon (177.59 ppm), and carbonylic carbon (179.34 ppm). The incorporation of an amine group

on to GMA matrix was estimated by shaking 0.5 g of p-DETA resin with 50 mL of 0.05M HCl for 24 h. The residual HCl was measured titrimetrically against 0.05 M NaOH. The number of moles of HCl that interacted with the amine group and consequently the amine content were found to be 1.95 mM g⁻¹ of the resin. Multi point BET surface area of p-DETAGO resin was found to be 51.063 m² g⁻¹. Structure morphology of p-DETAGO was evaluated by the SEM analysis along with EDS. GO sheets were decorated on the surface of the p-DETA resin beads as it can be seen from the SEM and TEM images (Figure 5.3). The presence of atom on pendant functional groups after immobilization of GO on GMA-DETA and metal ion complexation was confirmed by EDS analysis shown in figure 5.4 including their elemental % weight. The elemental analysis C, H, N and O resulted in % content as 55.6, 7.7, 7.0 and 29.7. TGA analysis shows that the resin was thermally stable up to 245 °C (5.4).

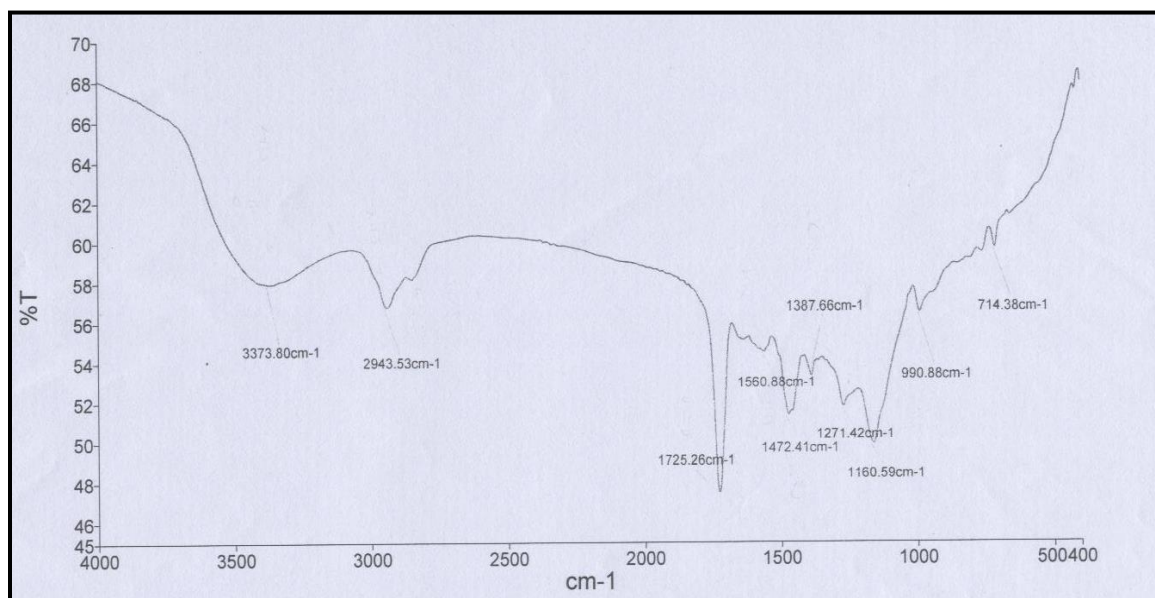


Figure 5.1 FTIR spectrum of p-DETAGO

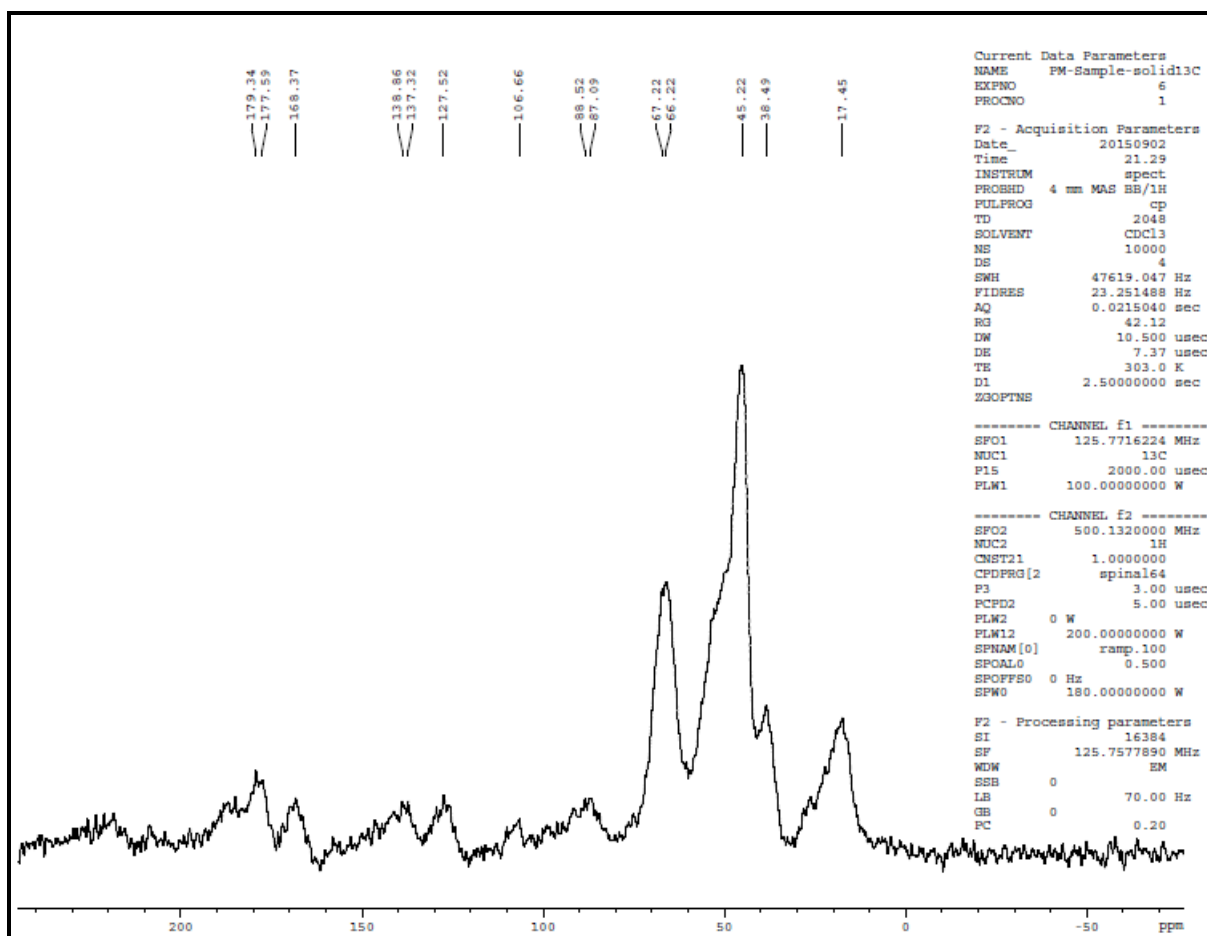


Figure 5.2: ^{13}C NMR spectrum of p-DETAGO

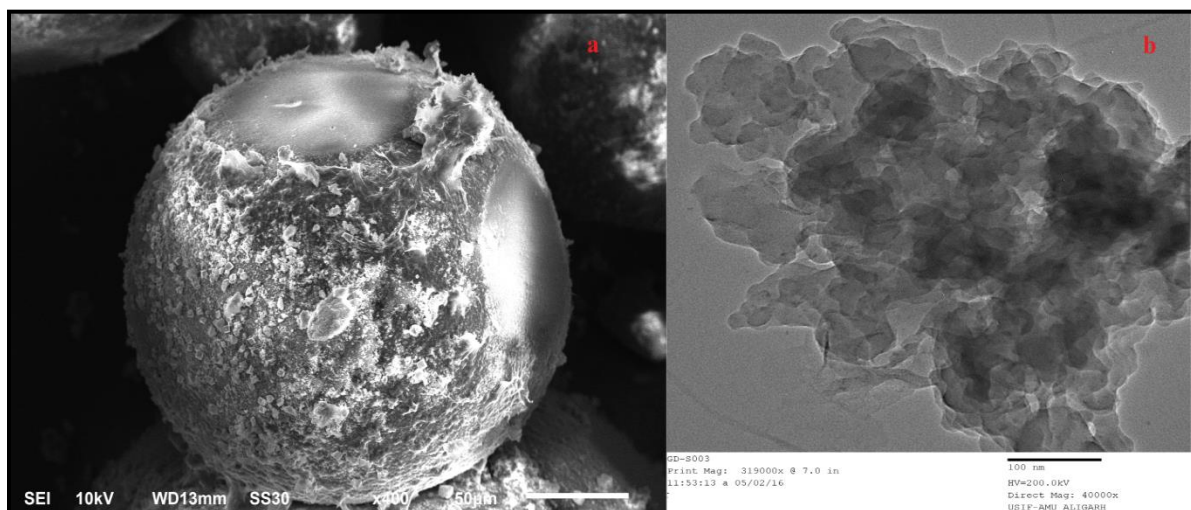


Figure 5.3 Micrographs of p-DETAGO (a) SEM and (b) TEM showing the decoration GO sheets onto the GMA matrix.

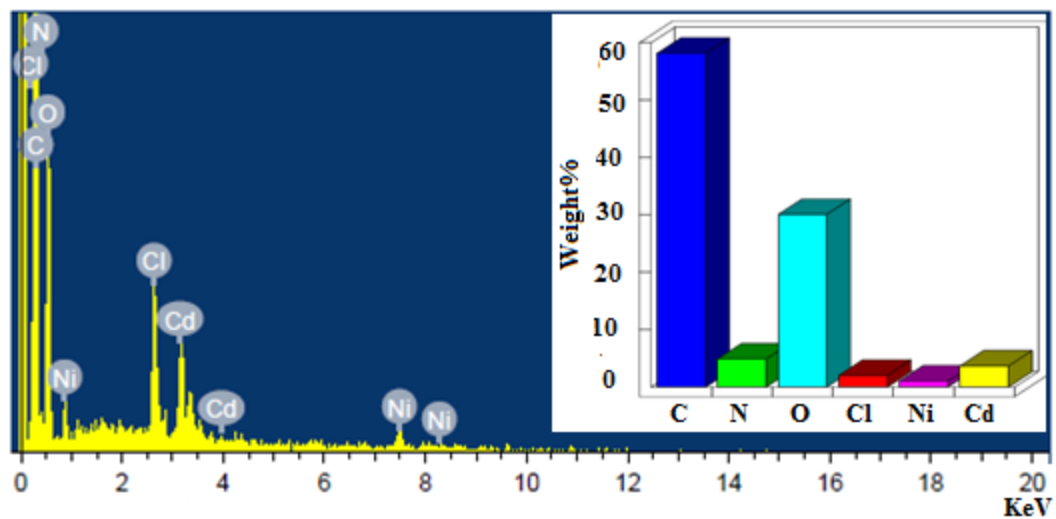


Figure 5.4 EDS investigation after Ni(II) and Cd(II) sorption.

