DOKUZ EYLUL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

PROGRESSING CHEMICALLY BOUND RESIN FOR PRECONCENTRATION AND DETERMINATION OF SOME METALS

by
Serap SEYHAN

May, 2007
İZMİR

PROGRESSING CHEMICALLY BOUND RESIN FOR PRECONCENTRATION AND DETERMINATION OF SOME METALS

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by
Serap SEYHAN

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Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"PROGRESSING CHEMICALLY BOUND RESIN FOR PRECONCENTRATION AND DETERMINATION OF SOME METALS"** completed by **SERAP SEYHAN** under supervision of **Prof. Dr. MELEK MERDİVAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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Serap SEYHAN

PROGRESSING CHEMICALLY BOUND RESIN FOR PRECONCENTRATION AND DETERMINATION OF SOME METALS

ABSTRACT

5,10,15,20 tetrakis-(phenoxy acetic acid) porphyrin and calix[4]crown-4 were synthesized and these ligands were chemically bound to Merrifield polymer resin. By FTIR, ^{1}H NMR and elemental analysis, the resulting resins were characterized. The amount of attached phenoxy acetic acid porphyrin and calix[4]crown-4 to the polymer resin and were found to be 0.242 and 0.489 mmol g^{-1} resin, respectively.

Several parameters, such as flow rate (sorption and desorption), concentration of acid for desorption, sorption capacity of the chelating resin, optimum pH, preconcentration factor and distribution coefficient for investigated metal ions were examined. The validities of the proposed methods were checked by analyzing these metal ions in standard soil samples. The most remarkable features of the modified resins were their high metal sorption capacities with good preconcentration factor values.

Using phenoxy acetic acid porphyrin in aqueous solution and sol-gel matrix, the fluorimetric determination of zirconium was studied. Phenoxy acetic acid porphyrin has highly luminescent property with emission wavelengths of 660 and 658 nm in aqueous solution and sol-gel matrix respectively. It was seen that optimum pH range in porphyrin-Zr complex was 5.0-7.0 for both medium. The complex became stable within 20 min in aqueous solution and 40 min in sol-gel matrix. The fluorescence intensity was linear for 2.0-25 μg mL^{-1} of zirconium in both medium. The proposed fluorimetric zirconium determination method was succesfully applied to the analysis of real and standard soil samples.

Keywords: Solid phase extraction, preconcentration, porphyrin, calixcrown, fluoresence quenching, sol-gel.

KİMYASAL BAĞLI SORBENT GELİŞTİREREK BAZI METALLERİN ÖNDERİŞTİRİLMESİ VE TAYİNİ

ÖZ

5,10,15,20 tetrakis(fenoksi asetik asit)porfirin ve kaliks[4]krown-4 sentezlendi ve bu ligantlar Merrifield polimerik reçineye kimyasal olarak bağlandı. Elde edilen reçinelerin yapıları FTIR, ^{1}H NMR ve elementel analiz ile karakterize edildi. Polimerik reçineye bağlanan fenoksi asetik asit porfirin ve kaliks[4]krown-4 miktarları sırasıyla 0,242 ve 0,489 mmol g^{-1} reçine'dir.

Metal iyonlarının tutunma ve geri alınma akış hızı, tutunma kapasitesi, geri alma çözeltisinin derişimi, optimum pH, önderiştirme faktörü ve dağılma katsayısı gibi parametreler incelendi. Yöntemin geçerliliği standart toprak örnekleri ile kontrol edildi. Modifiye edilen reçinelerin en göze çarpan özellikleri yüksek önderiştirme faktörü ve tutunma kapasitesiteleridir.

Fenoksi asetik asit porfirinin sulu çözelti ve sol-gel matriks içinde kullanılması ile zirkonyumun florimetrik tayini gerçekleştirildi. Porfirin sulu çözeltide 660 nm'de ve sol-gel matriksde 658 nm'de kuvvetli floresans özelliğie sahip olduğu görüldü. Zirkonyum-porfirin kompleksi için her iki ortamda da optimum pH aralığı 5-7olarak bulundu. Kompleks sulu çözeltide 20 dakikada sol-gel matriks ortamında ise 40 dakikada kararlı hale geldi. Her iki ortamda da zirkonyum için lineer aralık 2.0-25 μg mL^{-1} olarak bulundu. Geliştirilen florimetrik zirkonyum yöntemi gerçek ve standart toprak örneklerine başarı ile uygulandı.

Anahtar sözcükler : Katı faz eksraksiyonu, önderiştirme, porfirin, kalikskrown, floresans sönümleme, sol-gel.

CONTENTS

Page

THESIS EXAMINATION RESULT FORM ii
ACKNOWLEDGEMENTS iii
ABSTRACT iv
ÖZ v

CHAPTER ONE – INTRODUCTION 1
1.1 Preconcentration and Separation 1

1.2 Solid Phase Extraction (SPE) 2
1.2.1 Process of Solid Phase Extraction 3
1.2.2 Advantages of Solid Phase Extraction 5

1.3 Solid sorbents 6

1.4 Retention of Trace Elements on Sorbents 11
1.4.1 Adsorption 12
1.4.2 Ion Exchange 12
1.4.3 Chelation 13

1.5 Chelating Agents 15
1.5.1 Porphyrins 15
1.5.1.1 Application of Porphyrin as Complex-Forming Reagent in Modified Sorbents for Preconcentration 19
1.5.2 Calixarenes 20
1.5.3 Calixcrowns 22
1.5.3.1 Application of Calixarenes as Complex-Forming Reagent in Modified Sorbents for Preconcentration 24

1.6 Sol-Gel Processing 25

1.6.1 Application of Porphyrins in Sol-Gel Matrix…….27

1.7 The Metal Ions Determined.....28

1.8 Objectives of This Work 30

CHAPTER TWO – MATERIAL AND METHOD.....32

2.1 Synthesis of Chelating Agents and Modified Resins 32

2.1.1 Preparation of PAAP.....32

2.1.2 Preparation of PAAP Modified Resin.....33

2.1.3 Synthesis of 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26-dihydroxy-27,28-crown-4-calix[4]arene.....35

2.1.4 Synthesis of p-tert-butylcalix[4]arene-1,2-crown-4.....35

2.1.5 Synthesis of p-tert-butylcalix[4]arene-1,2-crown-4 Modified Resin..36

2.2 Characterization of Synthesized Chelating Agents and Modified Resins .. 37

2.2.1 FTIR Measurements.....37

2.2.2 ^{1}H NMR Measurements.....37

2.2.3 Elemental Analysis.....37

2.3 Metal Ions Preconcentrated 37

2.4 Recommended Procedure for Preconcentration and Determination of Metal Ions 38

2.4.1 Column Procedure 38

2.4.2 Batch Procedure.....39

2.5 Optimum Conditions Investigated..... 39

2.5.1 Effect of pH.....39

2.5.2 Effect of Flow Rate.....39

2.5.3 Stripping of Metal Ions.....40

2.5.4 Sorption Capacity.....40

2.5.5 Preconcentration Factor.....40
2.5.6 Distribution Coefficient.....40

2.6 Determination of Metal Ions Preconcentrated..... 41
2.6.1 Procedure for FAAS.....41
2.6.2 Procedure for Spectrophotometric Measurement.....42

2.7 Preparation of Electrolyte Solutions.....43

2.8 Fluorimetric Measurements of Zirconium in Aqueous Solution.....43
2.8.1 PAAP in Aqueous Solution.....43
2.8.2 PAAP in Sol-Gel Matrix..... 43
2.8.2.1 Sol-Gel Matrix and Film Preparation Process.....43
2.8.3 Calibration Graph.....45

2.9 Analysis of Soil Samples.....45
2.9.1 Standard Reference Materials (NIST SRM 2709 and NIST SRM 2711, San Joaquin Soil, Montana Soil).....45
2.9.2 Soil Sample from Kula Region.....46

CHAPTER THREE – RESULTS.....47
3.1 Characterization Studies for Chelating Agents and Modified Resins 47
3.1.1 Infrared Spectrum.....47
3.1.1.1 For PAAP and PAAP Modified Resin.....47
3.1.1.2 For calix[4]crown-4 Modified.Resin.....47
3.1.2 ^{1}H NMR Spectrum.....50
3.1.2.1 For PAAP and PAAP Modified Resin.....50
3.1.2.2 For calix[4]crown-4 and Modified Resin.....53
3.1.3 Elemental Analysis.....54
3.1.3.1 For PAAP Modified Resin 54
3.1.3.2 For calix[4]-crown-4 Modified Resin.....54

3.2 Preconcentration and Determination of Metal Ions with PAAP Modified Resin..... 54
3.2.1 Effect of pH 54
3.2.2 Effect of Flow Rate 56
3.2.3 Stripping of Metal Ions 56
3.2.4 Sorption Capacity 58
3.2.5 Preconcentration Factor.....59
3.2.6 Distribution Coefficient 59
3.2.7 Effect of Electrolytes.....61
3.2.8 Resin Stability.....61
3.2.9 Analysis of Standard Reference Materials.....62
3.2.10 Analytical Figures of Merit.....63

3.3 Preconcentration and Determination of Metal Ions calix[4]crown-4 Modified Resin 64
3.3.1 Effect of pH.....64
3.3.2 Effect of Flow Rate.....65
3.3.3 Stripping of Metal Ions.....65
3.3.4 Sorption Capacity.....67
3.3.5 Preconcentration Factor.....68
3.3.6 Distribution Coefficient.....68
3.3.7 Effect of Electrolytes.....69
3.3.8 Resin Stability 70
3.3.9 Analysis of Standard Reference Materials.....70
3.3.10 Analytical Figures of Merit.....71

3.4 Fluorimetric Determination for Zirconium 71
3.4.1 PAAP in Aqueous Solution 71
3.4.1.1 Excitation and Emission Spectra of PAAP..... 71
3.4.1.2 Effect of pH 72
3.4.1.3 Calibration Graph 75
3.4.2 PAAP in Sol-Gel Matrix 76

3.4.2.1 Emission Spectra of PAAP in Sol-Gel Matrix 76
3.4.2.2 Effect of pH 77
3.4.2.3 Calibration Graph 78
3.4.3 Selectivity 78
3.4.4 Applications.....79
3.4.5 Analytical Characteristics..... .80

CHAPTER FOUR – CONCLUSIONS 81
4.1 Preconcentration and Determination of Metal Ions.....81

4.2 Fluorimetric Determination for Zirconium.....86

REFERENCES.....88

CHAPTER ONE

INTRODUCTION

1.1 Preconcentration and Separation

The methods of analytical chemistry can be divided into two large gropus: (1) methods of separation and preconcentration of components, (2) methods of determination of components of the material to be analysed.

Separation is a process in which the components constituting the starting mixture are separated from each other. Preconcentration is a technique by which the ratio of concentration (or the amount) of trace components to the concentration (or the amount) of macrocomponent is increased. In separation, the components constituting the mixture may or may not differ in concentration from each other. In preconcentration, the components that have significantly different concentrations are treated.

Numerous methods are used for analytical preconcentration of trace elements. A large majority of them had previously been employed only for separation. Probably evaporation was first utilized for preconcentration; precipitation, extraction, electrochemical and other methods gained recognition at a later stage. Fire assay is one of the oldest methods of preconcentration.

By the nature of the separation methods used, they can be classified into 1) chemical and physico-chemical and 2) physical methods. With the first group may be classed extraction, sorption, precipitation and co-precipitation, partial dissolution of matrix, flotation, volatilization after chemical transformations, chemical transport reactions, fire assay, electrochemical methods and dialysis. The second group may include volatilization, crystallization, freezing out, filtration and gel filtration and ultracentrifugation.