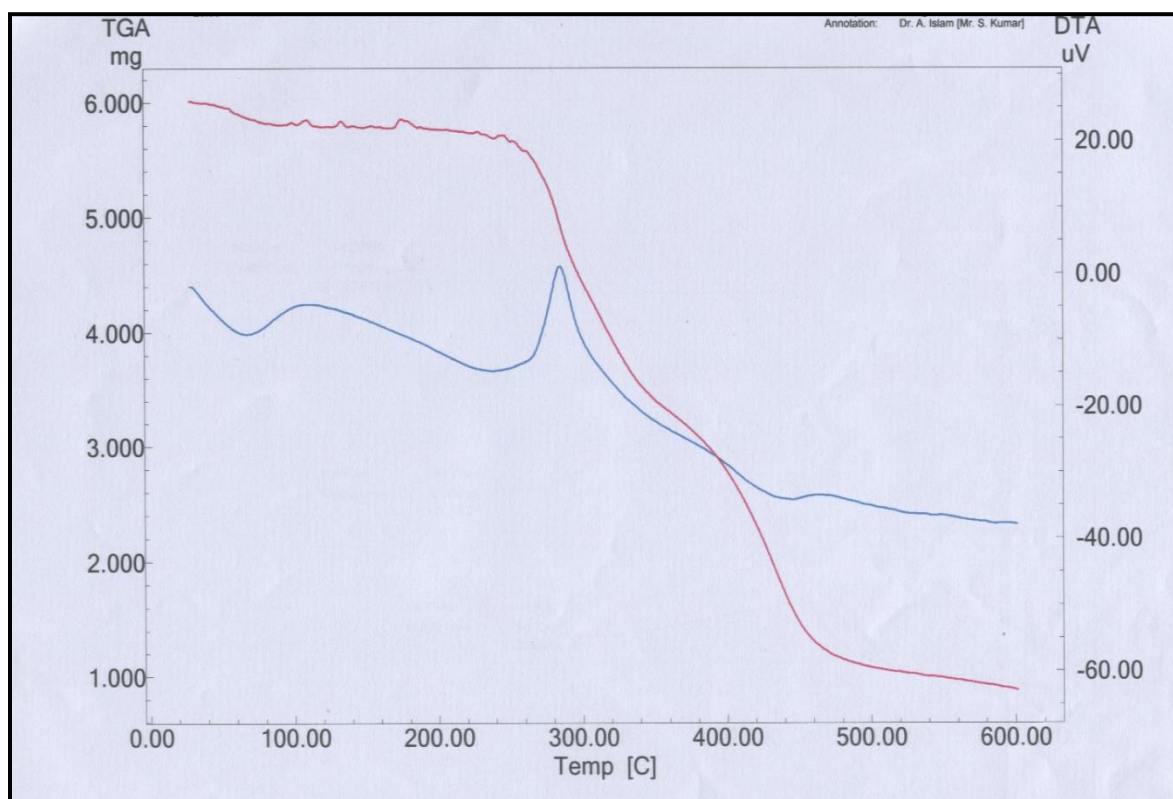


Figure 5.5 TGA/DTA analysis of p-DETAGO

5.3.2 Optimization of experimental parameters

The variables pH, time and eluent was optimized by batch mode while rest of with the recommended column procedure. For optimization of sample pH, 0.1 g of p-DETAGO resin was equilibrated with 50 ml of 0.05 mM Ni(II) and Cd(II) ions solution over a pH range of 2.0-10.0 for 180 min. It can be seen from the figure 5.6a that sorption capacity of both metal ions was found to be a maximum at pH 8.0. The slight decrease in the sorption capacity was observed at pH 9.0 and 10.0. The pH studies for p-EDAGO were also carried out as shown in a Table 5.1. It is clear from the data that the sorption capacity of p-EDAGO resin was less than that of p-DETAGO resin. Hence, p-DETAGO was selected as a final sorbent for rest of the experimental studies. The higher capacity of the p-DETAGO resin is because of deprotonation, which makes the oxygen-containing GO functional groups negatively charged, which facilitates complexation with Ni^{2+} and Cd^{2+} owing to the strong electrostatic interactions [20]. Hence, pH 8.0 was fixed as optimum for rest of the studies. Sorption behaviour is more clearly understood by the point of zero charged (pHpzc) studies which was carried out following the procedure as reported in some previous literature [21-22]. The 0.1g of the p-DETAGO resin and 50 ml solution of 0.1 M NaCl were taken into a series of conical flasks with their initial pH (pH_i) adjusted in the range 2.0-10.0. It was kept for 24hr with occasional shaking and the final pH (pH_f) was measured after decanting. The value of pHpzc was found to be 4.6 from point of intersection in pH_i versus ΔpH ($\text{pH}_i - \text{pH}_f$) as shown in figure (5.7). Since $\text{pHpzc} < \text{pH}$ it was concluded that the surface of the resin is negatively charged and favors the sorption of positive metal ions due to strong electrostatic interaction. For the optimization of contact time between resin and metal ions, the sorption of Ni(II) and Cd(II) on p-DETAGO resin was studied in the different time interval and it was observed from the experimental results that 40 min was sufficient for the complete sorption of Ni(II) and 30 min for Cd(II) figure 5.6b. Thus, Ni(II) takes more time than Cd(II) to reach the equilibrium because of high complexation rate between borderline acid Ni(II) and hard base O^- of GO while Cd(II) comes in the category of soft acids. Fast sorption kinetics is a great advantage for the preconcentration studies. Column flow rate was optimized by passing a 100 mL solution containing 5 μg of Ni(II) and Cd(II) buffered at $\text{pH } 8.0 \pm 0.1$ over the range of 2-7 mL min^{-1} . The results showed that the sorption of the analyte ions

on the column was unaffected up to a flow rate of 2.5 mL min⁻¹ for Ni(II) and 5.0 mL min⁻¹ for Cd(II) and it was maintained throughout the column experiment. HCl and HNO₃ were used as an eluent in order to check the quantitative recovery of sorbed Ni(II) and Cd(II). It was observed from the experimental studies that 5 mL of 2M HCl gives the recovery of >98% for both the metal ion. Thus, 5 mL of 2M HCl was selected as an eluting agent. The effect of temperature on the sorption of metal ions has been studied by batch method. The results are shown in the table (Table 5.2). Increase of sorption capacity as well as decrease of loading half time for both Ni(II) and Cd(II) were observed with the increase of temperature which are in agreement with reported studies [23,24]. However, 25±0.2°C was selected for further studies since application of SPE method involves real samples having temperature closed to it. The prepared p-DETAGO resin was tested for reusability by several loading and elution cycles and it was concluded that resin can be regenerated up to 36 cycles without loss in capacity indicating that GO remains immobilized on to resin matrix. The releases of GO sheets are potential health threat to ecosystems [25]. Hence, the method is environmentally green and has good application in the separation and preconcentration of metal ions.

Table. 5.1
Comparison of sorption capacity of different sorbent during
synthesis (Experimental conditions: 0.1 g resin, 50mL
solution of 0.05mM Ni(II) and Cd(II)

Sorbent	pH	Ni(II) SC ^a (mg g ⁻¹)	Cd(II) SC ^a (mg g ⁻¹)
GMA-DVB-EDA	8.0	1.76	10.12
GMA-DVB-EDA-GO	8.0	9.39	37.66
GMA-DVB-DETA	8.0	7.04	30.35
GMA-DVB-DETA-GO	8.0	17.02	38.78

^a sorption capacity

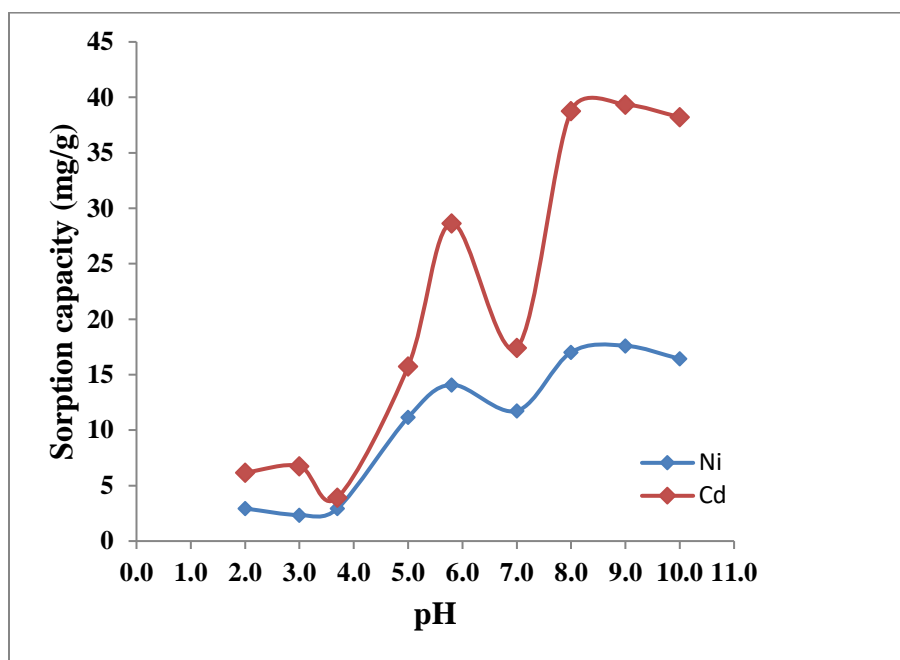


Figure 5.6a Effect of sample solution pH on the sorption of Ni(II) and Cd(II)

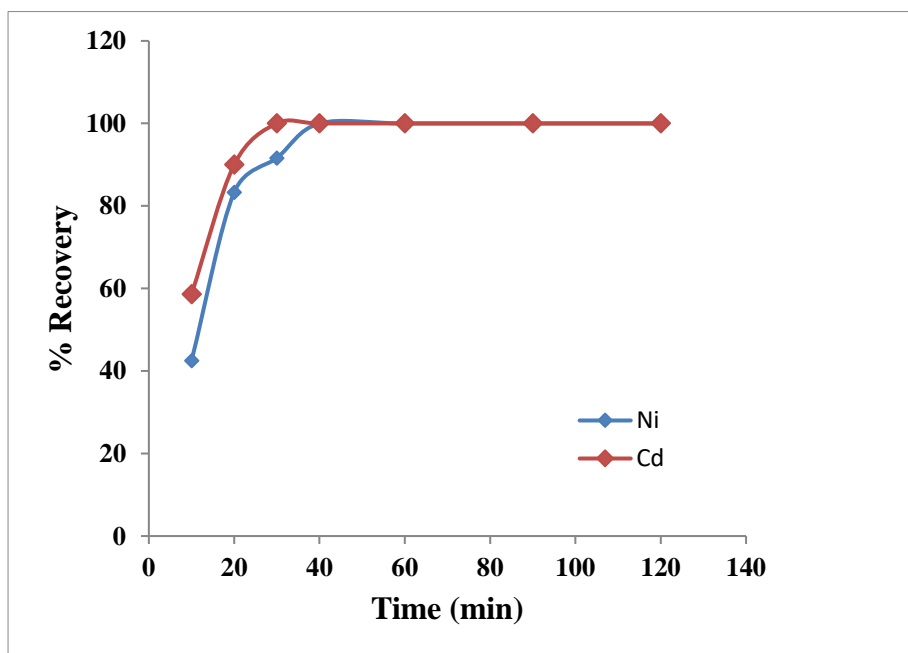


Figure 5.6b effect of contact time on the % recovery of Ni(II) and Cd(II)