The selection of a preconcentration method is dictated by (i) the practical problem being solved, the nature of the material to be analyzed, trace elements to be determined, the specified metrological parameters of the technique; (ii) the origin and previous history of the material to be analyzed; (iii) the combination of the selected method of preconcentration and subsequent method of determination of trace elements in a concentrate; (iv) the simplicity, the availability and the duration of the method; (v) the equipment available in the laboratory of the scientist and in those laboratories which will use the method; (vi) the specialization and qualification of the researcher developing the technique and of the analysts of the laboratories where this technique is to be employed; (vii) the need to ensure safe working conditions.

Solvent extraction is an effective and widely used method of preconcentration. It can be applied both for the removal of matrix and for the selective, group, or subsequent separation of trace elements. One of its variants extraction chromatography is an effective way of separating substances with almost similar properties and ensures high efficiency of preconcentration (Zolotov & Kuz'min; 1990).

Liquid-liquid extraction is a classical method for preconcentrating metal ions and/or matrix removal. Solid phase extraction is another approach that offers a number of important benefits. It reduces solvent usage and exposure, disposal costs and extraction time for sample preparation. Consequently, in recent years solid phase extraction has been successfully used for the separation and sensitive determination of metal ions (Camel; 2003).

1.2 Solid Phase Extraction (SPE)

The history of SPE dates back at least to the early 1970s, when columns packed with Rohm and Haas XAD resin particles were used to concentrate low concentrations of organic pollutants from water samples. However, activated carbons have been used several years prior to 1970 to accumulate organic solutes prior to

analysis. During the late 1980s and the 1990s, the development and analytical use of solid-phase extraction have expanded tremendously.

In SPE solutes are extracted from a liquid into solid phase. The solid phase typically consists of small, porous particles of silica with bonded organic phase or of an organic polymer, such as cross linked polystyrene.

The extraction can take place in a batch method in which the solid extract is intimately mixed with the liquid sample solution. In chemical analysis it is more common to take the packed solid extract into a small tube and pass the liquid sample through the tube. Typical apparatuses for SPE are shown in Figure 1.1.

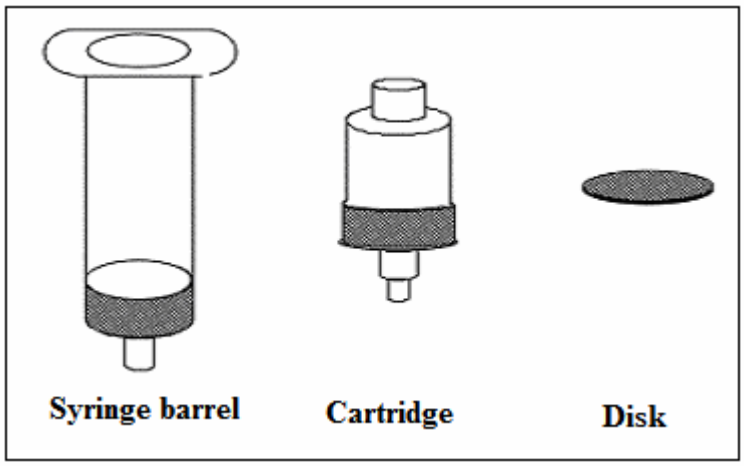


Figure 1.1 Disposable sorbent containers

Substances that have been extracted by the solid phase can be removed by washing up with an appropriate solvent. Usually, the volume of solvent needed for complete elution of the analytes is much smaller than the original sample volume. A concentration of the analytes is thus achieved.

1.2.1 Process of Solid-Phase Extraction

An SPE method always consists of three to four successive steps (Figure 1.2). First, the solid sorbent should be conditioned using an appropriate solvent, followed

by the same solvent as the sample solvent. This step is crucial, as it enables the wetting of the packing material and the solvation of the functional groups. In addition, it removes possible impurities initially contained in the sorbent or the packaging. Also, this step removes the air present in the column and fills the void volume with solvent.

The nature of the conditioning solvent depends on the nature of the solid sorbent. Typically, for reversed phase sorbent (such as octadecyl-bonded silica), methanol is frequently used, followed with water or aqueous buffer whose pH and ionic strength are similar to that of the sample. Care must be taken not to allow the solid sorbent to dry between the conditioning and the sample treatment steps, otherwise the analytes will not be efficiently retained and poor recoveries will be obtained. If the sorbent dries for more than several minutes, it must be reconditioned.

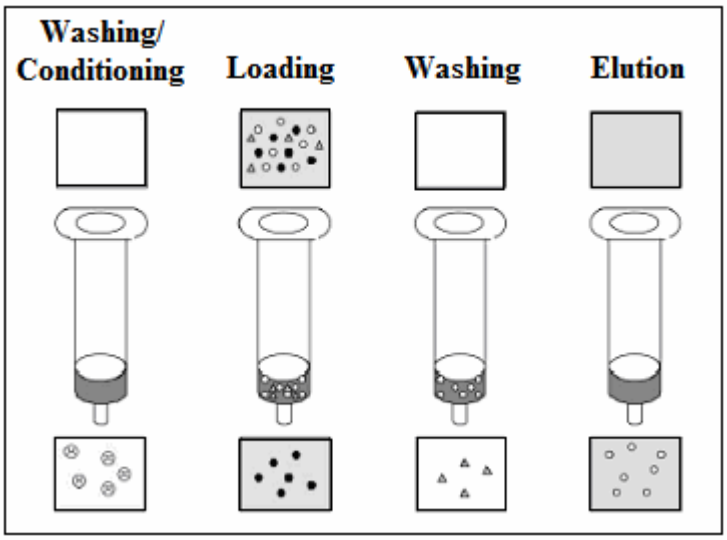


Figure 1.2 SPE Operation steps

The second step is the percolation of the sample through the solid sorbent. Depending on the system used, volumes can range from 1 mL to 1 L. The sample may be applied to the column by gravity, pumping, aspirated by vacuum or by an automated system. The sample flow-rate through the sorbent should be low enough to enable efficient retention of the analytes, and high enough to avoid excessive duration. During this step, the analytes are concentrated on the sorbent. Even though

matrix components may also be retained by the solid sorbent, some of them pass through, thus enabling some purification (matrix separation) of the sample.