Table 5.2. Effect of temperature on the sorption and loading half time of Ni(II) and Cd(II) (Experimental conditions: 0.1 g resin, 50mL solution of 0.05mM Ni(II) and Cd(II))

Temperature	Sorption capacity (mg g ⁻¹)		Loading half time $t^{1/2}$ /min	
	Ni(II)	Cd(II)	Ni(II)	Cd(II)
25±0.2°C	17.02	38.78	15	10
35±0.2°C	19.95	41.59	10	8
45±0.2°C	21.13	43.84	5	5

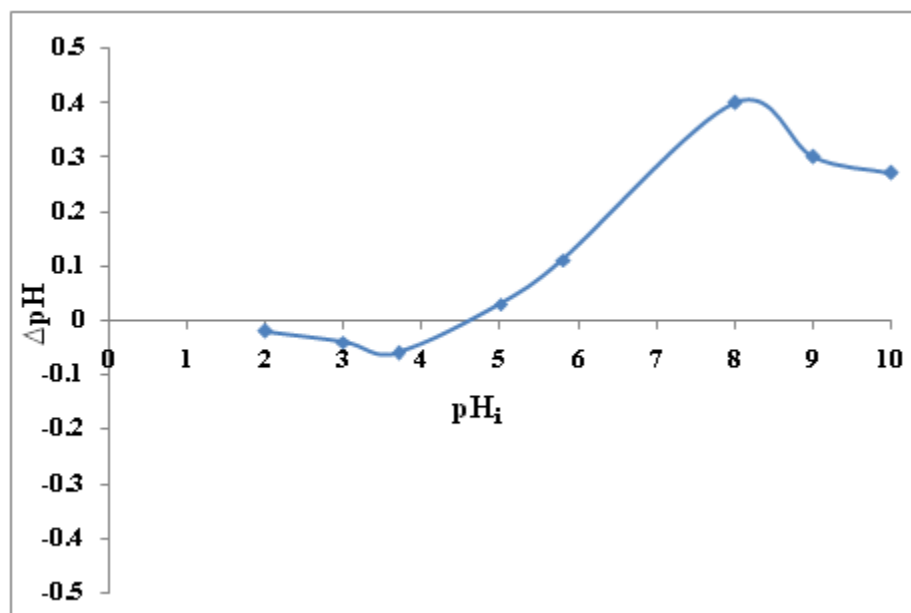


Fig 5.7: The point of zero charge (pHpzc) of p-DETAGO

5.3.3 Interference studies

The extraction efficiency of the p-DETAGO resin in the determination of Ni(II) and Cd(II) was affected by co-existing interferents. In order to see the interfering effect, 5 μ g of Ni(II) and Cd(II) was checked out with some transition, alkali and alkaline earth metal ions (Table 5.3). As it was evidenced from the results, that no significant interferences were observed in the determination of Ni(II) and Cd(II) up to an appreciable tolerance level. Hence, the prepared resin has good applicability in the trace preconcentration and determination of analyte ions in different samples. The tolerance limit is defined as the interfering ions concentration causing a relative error smaller than $\pm 5\%$ related to the preconcentration/determination of the analytes.

Table. 5.3

Interference studies of some commonly interfering ions in the determination of Ni(II) and Cd(II) by FAAS (Experimental conditions: 0.1g resin, 100 ml solution containing 5µg of Ni(II) and Cd(II))

Interfering ions	Added as	Tolerance limit	Tolerance limit
		Ni(II) (µg L ⁻¹)	Cd(II) (µg L ⁻¹)
Zn	ZnCl ₂	500	200
Cd	CdCl ₂	500	—
Ni	NiNO ₃	—	500
Fe	Fe(NO ₃) ₃	400	250
Cr	CrCl ₃	400	250
Cu	Cu(NO ₃) ₂	400	250
Co	Co(NO ₃) ₂	500	200
Pb	Pb(NO ₃) ₂	500	250
Na	NaCl	19×10 ⁵	19×10 ⁵
Mg	MgCl ₂	48×10 ⁴	48×10 ⁵
Cl	NaCl	30×10 ⁵	30×10 ⁶
HPO ₄ ⁻²	Na ₂ HPO ₄	11×10 ⁵	11×10 ⁶
CH ₃ COO ⁻	C ₃ H ₂ NaO ₂	11×10 ⁴	11×10 ⁵
NO ₃ ⁻	NaNO ₃	15×10 ⁴	15×10 ⁵
relative error ≤5%			

5.3.4 Preconcentration studies

Our ultimate object is to preconcentrate the trace metal ions in water samples before subjected to FAAS determination in order to improve the detection limit and reduction of matrix effects which further enhances the accuracy. Preconcentration studies were done by constant loading (5 μ g) of Ni(II) and Cd(II) with increasing volume of samples. As a result of this, preconcentration limits were found to be 5.56 μ g L⁻¹ and 6.25 μ g L⁻¹ along with the preconcentration factors of 180, 160 for Ni(II) and Cd(II), respectively.

5.4 Analytical Method Validation

Limit of detection (LOD), limit of quantification (LOQ), linearity and correlation coefficient (R^2) were important parameters for validation of method [26-27]. A calibration curve was

Table 5.4.
Validation of developed Column preconcentration SPE method by the analysis of SRM for Ni and Cd concentration

SRM	Certified value (μ g g ⁻¹)	Found value (μ g g ⁻¹) ^a	Calculated Student's t value ^b
Ni JSS 513-4 Cr Steel	1300	1289 \pm 2.0	3.198
Cd NIES-10c	1.82 \pm 0.06	1.79 \pm 0.03	0.5814

^a Mean value \pm 95 % confidence limit; N=3. ^b At 95% confidence limit

plotted using least square method under the optimized conditions which results regression equation $Y=0.054X_{Ni} + 0.038$ and $Y=0.264X_{Cd} + 0.015$ and correlation coefficient $R^2=0.998$ for Ni(II) and Cd(II). The LOD and LOQ were found to be 0.23, 0.76 μ g L⁻¹ for Ni(II) and 0.06, 0.20 μ g L⁻¹ for Cd(II). The inter-day and intra-day repeatability experiments were performed in order to check the precision of the method by running 3 replicates of 50 mg L⁻¹ Cd(II) and Ni(II) between three columns and for three consecutive days. The average column to column and day to day coefficient of variation of the method was found to be 2.96, 3.14% for Cd(II) and 3.46, 2.54% for Ni(II), respectively.

The accuracy of the method was tested by analyzing SRM by the recommended column procedure. Calculated Student's t (t-test) values for Ni(II) and Cd(II) ions were found to be less than the critical Student's t value of 4.303 at 95 % confidence level for $N=3$ (Table 5.4). Hence, the mean concentration values were not statistically significant from the certified values indicating that absence of systematic errors and demonstrate the accuracy of the proposed method. The reliability of the developed method was also investigated by the analysis of electroplating waste water, river water, tap water and fruit juice spiked with the known amount (5 μ g) of Ni(II) and Cd(II), resulting in percentage recoveries of 95-107.2% with a relative standard deviation (RSD) <5% (Table 5.5).

Table. 5.5**FAAS determination Ni(II) and Cd(II) in environmental water samples and fruit juice**

Sample	Element	Amount spiked ($\mu\text{g L}^{-1}$) ^a	Amount Found ($\mu\text{g L}^{-1}$) ^a	%RSD	%Recovery
River water (Ganga)	Ni(II)	0	4.85	2.61	-
		10	14.92	1.24	100.7
	Cd(II)	0	3.76	2.06	-
		10	13.26	3.54	94.97
Tap water	Ni(II)	0	2.39	3.60	-
		10	13.11	0.73	107.2
	Cd(II)	0	1.84	2.17	-
		10	11.85	0.34	100.1
Electroplating waste water	Ni(II)	0	30.26	0.49	-
		25	54.09	0.48	95.33
	Cd(II)	0	2.22	1.69	-
		25	27.27	0.06	100.2
Fruit juice	Ni(II)	0	14.34	0.27	-
		25	39.53	0.16	100.7
	Cd(II)	0	2.92	2.24	-
		25	27.61	0.71	98.77

^a Mean value \pm 95% confidence limit; N=3

5.5. Conclusion

A novel GO based solid phase extractant was used in a column SPE method which allowed the determination of trace metal ions by cheap and less sensitive technique such as FAAS. Toxic GO was not observed to escape from p-DETAGO even after 36 sorption/elution cycles and elution of metal ions do not involve use of any carcinogenic organic solvents. The proposed method was simple, economic, ecofriendly and successfully applied for the separation and preconcentration of Cd(II) and Ni(II) in electroplating waste water, river water, tap water and fruit juice samples without any significant interference from commonly occurring cations and anions. The validity and reliability of the method was indicated by good accuracy and precision. It is evident from the comparison (Table 5.6) that the presented method shows good analytical figure of merits over other GO based column SPE method. Potential application in the removal of Cd(II) and Ni(II) could further be explored since it possesses comparatively high sorption capacity.

Table. 5.6**Comparison of figure of merits with previously reported GO based column SPE method**

Sorbent	Analyte	SC ^a (mg g ⁻¹)	LOD ^b (µg L ⁻¹)	Flow rate (mL min ⁻¹)	PF ^c	Technique	Reference
GMA-DVB-DETA-GO	Ni, Cd	17.02, 38.78	0.23, 0.06	2.5, 5.0	180, 160, 250	FAAS	This work
Si-GO	Cu, Pb	4.9, 13.6	0.084, 0.27	7	200	FI-AAS	[8]
Si-GO	Mn, Co, Ni Cu, Cd, Pb	-		0.2	10	ICP-MS	[28]
GO-TiO ₂	Cu, Pb, La, Ce, Eu, Dy, Yb	0.8, 13.5, 3.8, 2.9, 2.8, 2.7, 3.2	0.48, 2.64, 0.41, 0.24, 0.13, 0.26, 0.21	0.5	10	ICP-OES	[29]
GO-MCNTs-DETA	Cr, Fe, Pb, Mn	5.4, 13.8, 6.6, 9.5	0.16, 0.50, 0.24, 0.38	1.5	75	ICP-OES	[30]
GO-MHE ^d	Co, Ni	6.8, 7.0	0.25, 0.18	10	250	FAAS	[31]
GO-MPPC ^e	Mn, Fe	21.6, 24.0	0.145, 0.162	8	325	FAAS	[32]
GO-TBCPA ^f	Fe, Ni, Cu, Zn	6.7, 6.4, 6.0, 6.1	0.39, 0.11, 0.63, 0.45	9	250	FAAS	[33]

^asorption capacity, ^blimit of detection, ^cpreconcentration factor, ^dN-(5-methyl-2hydroxyacetophenone)-N'-(2-hydroxyacetophenone) ethylene diamine, ^e3-(1-Methyl-1H-pyrrol-2-yl)-1H-pyrazole-5-carboxylic acid, ^f2-(tert-butoxy)-N-(3-carbamothioylphenyl) acetamide