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.

The final step consists in the elution of the analytes of interest by an appropriate solvent, without removing retained matrix components. The solvent volume should be adjusted so that quantitative recovery of the analytes is achieved with subsequent low dilution. In addition, the flow-rate should be correctly adjusted to ensure efficient elution. It is often recommended that the solvent volume be fractionated into two aliquots, and before the elution to let the solvent soak the solid sorbent.

1.2.2 Advantages of Solid Phase Extraction

Solid-phase extraction has several important advantages over liquid-liquid extraction (Pyrzynska & Trojanowicz; 1999).

1. Faster and easier manipulation. A sample can be quickly passed through a SPE column or cartridge by means of a pump or with gentle pressure of suction. After a quick rinse, the extracted substances can be washed from the column by a small amount of appropriate eluent. However, simple solvent extraction requires a considerable amount of manipulation in adding the extractive liquid, shaking, waiting for the emulsion to break, and carefully separating the two liquid phases. Often a washing step and a back extraction are required.

2. Much smaller amounts of organic solvents are used. The large volumes of organic solvents used in analytical separations have become an important environmental concern. Aqueous samples have become contaminated with organic solvents and an evaporative concentration of the extracts pollutes the air with organic vapors. Proper disposal of used organic solvents has become troublesome and expensive. Today, analytical chemists are working to replace liquid-liquid extraction with solid-phase extraction in analytical procedure in control laboratories.

3. Less stringent requirements for separation. SPE is a multistage separation method and as such requires only a reasonable difference at least %80 in extractability to separate two solutes.

4. Higher concentration factor. The concentration factor is how many times more concentrated a substance is in the extract than it is in the original sample. In SPE, concentration factors of 100 or more are possible. The concentration factor in liquid-liquid extraction depends in part on the volume ratio of the liquids.

1.3 Solid Sorbents

The nature and properties of the sorbent are of prime importance for effective retention of metallic species. Careful choice of the sorbent is thus crucial to the development of SPE methodology. In practice, the main requirements for a solid sorbent are:

(1) The possibility to extract a large number of trace elements over a wide pH range (along with selectivity towards major ions);
(2) The fast and quantitative sorption and elution;
(3) High capacity;
(4) Regenerability;
(5) Accessibility.

It is convenient to divide sorbents for SPE into three groups: inorganic oxides; low-specificity sorbents; and, compound-specific and class-specific sorbents (Poole, 2003).

The most important inorganic oxides adsorbents for SPE are silica gel, alumina, Florisil (synthetic magnesium silicate) and diatomaceous earth (Barker; 2000).

Adsorbent properties that increase retention are a larger surface area and a high activity. Hydrogen-bonding functional groups are strongly retained (e.g. sulfonic acid, carboxylic acid, phenol and hydroxyl). Coating inorganic oxides with a complexing agent (e.g. silver nitrate, and caffeine) or an acid or base (an acid can be used for the selective isolation of bases and vice versa) can be used to modify selectivity for the isolation of target compound (Thurman, & Mills; 1998).

Low-specificity sorbents, which include chemically-bonded silicas, porous polymers and carbon, are commonly used for the isolation of contaminants from aqueous solution. Silica-based, siloxane-bonded sorbents can be prepared with a wide range of bonding density, pore size and functional group types. Silica gels of high surface area, 500-600 m^2 / g, are generally used to prepare sorbents for the isolation of small molecules. Siloxane-bonded sorbents with high surface areas, long alkyl chains and high phase loading maximize retention of small molecules from aqueous solution, while wide-pore materials with a low phase loading short alkyl chains are used to isolate macromolecules. Porous polymer sorbents are generally copolymers of styrene and divinylbenzene processed to enhance their properties for SPE. They are either similar in chemistry to porous polymers developed for HPLC and have moderate surface area (< 600 m^2 / g) or are biporous and highly crosslinked with surface areas of 700-1200 m^2/ g. The modern forms of carbon used for SPE are graphitized carbon blacks and porous graphitic carbon. Graphitized carbon blacks are (largely) non-porous with moderate surface area of 100-210 m^2 / g (Poole, 2003).

Various selective sorbents based on ion exchange, bioaffinity, molecular recognition, and restricted access material are used. Ion-exchange sorbents are

usually classified as weak or strong, depending on the identity of the ionic group and whether its charge is independent of the sample pH (strong ion exchanger) or can be manipulated by changing pH (weak ion exchanger) (Poole, 2003).

Restricted access sorbents were initially developed for the isolation of low-molecular-mass drugs from biological fluids with minimum sample pretreatment and now also find use in the isolation of herbicides from surface waters containing high levels of humic substance (Simpson; 2000).

Immunosorbents (or immunoaffinity sorbents) have long been used for sample pretreatment in medicine, biology and food science, but more general applications, such as to environmental samples. Immunosorbents are prepared by covalently bonding a suitable antibody to an appropriate sorbent (Delaunay, Pichon & Hennion; 2000).