References

- [1] W. S. Hummers, R. E. Offeman, Preparation of Graphitic Oxide, *J. Am. Chem. Soc.* 80 (1958) 1339–1339.
- [2] A. Islam, H. Ahmad, N. Zaidi, S. Kumar, Graphene oxide sheets immobilized polystyrene for column preconcentration and sensitive determination of lead by flame atomic absorption spectrometry, *ACS Appl. Mater. Interfaces*, 6 (2014) 13257-13265.
- [3] B. Zawisza, A. Baranik, E. Malicka¹, E. Talik, R. Sitko, Preconcentration of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Pb(II) with ethylenediamine-modified graphene oxide, *Microchim. Acta* 183 (2016) 231-240.
- [4] R. Sitko, B. Zawisza, E. Malicka, Graphene as a new sorbent in analytical chemistry, *Trac, Trends in Analytical Chemistry*, 51 (2013) 33-43.
- [5] M. Ghazaghi, H.Z. Mousavi, A. M. Rashidi, H. Shirkhanloo, R. Rahighi, Innovative separation and preconcentration technique of coagulating homogenous dispersive micro solid phase extraction exploiting graphene oxide nanosheets, *Anal. Chim. Acta* 902 (2016) 33-42.
- [6] G. X. Zhao, J. X. Li, X. M. Ren, C. L. Chen, X. K. Wang, Few-Layered Graphene Oxide Nanosheets As Superior Sorbents for Heavy Metal Ion Pollution Management, *Environ. Sci. Technol.* 45 (2011) 10454–10462.
- [7] A. Lerf, H. He, M. Forster, Klinowski, Structure of Graphite Oxide Revisited, *J. Phys. Chem. B* 102 (1998) 4477–4482.
- [8] R. Sitko, B. Zawisza, E. Talik, P. Janik, G. Osoba, B. Feist, E. Malicka, Spherical silica particles decorated with graphene oxide nanosheets as a new sorbent in inorganic trace analysis, *Anal. Chim. Acta* 834 (2014) 22–29.
- [9] K. Pytlakowska, V. Kozik, M. Matussek, M. Pilch, B. Hachuła, K. Kocot, Glycine modified graphene oxide as a novel sorbent for preconcentration of chromium, copper, and zinc ions from water samples prior to energy dispersive

X-ray fluorescence spectrometric determination, RSC Adv. 6 (2016) 42836-42844.

- [10] A. Islam, H. Ahmad, N. Zaidi, S. Kumar, A graphene oxide decorated with triethylenetetramine-modified magnetite for separation of chromium species prior to their sequential speciation and determination via FAAS, Microchim. Acta 183 (2016) 289-296.
- [11] E. Kazemi, S. Dadfarnia, A. M. H. Shabani, Dispersive solid phase microextraction with magnetic graphene oxide as the sorbent for separation and preconcentration of ultra-trace amounts of gold ions, Talanta 141 (2015) 273-278.
- [12] D. Deng, X. Jiang, L. Yang, X. Hou, C. Zheng, Organic Solvent-Free Cloud Point Extraction-like Methodology Using Aggregation of Graphene Oxide, Anal. Chem. 86 (2014) 758-765.
- [13] H. Kumagai and Y. Inoue, Chromatographic Selectivity of Rare Earth Elements on Iminodiacetate-Type Chelating Resins Having Spacer Arms of Different Lengths: Importance of Steric Flexibility of Functional Group in a Polymer Chelating Resin, Anal. Chem. 70 (1998) 4070-4073.
- [14] M. A. L. Tanco, D. A. P. Tanaka, V. C. Flores, T. Nagase, T. M. Suzuki, Preparation of porous chelating resin containing linear polymer ligand and the adsorption characteristics for harmful metal ions, React. Funct. Polym. 53 (2002) 91-101.
- [15] A. Islam, N. Zaidi, H. Ahmad, S. Kumar, Amine-functionalized mesoporous polymer as potential sorbent for nickel preconcentration from electroplating wastewater, Environ Sci Pollut Res. 22 (2015) 7716-7725.
- [16] A. Islam, H. Ahmad, N. Zaidi, S. Yadav, Selective Separation of Aluminum from Biological and Environmental Samples Using Glyoxal-bis(2-hydroxyanil) Functionalized Amberlite XAD-16 Resin: Kinetics and Equilibrium Studies, Ind. Eng. Chem. Res. 52 (2013) 5213-5220.
- [17] A. Islam, N. Zaidi, H. Ahmad, S. Kumar, Efficacy of dihydroxy-mercaptopurine functionalized polymeric resin for the trace determination

- of Cd by SPE coupled flame atomic absorption spectrometry, RSC Adv.5 (2015) 46662-46671.
- [18] R. S. Drago, Physical Methods in Inorganic Chemistry, Litton Educational Publishing: USA, 1965.
- [19] G. Socrates, Infrared Characteristics Group Frequencies, John Wiley & Sons: New York, 1980.
- [20] R. Sitko, E. Turek, B. Zawisza, E. Malicka, E. Talik, J. Heimann, A. Gagor, B. Feista, R. Wrzalik, Adsorption of divalent metal ions from aqueous solutions using graphene oxide, Dalton Trans. 42 (2013) 5682-5689.
- [21] A.H. Gedam, R. S. Dongre, Adsorption characterization of Pb(II) ions onto iodate doped chitosan composite: equilibrium and kinetic studies, RSC Adv.5 (2015) 54188-54201.
- [22] S. Jayanthi, N. K. R. Eswar, S. A. Singh, K. Chatterjee, G. Madras, A. K. Sood, Macroporous three-dimensional graphene oxide foams for dye adsorption and antibacterial applications, RSC Adv. 6 (2016) 1231-1242
- [23] H. H. Najafabadi, M. Irani, L. R. Rad, A. H. Haratameh, I. Haririan, Removal of Cu²⁺, Pb²⁺ and Cr⁶⁺ from aqueous solutions using a chitosan/graphene oxide composite nanofibrous adsorbent, RSC Adv. 5 (2015) 16532-16539.
- [24] A. Islam, M. A. Laskar, A. Ahmad, Characterization and Application of 1-(2-Pyridylazo)-2-naphthol Functionalized Amberlite XAD-4 for Preconcentration of Trace Metal Ions in Real Matrices, J. Chem. Eng. Data 55 (2010) 5553-5561.
- [25] Y. L. Chang, S. T. Yang, J. H. Liu, E. Dong, Y.W. Wang, A. N Cao, Y. F Liu, H. F Wang, In vitro toxicity evaluation of graphene oxide on A549 cells, Toxicol. Lett. 200 (2011) 201-210.
- [26] S. Patra, E. Roy, R. Madhuri, P. K. Sharma, Fast and Selective Preconcentration of Europium from Wastewater and Coal Soil by Graphene Oxide/Silane@Fe₃O₄ Dendritic Nanostructure, Environ. Sci. Technol. 49 (2015) 6117-6126.
- [27] A. Islam, H. Ahmad, N. Zaidi, S. Kumar, Copper selective self-sorting polymeric resin with mixed-mode functionality for column preconcentration and atomic absorption spectrometric determination, RSC Adv. 6 (2016) 5590-5598.

- [28] S. Su, B. Chen, M. He, B. Hu, Graphene oxide–silica composite coating hollow fiber solid phase microextraction online coupled with inductively coupled plasma mass spectrometry for the determination of trace heavy metals in environmental water samples, *Talanta* 123 (2014) 1-9.
- [29] Y. Zhang, C. Zhong, Q. Zhang, B. Chen, M. He, Bin Hu, Graphene oxide–TiO₂ composite as a novel adsorbent for the preconcentration of heavy metals and rare earth elements in environmental samples followed by on-line inductively coupled plasma optical emission spectrometry detection, *RSC Adv.* 5 (2015) 5996-6005.
- [30] X. Zhu, Y. Cui, X. Chang, H. Wang, Selective solid-phase extraction and analysis of trace-level Cr(III), Fe(III), Pb(II), and Mn(II) Ions in wastewater using diethylenetriamine-functionalized carbon nanotubes dispersed in graphene oxide colloids, *Talanta* 146 (2016) 358-363.
- [31] M. R. Pourjavid, M. Arabieh, S. R. Yousefi, M. R. Jamali, M. Rezaee, M. H. Hosseini, A. A. Sehat, Study on column SPE with synthesized graphene oxide and FAAS for determination of trace amount of Co (II) and Ni (II) ions in real samples, *Mater. Sci. Eng.*, C47 (2015) 114-122.
- [32] M. R. Pourjavid, A. A. Sehat, M. Arabieh, S. R. Yousefi, M. H. Hosseini, M. Rezaee, Column solid phase extraction and flame atomic absorption spectrometric determination of manganese (II) and iron (III) ions in water, food and biological samples using 3-(1-methyl-1H-pyrrol-2-yl)-1H-pyrazole-5-carboxylic acid on synthesized graphene oxide, *Mater. Sci. Eng.*, C35 (2014) 370-378.
- [33] M. R. Pourjavid, A. A. Sehat, M. H. Hosseini, M. Rezaee, M. Arabieh, S. R. Yousefi, M. R. Jamali, Use of 2-(tert-butoxy)-N-(3-carbamothioylphenyl) acetamide and graphene oxide for separation and preconcentration of Fe (III), Ni (II), Cu (II) and Zn (II) ions in different samples, *Chin. Chem. Lett.* 25 (2014) 791-793.

Chapter 6

***Graphene Oxide
Supported on Amberlite
Resin for the Development
of Analytical Method
Based on Systematic
Approach of Column
Preconcentration/Sensitiv
e FAAS Determination of
Toxic Metal Ions in
Environmental Samples***

6.1 Introduction

The increasing levels of heavy metals, a major category of persistent, bio accumulative and toxic chemicals in the natural ecosystem have been the subject of great concern due to their environmental impact and pose a significant threat to biota. Their biochemical action includes the inhibition and reduction of various enzymes, complexation with certain ligands of amino acids and substitution of essential metal ions from enzymes [1-3]. Development of sensitive, accurate, selective and economical methods for the trace determination of heavy metals in industrial wastewater, various water bodies and biological samples is essential in environment monitoring and assessment of its level of exposure and effects onto biota. Despite the availability of highly sensitive, selective and rapid atomic spectroscopic and electro analytical techniques with simultaneous capability, direct trace determination of heavy metals in real samples with complex matrix can be complicated due to potential concomitant matrix effects [4-5]. Moreover, high initial capital investment, maintenance and running cost of sophisticated instruments render the affordability of a routine analytical laboratory. Flame atomic absorption spectrometry being the cheaper technique amongst them also has limitation of having low detection limit. Solid- phase extraction (SPE) [6-8] addresses these two problems by virtue of its ability to concentrate the desired trace elements and remove interfering constituents thereby improving the detection limits, precision, accuracy of the analytical methods and allowing the use of less sensitive and cost-effective technique. The focus of much current research in SPE as metal ion extractants is the development of functionalized polymers of high surface area and sorption capacity, by increasing the number of chelating sites on the material as well as their accessibility for metal ions. Recently, carbon based nanomaterials [9-11] as SPE sorbent have become an active area of research in the field of separation science due to their unique properties, like large surface area and high mechanical strength. Carbon nanotubes (CNT) possess great potential to remove metal ions and proved to be a good adsorbent for waste water treatment. However, presence of large amount of residual metallic constituents as impurities which exert negative influence and relatively higher cost restrict their large-scale applications [12-14]. Graphene oxide (GO) [15,16] with two-dimensional (2D) honeycomb carbon lattice having high specific surface area and abundant epoxy (–COC), hydroxyl (–OH), carbonyl (–CO) and carboxyl (–COOH) groups that can be synthesized oxidizing graphite in a single step without the use of metal catalyst has

been considered a material of choice and substitute for CNT due to its hydrophilic character facilitating better surface contact with aqueous phase and active sites for metal ion complexation.

Currently, sorption studies of metals using GO, magnetite GO and GO-nanocomposites dealt with only batch method using either 0.22 μm membrane filters, centrifugation, addition of sodium chloride, and/or magnetic separation to separate the GO from the sample solution [17-21]. Such separation method restricts the use of large sample volumes and multiple use of GO since it tends to aggregate which requires sonication to re-disperse before its use in the next sorption cycle. Moreover, GO miniscule may escape during filtration which pose a potential health threat to ecosystems. A radical way circumvent these obstacles is to immobilize GO onto a porous polymeric beads through azo spacer so that it can be used in column operation to achieve the benefits of higher number of theoretical plates for better extraction and high preconcentration factor.

6.2 Experimental

Prepared XAD-GO (**Chapter 2**, section 2.1.3) resin has been characterized by solid state ^{13}C NMR, FT-IR, TGA/DTA, SEM along with EDS, TEM analysis by using the recommended instruments as reported in **Chapter 2**, section 2.2 Sample solution adjusted to pH 8.0 ± 0.1 of optimum concentration was passed through the column at a flow rate of 5 mL min^{-1} . Sorbed Pb(II), Cd(II) and Zn(II) were eluted with 5.0 mL of 2M HCl was determined subsequently by FAAS.

6.3 Result and discussion

Characterization

FT-IR spectrum of XAD-GO (figure 6.1) shows a broad band at 3436 cm^{-1} due to structural O-H (-COOH and -OH) stretching vibrations of GO. Peaks at 2925.89 and 2856 cm^{-1} indicate the presence of sp^3 C-H and sp^2 C-H stretching vibrations, respectively. The peak appearing at 1698.25 cm^{-1} corresponds to carbonyl (C=O) stretching vibrations [22]. The azo (-N=N-) spacer band appeared at 1606.96 cm^{-1} [23] indicates the immobilization of GO onto XAD resin. The band at 1447.65 and 1488.39 cm^{-1} may be assigned as C=C bond stretching. Peaks at 1276.7 and 1117.93 cm^{-1} , and 1350 cm^{-1} may be attributed to C-O stretching vibrations and O-H deformation. The peaks at 39.60 , 96.87 , 174.96 , 223.97 and 145.06 in the solid state

^{13}C NMR (figure 6.2) spectrum corresponding to styrenic $-\text{CH}$ and $-\text{CH}_2$, aromatic carbon, carboxylic carbon, carbonylic carbon and carbon of azo group ($\text{C}=\text{N}=\text{N}-$), respectively confirm the successful immobilization of GO onto the XAD matrix [24]. The XRD pattern of graphite and GO was shown in figure 6.3. In the XRD pattern of graphite a sharp and highly symmetrical diffraction peak was appeared at $2\Theta=26.5^\circ$ which was assigned to the (002) reflection plane with interlayer spacing of 0.34 nm. This interprets the crystalline nature of graphite. In GO XRD pattern, a strong peak was observed at $2\Theta=10.6^\circ$ corresponds to interlayer spacing of 0.834 nm. The shifting in 2Θ from 26.5° to 10.6° and increase in the layer to layer distance from 0.34 nm to 0.834 nm depicts the conversion of graphite to GO [25]. It is further supported by Raman spectra (figure 6.4), shows the characteristics D and G bands of GO at 1344.6 and 1588.6 cm^{-1} , respectively. The multipoint BET surface area of XAD-GO was found to be $772.83\text{ m}^2\text{ g}^{-1}$ and comparable to the previous reported literature as in GO immobilized polystyrene (PS-GO) it was $12.06\text{ m}^2\text{ g}^{-1}$.