Polymeric adsorbents, which are sub-groups of low-specificity sorbents, are highly porous structures whose internal surfaces can adsorb and then desorb a wide variety of different species depending on the environment in which they are used. For example, in polar solvents such as water, polymeric adsorbents exhibit non-polar or hydrophobic behaviour and so can adsorb organic species that are sparingly soluble. This hydrophobicity is most pronounced with the styrenic adsorbents. In non-polar solvents, such as hydrocarbons, etc. most adsorbents exhibit slightly polar or hydrophilic properties and so will adsorb species with some degree of polarity. This polarity is most pronounced with the acrylic adsorbents and the phenolic adsorbents.The efficiency of the polymeric sorbents depends on various physico-chemical parameters such as particle size, surface area, pore diameter, pore volume, degree of crosslinking and particle size distribution. The highly porous materials have higher real activity surface available which allows higher π- π interactions and so higher adsorption capacity. The band broadening with highly porous sorbents is only slightly greater than with other sorbents because porosity minimizes band dispersion. Band broadening is also minimized when working with sorbents with a narrower particle size distribution (Puig & Barceló; 1996).

Macroporous hydrophobic resins of the Amberlite XAD series are good supports for developing chelating matrices. Amberlite XAD-1, XAD-2, XAD-4 and XAD-16 (Table 1.1) are polystyrene-divinylbenzene (PS-DVB) resins with a high hydrophobic character and no ion-exchange capacity. The broader range of pH stability of these resins increases the flexibility of the method, and they have no silanol groups (Bouvier, Martin, Iraneta, Capparella, Cheng & Phillips., 1997).

XAD-1, XAD-2 and XAD-4 are PS-DVB with a highly hydrophobic character. XAD-7 and XAD-8 are acrylic ester resin with a higher affinity for solutes. XADs based on PS-DVB have some drawbacks including lack of selectivity, low breakthrough volumes for very polar compounds, and sampling rate, and they require extensive cleaning before use (Akerblom; 1995).

All those properties mentioned above make the macroporous polymers have higher capacities and be more highly selective than microporous resins. In addition; use of microporous matrix during preparation of polymers exhibit some problems, as the swelling of this kind of matrix is highly dependent on the consumption of the sample solution, thus making it difficult for data to be reproduced. However, macroporous resins are not affected by the concentration of the external solution or by the solvent.

Hypercrosslinked resin sorbents have also gained increasing interest. These sorbents are produced by crosslinking polymers of macroporous resin in a good solvent. The crosslinking tightens the structures and modifies the surface properties of the porous polymeric network.

One of the advantages of macroporous and hypercrosslinked resin sorbents is the possibility of controlling their resin structure, internal surface area, and pore size distribution. This can be achieved by varying the polymerization conditions, the most crucial of which are the amounts of monomers and pore-forming agent used in the polymerization reaction.

The term “macroporous resin sorbents” in the literature can be found as polymeric resin, nonionic polymeric resin, macroporous resin, macroporous polymeric adsorbent, macroporous copolymer, organic adsorption polymer, synthetic adsorber polymer, polymeric sorbent, nonionic macroreticular polymeric resin, macroporous synthetic adsorbent, etc. The most common type of macroporous sorbent is styrene and divinylbenzene copolymer, which may be found as polystyrene, styrene-divinylbenzene copolymer or simply St-DVB copolymer.

The most widely used polymeric sorbents are styrene-divinyl benzene copolymers with a hydrophobic surface (Prasada Rao, Praveen & Daniel; 2004). Polystyrene cross-linked with divinylbenzene with low cross-linkage developed by Merrifield in 1960 is the most commonly used polymer support for solid phase extraction because of the advantages such as good mechanical and chemical stabilities. Chelate immobilized Merrifield chloromethylated styrene-divinyl benzene resin offer better retention capacities due to its small pore diameter and low bead surface area over Amberlite XAD resins (Praveen, Metilda, Daniel & Prasada Rao; 2005).

Table 1.1 Some properties of Amberlite resins

Amberlite	**Chemistry**	**Surface area (m^2/g)**	**Av. pore diameter (Å)**	**Mean diameter (µ)**	**Applications**
XAD-2	pStyDVB	300	90	500	Separation of metals, antibiotic recovery
XAD-4	pStyDVB	750	100	640	Removal of solvents and low MW species
XAD-16	pStyDVB	800	150	700	Small molecules, antibiotic recovery
XAD-1180	pStyDVB	500	400	530	Plant extracts, large MW product recovery
XAD-7	Aliphatic ester	500	450	560	Plant extract, enzyme purification, peptides

pStyDVB: polystyrene divinylbenzene

1.4 Retention of Trace Elements on Sorbents

Adsorption of trace metals on the solid sorbent is required for preconcentration (Figure 1.3). The mechanism of retention depends on the nature of the sorbent, and may include simple adsorption, ion-exchange or chelation.