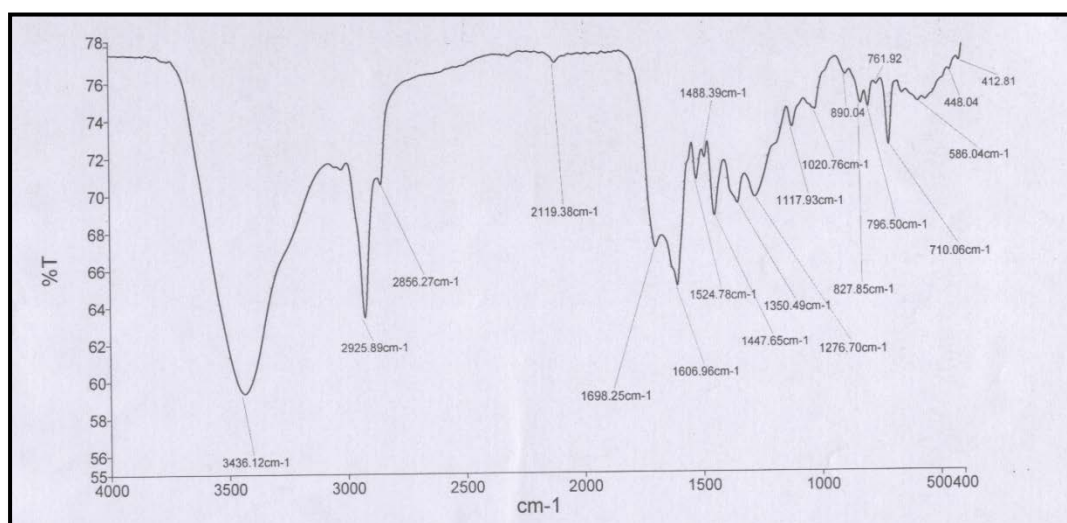


Figure 6.1: FTIR spectrum of XAD-GO

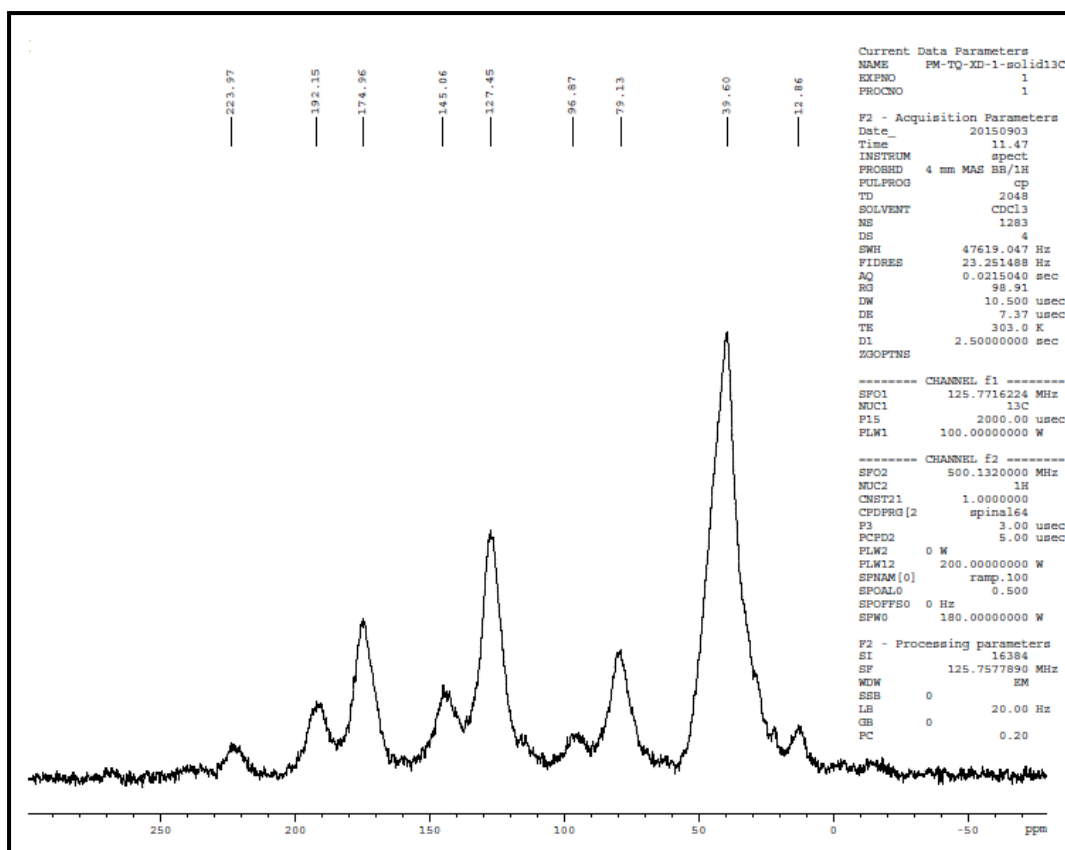


Figure 6.2: ^{13}C NMR spectrum of XAD-GO

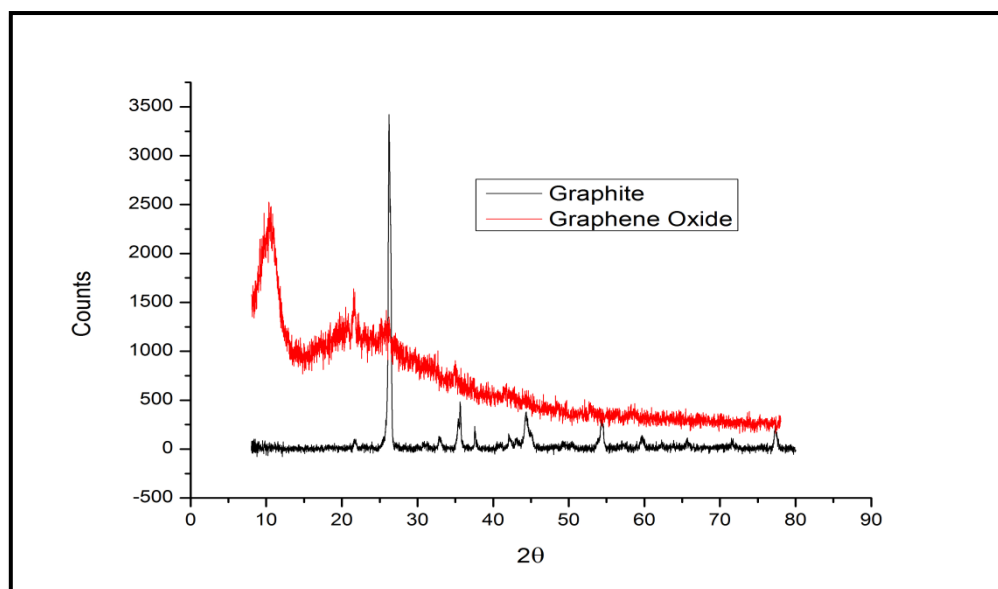


Figure 6.3: XRD pattern of graphite and GO

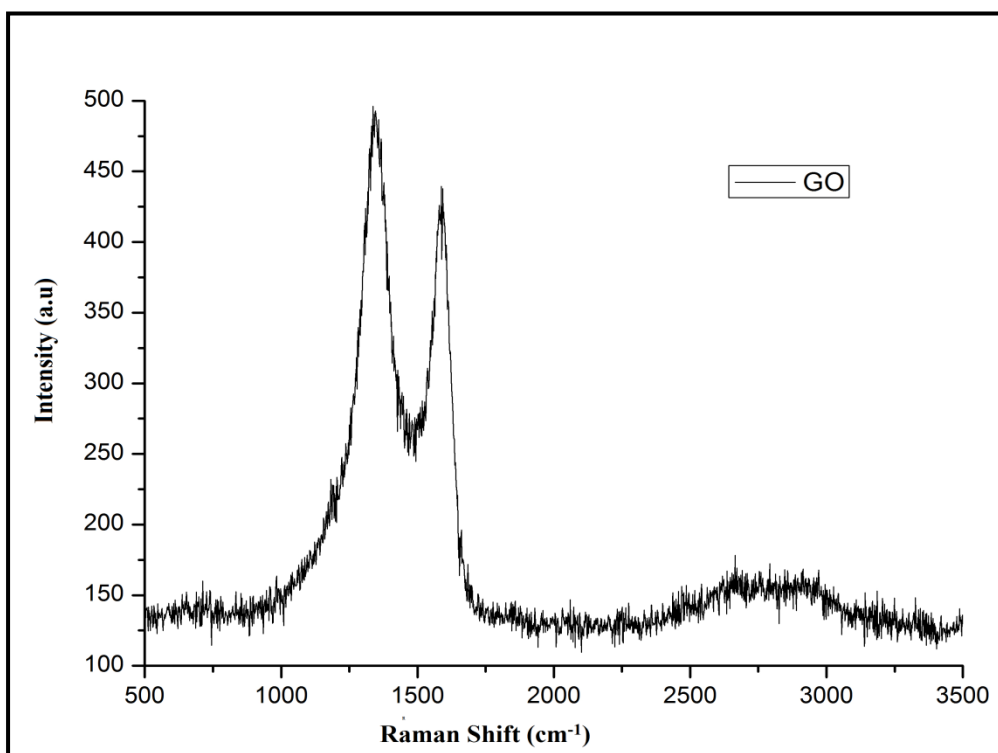


Figure 6.4: Raman spectra of GO

[24], glycidylmethacrylate-diethylenetriamine-GO (p-DETAGO) gives $51.063 \text{ m}^2 \text{ g}^{-1}$ [26] and in case of chitosan-GO it was found to be $4.232 \text{ m}^2 \text{ g}^{-1}$ [27]. TGA curve (figure 6.5) shows that the material can be used up to 260°C without any degradation. Decoration of XAD resin surface with GO sheets can be seen from the SEM images while disordered stacking of GO layers evidenced by different degree of transparency was observed in TEM images figure 6.6. Metal ion complexation with the donar atom of GO were indicated by the M-O vibrational frequency band in the region of $120\text{-}290 \text{ cm}^{-1}$ in FIR spectrum (figure 6.7) [28]. The presence of Pb, Cd and Zn in energy-dispersive X-ray analysis (EDS) spectra figure 6.8, obtained from SEM for micro-compositional analysis also supports of their chelation.

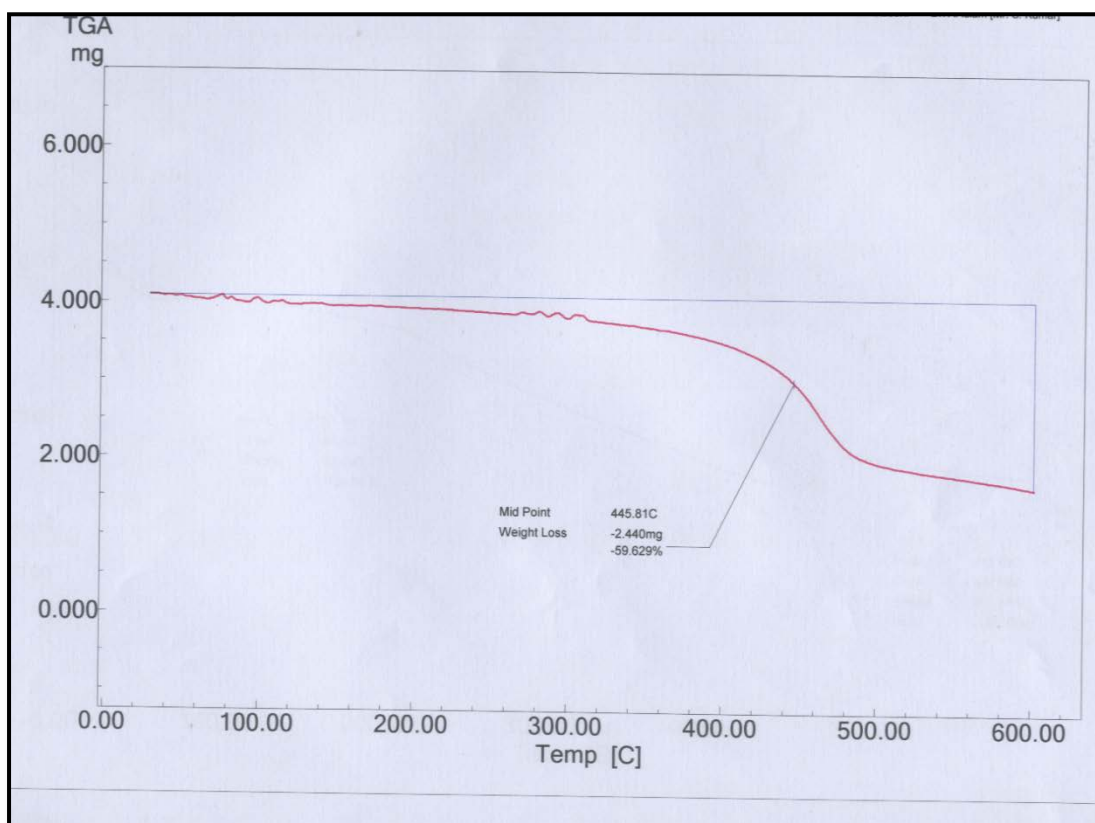


Figure 6.5: TGA curve of XAD-GO

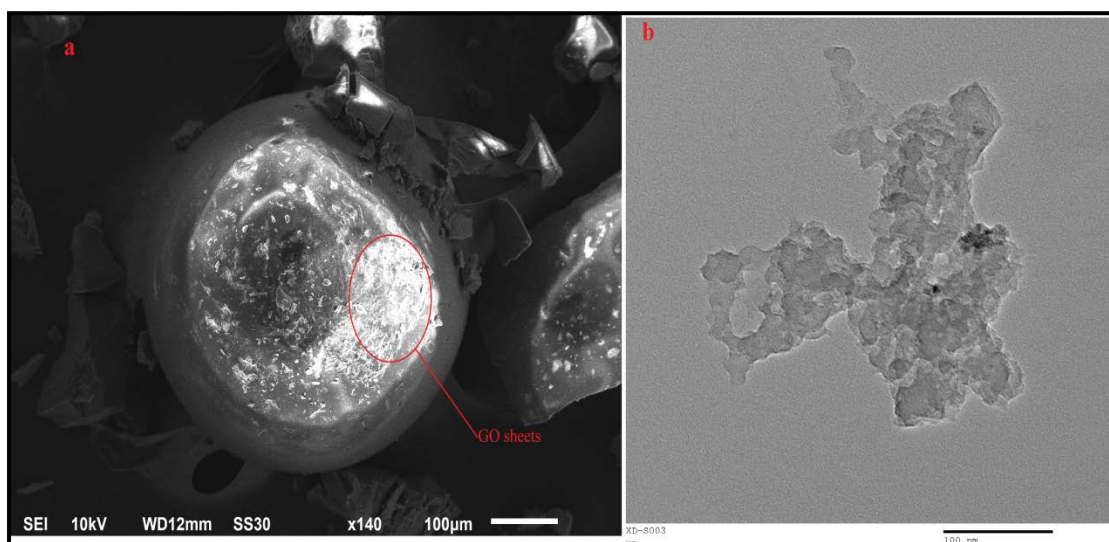


Figure 6.6: a) SEM and b) TEM image of XAD-GO

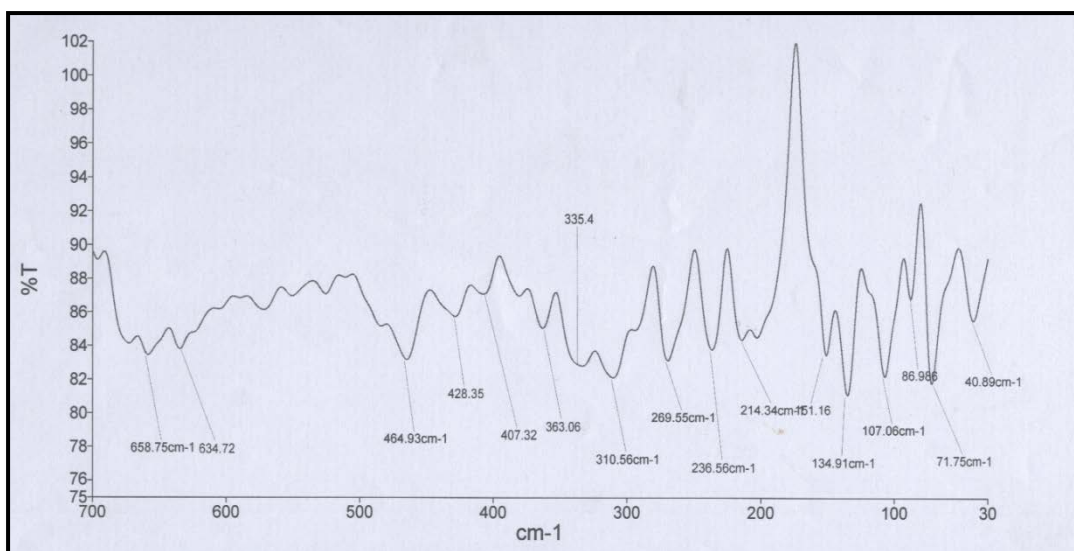


Figure 6.7: FIR spectrum of XAD-GO

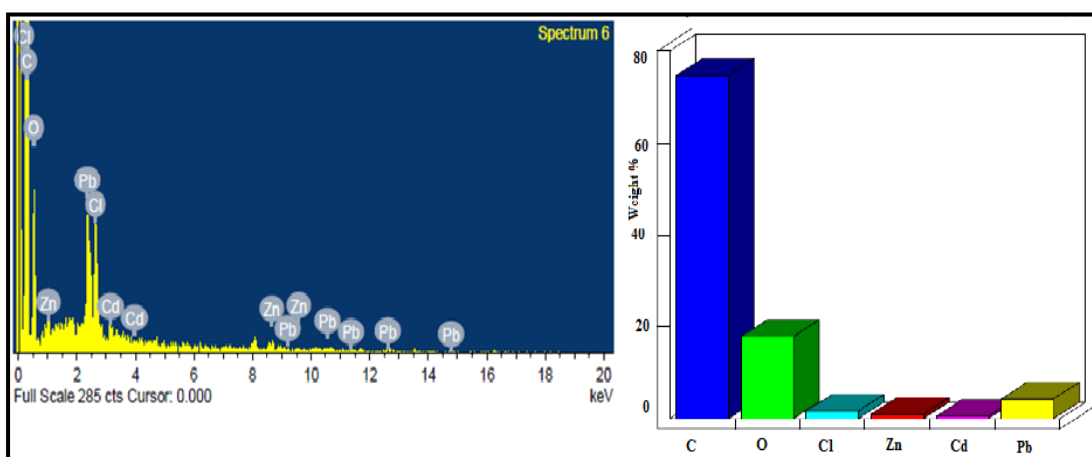


Figure 6.8: EDS spectra of XAD-GO

Optimization of experimental parameters

The experimental variables such as pH, column flow rate, eluent type and volume, and loading concentration of metal ions were optimized by adopting a univariate approach (one parameter kept constant and other one is varied, **Chapter 3**, section 3.8) for preconcentration of metal ions. Sorption of metal ions onto chelating resin was affected by the acidity/basicity of the sample solution. It is important to optimize