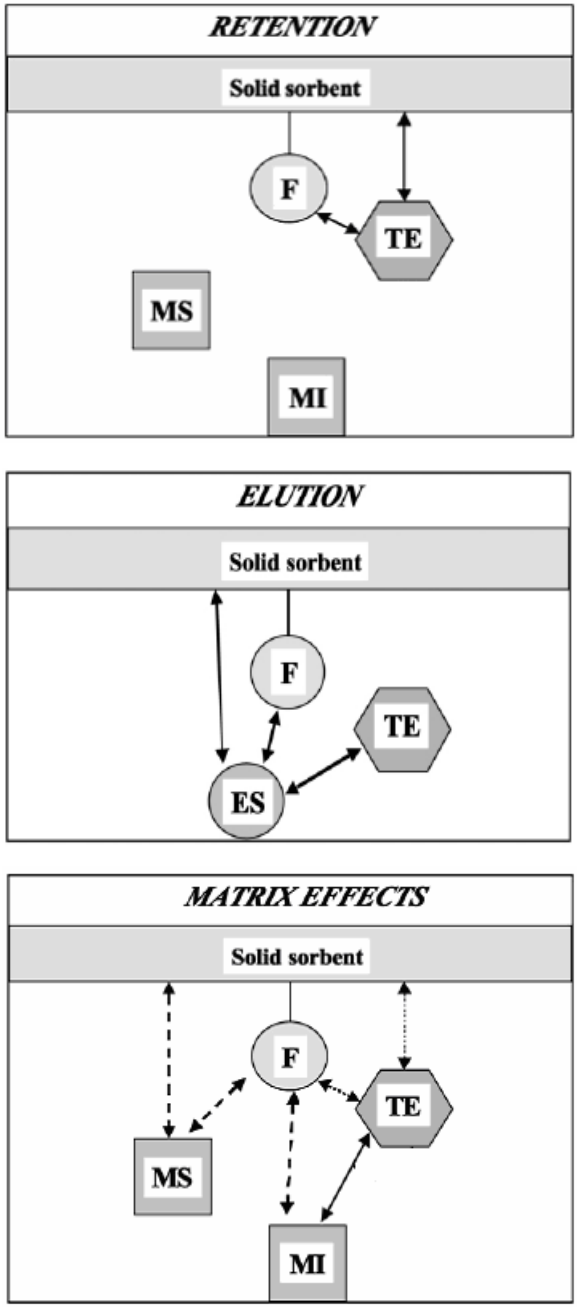


Figure 1.3 Interactions occuring at the surface of the solid sorbent. F,functional group; TE, trace element; MS, matrix solvent; MI, matrix ions; ES, elution solvent.

1.4.1 Adsorption

Trace elements are usually adsorbed on solid phases through van der Waals forces or hydrophobic interaction. Hydrophobic interaction occurs when the solid sorbent is highly non-polar (reversed phase). The most common sorbent of this type is octadecyl-bonded silica (C_{18}-silica). More recently, reversed polymeric 18 phases have appeared, especially the styrene-divinylbenzene copolymer that provides additional π-π interaction when π-electrons are present in the analyte (Camel, 2003). Elution is usually performed with organic solvents, such as methanol or acetonitrile. Such interactions are usually preferred with online systems, as they are not too strong and thus they can be rapidly disrupted. However, because most trace element species are ionic, they will not be retained by such sorbents.

1.4.2 Ion Exchange

Ion-exchange sorbents usually contain cationic or anionic functional groups that can exchange the associated counter-ion. Strong and weak sites refer to the fact that strong sites are always present as ion-exchange sites at any pH, while weak sites are only ion-exchange sites at pH values greater or less than the pK. Strong sites are sulfonic acid groups (cation-exchange) and quaternary amines (anion-exchange), while weak sites consist of carboxylic acid groups (cation-exchange) or primary, secondary and tertiary amines (anion-exchange).

Functional groups can be chemically bound to silica gel or polymers (usually a styrene-divinylbenzene copolymer), the latter allowing a wider pH range. An ion-exchanger may be characterized by its capacity, resulting from the effective number of functional active groups per unit mass of the material. The theoretical value depends upon the nature of the material and the form of the resin. However, in the column operation mode, the operational capacity is usually lower than the theoretical one, as it depends on several experimental factors, such as flow-rate, temperature, particle size and concentration of the feed solution. As a matter of fact, retention on ion-exchangers depends on the distribution ratio of the ion on the resin, the stability

constants of the complexes in solution, the exchange kinetics and the presence of other competing ions.

1.4.3 Chelation

Several functional group atoms are capable of chelating trace elements. The atoms most frequently used are nitrogen (e.g. N present in amines, azo groups, amides and nitriles), oxygen (e.g. O present in carboxylic, hydroxyl, phenolic, ether, carbonyl and phosphor groups) and sulfur (e.g. S present in thiols, thiocarbamates, thioethers). The nature of the functional group will give an idea of the selectivity of the ligand towards trace metals (Camel; 2003).

In practice, inorganic cations may be divided into 3 groups:

Group I- 'hard' cations: These preferentially react via electrostatic interactions due to a gain in entropy caused by changes in orientation of hydration water molecules; this group includes alkaline and alkaline-earth metals (Na^{+}, Li^{+} , Ca^{2+}, Mg^{2+}, etc.) that form rather weak outer-sphere complexes with only hard oxygen ligands.

Group II- 'borderline' cations: These have an intermediate character; this group contains Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}. They possess affinity for both hard and soft ligands.

Group III- 'soft' cations (*Soft acids or class b*): These tend to form covalent bonds. Hence, Cd^{2+} and Hg^{2+} possess strong affinity for intermediate (N) and soft (S) ligands.

For soft metals, the following order of donor atom increasing affinity is observed: O>N>S. A reversed order is observed for hard cations. For a bidentate ligand, affinity for a soft metal increases with the overall softness of the donor toms: (O, O)>(O, N)>(N, N)>(N, S).

The order is reversed for hard metals. In general, the competition for a given ligand essentially involves Group I and Group II metals for O sites, and metals of Group II and Group III for N and S sites. The competition between metals of Group I and Group III is weak**.**

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

(1) the synthesis of new sorbents containing such groups (new sorbents)

(2) the chemical bonding of such groups on existing sorbents (functionalized sorbents)

(3) the physical binding of the groups on the sorbent by impregnating the solid matrix with a solution containing the chelating ligand (impregnated, coated or loaded sorbents).

The last one remains the most simple to be used in practice. Impregnated resins have been proposed by Warshawsky and used successfully in the extraction of metals from a multicomponent mixture in various analytical applications (Cortina, Arad-Yellin, Miralles, Sastre & Warshawsky; 1998). Different ligands (dithizone, thiocarbamate, calixarene, resorcinarene, chromotropic acid, etc.) impregnated on a variety of solid matrices have been successfully used for preconcentration, separation and determination of trace metal ions. Its main drawback is the possible flush of the chelating agent out of the solid sorbent during sample percolation or elution that reduces the lifetime of the impregnated sorbent.

Due to their high capacity and operational efficiency ligand functionalized sorbents have been used for matrix isolation and preconcentration of trace metal ions prior to their determination by atomic spectrometric methods (Sabermahani & Taher;

2006). The application of functionalized resins has the additional advantage of controlling the capacity and selectivity of metal sorption by the appropriate choice of loading organic reagents, thus controlling the efficiency of the process.