the solution pH in order to achieve effective sorption. For this, 0.1 g of XAD-GO resin were shaken for 120 minute with 50 ml of solution containing 0.5 mM of Pb, Cd and Zn ions individually at the pH range of 2.0-9.0 (figure 6.9). Extent of sorption of all the studied metal ions increases from pH 2.0 onward. For Pb and Cd, sorption attains its maximum at pH 8.0 and decreases thereafter while for Zn it increases continuously up to pH 9.0. This is because the oxygen ($-\text{COO}^-$) of the GO was negatively charged after deprotonation while the metal ions were positively charged and hence, strong electrostatic interaction takes place [29]. It was observed that Pb(II) forms precipitate at pH 9.0 and hence, pH > 9.0 for Pb(II) was not studied. The point of zero charge (pHpzc) has been calculated for better understanding the sorption behaviour [26] and the value of pHpzc was found to be 6.2 (figure 6.10) which is less than that of pH (8.0). Thus, it gives the idea of negatively charged surface groups at above pHpzc, favoured the strong electrostatic interaction with metal ion [30].

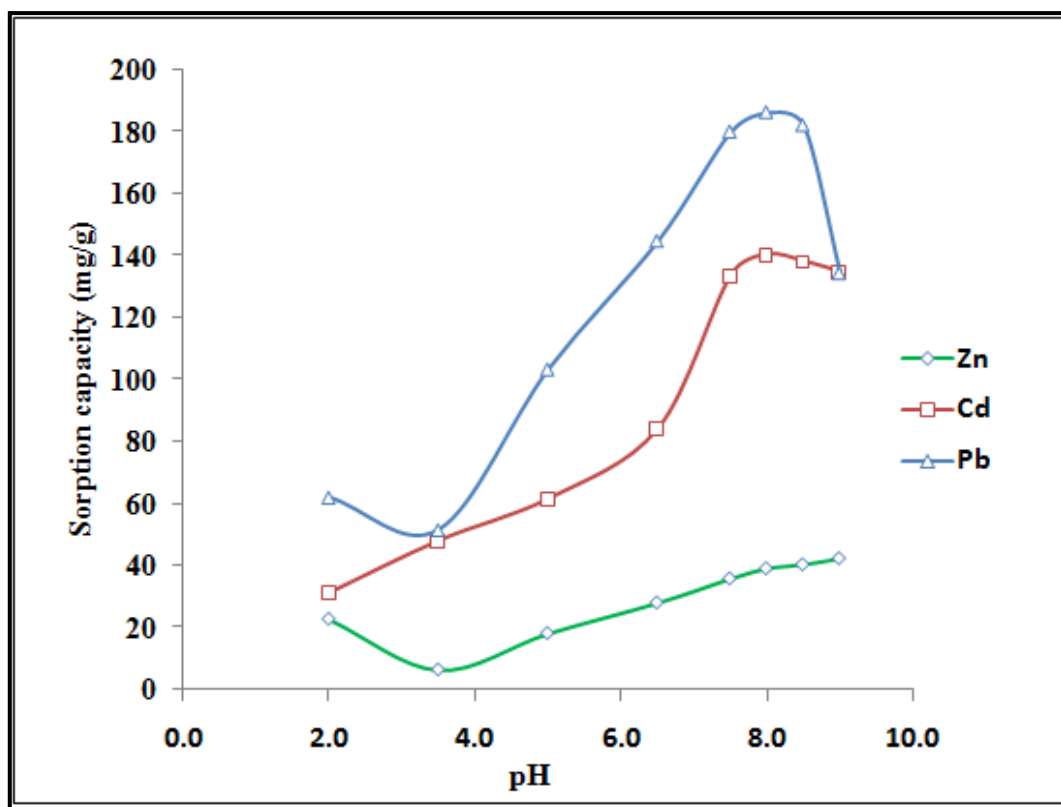


Figure 6.9: Effect of solution pH on sorption of Pb, Cd and Zn ions on to XAD-GO

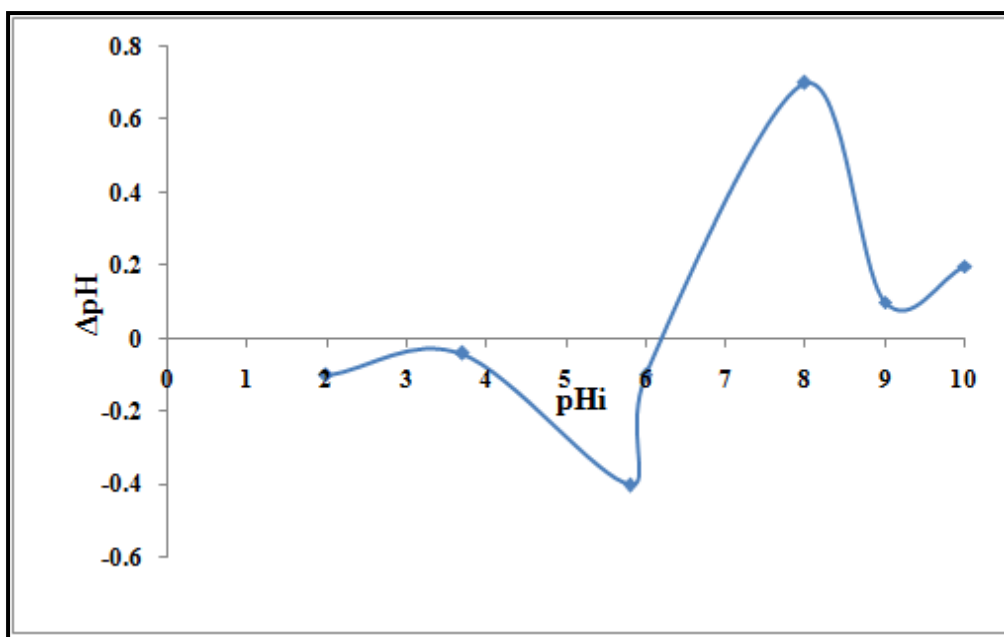


Figure 6.10: The point of zero charge (pH_zc)

Column flow rate was optimized (using recommended column procedure) by passing 100 ml of solution contain 5 μg of metal ion at a flow rate range of 2-6 mL min^{-1} . It was observed that quantitative sorption of metal ions was unaffected up to a flow rate of 5.0 mL min^{-1} . Above that a slight decrease in sorption was observed. Hence, a flow rate of 5.0 mL min^{-1} was set for further subsequent column experiments. In order to check the quantitative recovery of sorbed Pb, Cd and Zn ions, varying concentration and volume of HCl and HNO_3 were tested out as eluent. Experimentally it was found that 5 mL of 2M HCl gives the recovery $>98\%$ for all the three metal ions. Hence, 5 mL of 2.0M HCl was selected as an eluent in further studies. The XAD-GO can be regenerated up to 53 sorption and elution cycles without loss of sorption capacity. After that a slight loss of sorption capacity was observed. Hence, the resin has been multiply use in the preconcentration and determination of metal ion from different environmental samples.

Effect of Foreign Ions

Various cations and anions mostly present in water samples were studied in order to check their interference on the selective sorption of Pb, Cd and Zn ions onto column. For this a 50 mL solution containing 5 μg of analyte ion and varying concentration of interfering ions, at pH 8.0 were preconcentrated using XAD-GO column at a flow rate

of 5mL min⁻¹. The sorbed analyte was subsequently eluted and determined by FAAS. The results are shown in table 6.1. It is evident that these interfering ions do not affect the sorption of Pb, Cd and Zn ions in the preconcentration studies up to a certain tolerance level. The tolerance limit is defined as the concentration of foreign ion causing a relative error smaller than $\pm 5\%$ in the determination of analytes. Hence, the prepared resin shows a great application in the field of preconcentration/determination of metal ions using SPE coupled with FAAS.

Table 6.1 Effect of interfering ions

Interfering Ions	Added as	Tolerance ratio of interfering ions ($\mu\text{g L}^{-1}$)/ analyte ions ($\mu\text{g L}^{-1}$)		
		Pb (RSD) ^a	Zn(RSD) ^a	Cd(RSD) ^a
Cd ²⁺	CdCl ₂	25 (3.87)	15 (2.91)	-
Zn ²⁺	ZnCl ₂	25 (1.22)	-	15 (2.59)
Pb ²⁺	Pb(NO ₃) ₂	-	10 (3.07)	10 (3.14)
Co ²⁺	CoNO ₃	25 (2.30)	10 (1.73)	15 (2.51)
Cr ³⁺	CrCl ₃	25 (4.29)	10 (4.89)	15 (4.69)
Cu ²⁺	CuNO ₃	30 (4.85)	10 (4.26)	15 (2.86)
Fe ³⁺	Fe(NO ₃) ₃	30 (1.03)	15 (3.43)	15 (2.54)
Ni ²⁺	NiNO ₃	30 (4.25)	15 (2.99)	15 (3.55)
Na ⁺	NaCl	5.0×10^4 (4.52)	4.0×10^4 (1.50)	4.0×10^4 (2.57)
K ⁺	KCl	3.8×10^4 (1.33)	3.2×10^4 (2.50)	3.2×10^4 (4.06)
Mg ²⁺	MgCl ₂	9.8×10^2 (4.58)	4.8×10^2 (4.62)	4.8×10^2 (2.25)
SO ₄ ²⁻	Na ₂ SO ₄	1.9×10^4 (1.72)	1.5×10^4 (4.71)	1.5×10^4 (2.60)
Cl ⁻	NaCl	7.6×10^4 (0.55)	6.2×10^4 (2.04)	6.2×10^4 (4.92)
PO ₄ ²⁻	Na ₂ HPO ₄	1.8×10^2 (1.13)	1.2×10^2 (4.49)	1.2×10^2 (1.37)
Br ⁻	NaBr	8.0×10^4 (4.40)	6.4×10^4 (1.69)	6.4×10^4 (4.74)

^aN=3

Preconcentration Studies

The object of this study is to preconcentrate the analyte ions from large sample volumes into the limits of calibration range of FAAS prior to its determination. Since, direct determination of metal ions in the dilute samples is too challenging because of their low concentration. Hence, it requires a preconcentration step before subjected to FAAS determination. Following the recommended column procedure an increasing sample volume in the range of 750-2600 mL containing a constant amount of analyte ion (5 μg) were preconcentrated. The results obtained are shown in table 6.2, gives the preconcentration limits of 2.0, 5.88 and 5.88 $\mu\text{g L}^{-1}$ for the maximum volume of 2.5 L, 0.85 L and 0.85 L for Pb, Cd and Zn ions, respectively, where quantitative recovery of analytes were achieved.

Table 6.2 Preconcentration of metal ions by SPE-FAAS

Volume (L)	Pb	Cd	Zn
	% Recovery	% Recovery	% Recovery
0.75	NS ^a	103	100
0.85	NS ^a	96.4	99.8
1.0	106	75.5	71.9
2.0	111	NS ^a	NS ^a
2.5	100	NS ^a	NS ^a
2.6	78.1	NS ^a	NS ^a
3.0	65.7	NS ^a	NS ^a

^aNS= Not Studied

6.4 Analytical Method Validation

In order to validate the proposed method, several parameters such as limit of detection (LOD), limit of quantification (LOQ), correlation coefficient (R^2) were investigated. Under the optimized conditions, calibration curves were plotted using least square method which results regression equation and correlation coefficient of $Y = 0.042X_{Pb} - 0.008$ ($R^2 = 0.986$) for Pb, $Y = 0.274X_{Cd} - 0.057$ ($R^2 = 0.989$) for Cd and $Y = 0.311X_{Zn} - 0.077$ ($R^2 = 0.990$) for Zn. The LOD (3s/m) and LOQ (10s/m) [31] were found to be 0.41, 0.41, 0.13 and 1.38, 1.36, 0.42 for Pb, Cd and Zn ions, respectively. The accuracy of the method was tested by analyzing SRM, following recommended column procedure. Calculated Student's t (t-test) values for Pb, Cd and Zn ions were found to be less than the critical Student's t value of 4.303 at 95 % confidence level for $N=3$ (Table 6.3). Hence, the mean concentration values were not statistically significant from the certified values indicating that absence of systematic errors and demonstrate the accuracy of the proposed method. The reliability of the developed method was also investigated by the analysis of spiked river water, tap water and city drinking water, with the known amount ($5\mu\text{g}$) of Pb, Cd and Zn ions. The percentage recoveries of spiked analyte were found to be 95-103 with a relative standard deviation (RSD) $<5\%$ (Table 6.4).

Table 6.3 Analysis of SRM for metal ion contents (column parameters: pH 8.0; sorption flow rate, 5.0 mL min^{-1} ; 0.2 g, resin)

SRM	Certified value ($\mu\text{g g}^{-1}$)	Found value ($\mu\text{g g}^{-1}$) ^a (RSD)	Calculated Student's t value ^b
Vehicle exhaust particulates NIES ^c 8	Pb: 219	Pb: 213.6 (1.14)	Pb: 3.867
	Cd: 1.1	Cd: 1.08 (3.39)	Cd: 1.113
	Zn:1040	Zn:1023 (0.77)	Zn: 3.660

^aMean value, ^b95% confidence limit; $N=3$, ^cNational Institute of Environmental Studies

Table 6.4 FAAS determination of Pb, Cd and Zn ions in environmental water samples by XAD-GO resin

Sample		Pb		Cd		Zn	
	Amount spiked ($\mu\text{g L}^{-1}$)	Amount found ($\mu\text{g L}^{-1}$)	%Recovery (RSD)	Amount found ($\mu\text{g L}^{-1}$)	%Recovery (RSD)	Amount found ($\mu\text{g L}^{-1}$)	%Recovery (RSD)
Tap water	0	2.91	- (3.49)	ND ^a	-	5.48	- (2.38)
	10	13.0	100.6 (0.96)	10.13	100.1 (4.97)	15.4	99.13 (4.74)
River water	0	2.63	- (3.96)	2.44	- (4.33)	3.46	- (2.46)
	10	12.7	100.4 (4.06)	12.0	95.27 (1.05)	13.3	97.90 (4.36)
City drinking water	0	2.85	- (3.19)	1.22	- (4.22)	4.11	- (1.84)
	10	12.8	99.27 (1.45)	11.6	103.6 (3.87)	13.8	97.13 (1.01)

^aNot Detected

6.5 Conclusion

The prime objective to design a new solid-phase extractant is to develop an environmentally safe column preconcentration/separation method for monitoring the level of contamination by toxic environmental pollutants. Thus, developed method do not involve the use of large volumes of carcinogenic organic solvent, and do not releases toxic GO bound to the XAD resin up to 53 sorption/elution cycles. Therefore, the method considered as eco-friendly and environmentally green. The high preconcentration factor and preconcentration limit for metal ions shows the good applicability of XAD-GO resin.

References

- [1] L.W. Chang, L.G. Cockerham, in L.G. Cockerham and B.S. Shane (Eds.), Basic Environmental Toxicology, CRC Press, Florida, 1986, pp. 121-128.
- [2] U.Ewers and H.W. Schlipkoter, E. Merian (Ed.), Metals and their Compounds in the Environment, VCH, Weinheim, 1991, pp. 971.
- [3] H. Hu, Heavy metal poisoning, Harrison's Principles of Internal Medicine, McGraw-Hill, Medical Publishing Division, New York, Vol. 2, 16th edition, 2005, pp. 2577.
- [4] Review of procedures involving separation and preconcentration for the determination of cadmium using spectrometric techniques, S.L.C. Ferreira et al., J. Hazard. Mater. 145 (2007) 358–36.
- [5] J. L. Todol, L. Gras, V. Hernandis and J. Mora, Elemental matrix effects in ICP-AES, J. Anal. At. Spectrom. 17 (2002) 142–169.
- [6] Y. Wen, L. Chen, J. Li, D. Liu, L. Chen, Recent advances in solid-phase sorbents for sample preparation prior to chromatographic analysis, Trends Anal. Chem. 59 (2014) 26–41.
- [7] B. H. Fumes *et al*, Recent advances and future trends in new materials for sample preparation, Trends Anal. Chem. 71 (2015) 9-25.
- [8] A. Tobiasz and S. Walas, Solid-phase-extraction procedures for atomic spectrometry determination of copper, Trends Anal. Chem. 62 (2014) 106–122.
- [9] K. Scida, P. W. Stege, G. Haby, G. A. Messina, C. D. Garcia, Recent applications of carbon-based nanomaterials in analytical chemistry: Critical review Anal. Chim. Acta 691 (2011) 6.
- [10] X. Jiang, K. Huang, D. Deng, H. Xia, X. Hou, C. Zheng, Nanomaterials in analytical atomic Spectrometry, Trac Trends Anal. Chem. 39 (2012) 38.
- [11] Z. Ding, X. Hu, Y. Wan, S. Wang, B. Gao, Removal of lead, copper, cadmium, zinc, and nickel from aqueous solutions by alkali-modified biochar: Batch and column tests, J. Ind. Eng. Chem. 33 (2016) 239-245.
- [12] X. Tian, S. Zhou, Z. Zhang, X. He, M. Yu, D. Lin, Metal impurities dominate the sorption of a commercially available carbon nanotube for Pb(II) from water, Environ. Sci. Technol. 44 (2010) 8144–8149.

- [13] M. Pumera, Y. Miyahara, What amount of metallic impurities in carbon nanotubes is small enough not to dominate their redox properties? *Nanoscale* 1 (2009) 260–265.
- [14] C. E Banks, E. Crossley, C. Salter, S. J. Wilkins, Compton, R.G. Carbon Nanotubes Contain Metal Impurities Which are Responsible for the “Electrocatalysis” Seen at Some Nanotube- Modified Electrodes. *Angew. Chem., Int. Ed.* 2006, 45, 2533–2537
- [15] D.R. Dreyer, S. Park, C.W. Bielawski, R.S. Ruoff, The chemistry of graphene oxide, *Chem. Soc. Rev.* 39 (2010) 228–240.
- [16] S. Yu, X. Wang, X. Tan, X. Wang, Sorption of radionuclides from aqueous systems onto graphene oxide-based materials: a review, *Inorganic Chemistry Frontiers* 2 (2015) 593-612.
- [17] V. Chandra, J. Park, Y. Chun, J. W. Lee, I. Hwang, K. S. Kim, Water-dispersible magnetite-reduced graphene oxide composites for arsenic removal. *ACS Nano* 2010, 4, 3979–3986.
- [18] Y. Sun, Q. Wang, C. Chen, X. Tan, X. Wang, Interaction between Eu(III) and graphene oxide nano sheets investigated by batch and extended x-ray absorption fine structure spectroscopy and by modeling techniques, *Environ. Sci. Technol.* 46 (2012) 6020-6027.
- [19] D. Deng, X. Jiang, L. Yang, X. Hou, C. Zheng, Organic solvent-free cloud point extraction-like methodology using aggregation of graphene oxide. *Anal. Chem.* 86 (2014) 758–765.
- [20] K. Bhowmik, A. Mukherjee, M. K. Mishra, G. De, Stable Ni nanoparticle-reduced graphene oxide composites for the reduction of highly toxic aqueous Cr(VI) at room temperature, *Langmuir.* 30 (2014) 3209–3216.
- [21] D. Dinda, A. Gupta and S.K. Saha, Removal of toxic Cr(VI) by UV-active functionalized graphene oxide for water purification, *J. Mater. Chem. A* 1 (2013) 11221–11228.
- [22] G. Socrates, *Infrared characteristics group frequencies*, (3rd ed.), New York: Wiley Inter Science (1980).
- [23] J. Coates *Interpretation of Infrared Spectra, A Practical Approach in Encyclopedia of Analytical Chemistry* R.A. Meyers (Ed.) 2000 pp. 10815–10837.

- [24] A. Islam, H. Ahmad, N. Zaidi and S. Kumar, Graphene oxide sheets immobilized polystyrene for column preconcentration and sensitive determination of lead by flame atomic absorption spectrometry, *ACS Appl. Mater. Interfaces* 6 (2014) 13257–13265
- [25] R. Dong, L. Liu, Preparation and properties of acrylic resin coating modified by functional graphene oxide, *Applied Surface Science* 368 (2016) 378–387.
- [26] A. Islam, S. Kumar, Glycidylmethacrylate based resin functionalized with graphene oxide for column preconcentration and trace determination of Cd(II) and Ni(II) in environmental and food samples. *RSC Adv.* 6 (2016) 6 77629-77635.
- [27] L. Liu, C. Li, C. Bao, Q. Jia, Pengfei Xiao, Xiaoting Liu, Qiuping Zhang Preparation and characterization of chitosan/graphene oxide composites for the adsorption of Au(III) and Pd(II), *Talanta* 93 (2012) 350-357.
- [28] D. Prabhakaran, M. S. Subramanian, A new chelating sorbent for metal ion extraction under high saline conditions, *Talanta* 59 (2003) 1227-1236.
- [29] R. Sitko, E. Turek, B. Zawisza, E. Malicka, E. Talik, J. Heimann, A. Gagor, B. Feista and R. Wrzalik, Adsorption of divalent metal ions from aqueous solutions using graphene oxide, *Dalton Trans.*, 2013, 42, 5682–5689
- [30] A. H. Gedam and R. S. Dongre, Adsorption characterization of Pb(II) ions onto iodate doped chitosan composite: equilibrium and kinetic studies, *RSC Adv.*, 2015, 5, 54188–54201.
- [31] G. L. Long and J. D. Winefordner, Limit of detection a closer look at the IUPAC definition, *Anal. Chem.* 55 (1983) 712A–724A.