Recently, chemically modified resins have been more used in the extraction of metals from a multicomponent mixture in various analytical applications. A number of chelating resins have been prepared by incorporating various functional groups (e.g. ethylenediamine tetraacetic acid, iminodiacetic acid, 8-hydroxyquinoline, dithiocarbamate, pyrocatechol, etc) and their analytical properties investigated. In this work, porphyrin and calixcrown have been used as functional group.

1.5 Chelating Agents

1.5.1 Porphyrins

The porphyrins are a class of naturally occurring macrocyclic compounds. The porphyrin molecule contains four pyrrole rings linked via methane bridges (Figure 1.4).

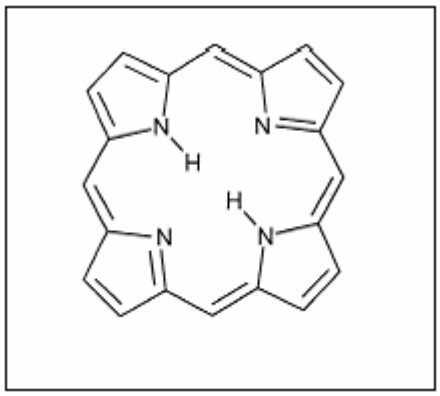


Figure 1.4 Porphyrin macrocyclic system

When coordination occurs, two protons are removed from the pyrrole nitrogen atoms (Figure 1.5), leaving two negative charges. The porphyrin ring system is very stable and exhibits aromatic character. The porphyrin complexes with transition metal ions are very stable.

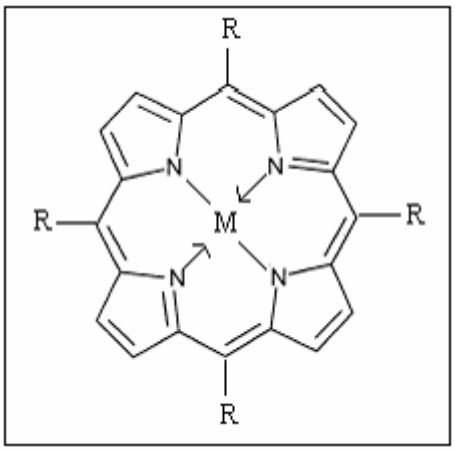


Figure 1.5 Porphyrin-metal complex

Almost all metals form complexes 1:1, although Na, K, Li complexes are 2:1 in which the metal atoms are incorporated slightly below and above the porphyrin macrocycle plane. When divalent metal ions (e.g. Co(II), Ni(II), Cu(II)) are chelated, the resulting tetracoordinate chelate has no residual charge. While Cu(II) and Ni(II) in their porphyrin complexes have generally low affinity for additional ligands, the chelates with Mg(II), Cd(II) and Zn(II) readily combine with more ligand to form pentacoordinated complexes with square-pyramidal structure. Some metalloporphyrins (Fe(II), Co(II) Mn(II)) are able to form distorted octahedral with two extra ligand molecules.

Porphyrin metal complexes play an important role in biological activities as for instance iron complex in the haemoproteins, magnesium complexes in the chlorophylls and a cobalt complex in Vitamin B_{12}. Complexes of many metals with various porphyrins have been extensively studied in order to understand the biosynthetic formation and biological activity of natural compounds.

It is well known that porphyrin is also a high sensitive chromogenic reagent. Porphyrins and their metal chelates generally exhibit characteristic sharp and intensive absorption bands in the visible region. The region from 400 to 500 nm, which is called the Soret bant, shows the most intensive absorption and molar absorptivities of the order of 10^5 are often found. The Soret band is widely used for spectrophotometric determination of metalloporphyrins.

Owing to its well known photochemical and redox activity, the porphyrin macrocycle is an attractive building block on which to append additional recognition sites for anion binding. The combination with Lewis acid, such as zinc, complexed in the porphyrin macrocycle cavity, may produce new selective redox active reagents for anions. Indeed various metalloporphyrins have shown a potentiometric response to anions with selectivity sequences solely dependent on the centrally bonded metal (Bakker, Malinowska, Schiller & Meyerhoff; 1994). The metalloporphyrins have rich redox chemistry since they have the advantage of including coordination of additional ligands above and below the porphyrin plane.

Due to strong complexing properties and catalytic behaviour of metalloporphyrins, these compounds have found numerous applications in chemical analysis (Table 1.2).

Table 1.2 Applications of porphyrins

Porphyrin	Application	Reference
Tetracarboxyphenyl porphyrin	Spectrophotometric determination of Zn(II)	(Kilian & Pyrzynska; 2003)
Meso-tetrakis(4-N-trimethylaminophenyl)porphyrin	Spectrophotometric determination of Cu(II)	(Watanabe & Ohmori; 1981)
Meso-tetrakis(4-sulfophenyl)porphyrin	Spectrophotometric determination of Co(II)	(Igarashi, Aihara & Yotsuyanagi; 1996)
Tetraphenyl porphyrin	Potentiometric determination of Ni(II)	(Gupta, Jain, Singh & Khurana; 1997)
Poly[tetra(4-aminophenyl)porphyrin	Potentiometric pH sensor	(Yuan, Chai, Shen & Yu; 1993)
Tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin	Voltammetric determination of Ni(II)	(Malinski, Ciszewski, Fish & Czuchajowski; 1990)
Meso-tetrakis(4-N-trimethylaminophenyl)porphyrin	Voltammetric determination of lanthanidies	(Itoh, Liu & Komata; 2006)
Tetraphenyl porphyrin	HPLC analysis for Ni(II), Cu(II) and Zn(II)	(Saitoh & Suzuki; 1985)
Tetra-(4-bromophenyl)-porphyrin	HPLC analysis for Pb(II), Cd(II) and Hg(II)	Hu, Yang, Yin & Yao, 2002)

Several porphyrins become useful as the spectrophotometric reagents for the determination of metal ions. Their disadvantage is that the incorporation reaction of the metal ion in the porphyrin ring is very low and the rate of metalloporphyrin formation is several orders of magnitude lower than with the common ligands. The slow reaction rate is the result of difficulty in deforming of porphyrin ring. The porphyrins with substituents at the pyrrole nitrogen, such as N-alkyl or N-benzyl, are more reactive than their non-substituted analogues. Alkylation of any of the pyrrole nitrogen will lead to an increase in the distortion of the macrocycle and this in turn leads to an increase in the basicity of particular pyrrole nitrogen. To enhance the reaction rate, several procedures, except heating, have been employed.

1.5.1.1 Application of Porphyrin as Complex-Forming Reagent in Modified Sorbents for Preconcentration

The porphyrins, which act as a tetradentate ligand forming strong complexes with transition metal ions, have gained increasing interest in analytical chemistry.

By Kilian et.al., the preconcentration of Pb(II),Ni(II), Cu(II), Cd(II), and Mg(II ions by complexation with carboxyphenylporphyrin (TCPP) and retention on anion-exchange resins Amberlite IRA-904 and Amberlite IRA-401 and a non-ionic sorbent Amberlite XAD-2 were examined. Two different procedures - ligand in solution or immobilised on the solid sorbents - were evaluated as a function of pH. Physical interactions as well as ion-pair formation are involved in the immobilization of such organic ligands on a polymeric matrix. In addition, sorption of TCPP may occur due to π- π dispersion forces arising from the aromatic nature of the resin and reagent. Amberlite IRA-904 modified with TCPP displays much better resistance towards mineral acids and alkaline solutions, which are usually employed for the elution processes, than microporous Amberlite IRA-401 loaded with the same ligand (Kilian & Pyrzynska, 2001; Kilian & Pyrzynska, 2002).

By Pyrzynska et. al., the sorption of metal ions (Cd(II), Cu(II), Ni(II), and Pb(II)) from aqueous solution on the anion exchange resin Amberlite IRA-904 modified

with TCPP was studied in batch equilibrium experiments. The influence of operating variables such as initial pH and contact time between solution and the resin on the equilibrium parameters was measured (Pyrzynska & Kilian; 2003).

By Pyrzynska et.al., Amberlite IRA-904 was modified with tetrakis (p-carboxyphenyl) porphyrin and used to preconcentrate vanadium species. Several parameters, such as sorption capacity of the chelating resin, pH for retention of V (IV) and V (V), volume of sample and eluent were evaluated (Pyrzynska & Wierzbicki; 2004).

By Yang et. al., a new method for the simultaneous determination of heavy metal ions in Chinese herbal medicine by microwave digestion and reversed-phase high-performance liquid chromatography (RP-HPLC) has been developed. Lead, cadmium, mercury, nickel, copper, zinc, and tin in the digested samples were pre-column derivatized with tetra-(4-chlorophenyl)-porphyrin (T-4-CPP) to form the colored chelates which were then enriched by solid phase extraction with C-18 cartridge and eluted from the cartridge with tetrahydrofuran (Yang, Yan & Lin; 2004).

1.5.2 Calixarenes

The name calixarenes was introduced by Gutsche (Gutsche; 1989) for the cyclic oligomers which are obtained from the condensation of formaldehyde with *p*-alkylphenols under alkaline conditions. The use of this word was suggested in particular by the shape of the tetramer, which can adopt a bowl-or beakerlike conformation which indicates the possibility of the inclusion of “guest” molecules.

The word calixarene is derived from calix or chalice because this type of molecule resembles a vase and from the word arene that refers to the aromatic building block. Calixarenes have hydrophobic cavities that can hold smaller molecules or ions and belong to the class of cavitands known in host-guest chemistry. Calixarene nomenclature is straightforward and involves counting the

number of repeating units in the ring and includes it in the name. A calix [4] arene has 4 units in the ring and a calix [6] arene has 6. The different types of calix [4] arene (Böhmer; 1995) are given in Figure 1.6**.**

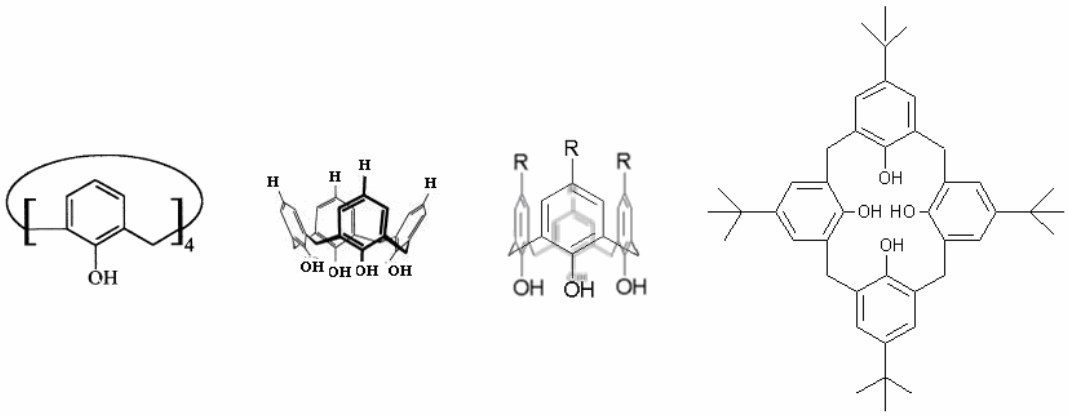


Figure 1.6 Various structure of calix [4] arene

One of the most fascinating aspects of calixarenes lies in the variety of conformations which they can assume; these result from the (more or less) free rotation about σ bonds of the Ar-CH_2-Ar groups.

In the case of calix[4]arenes, the relative orientations of the phenol units shown in Figure 1.7 can in principle be assumed. Gutsche has introduced the terms “cone”, “partial cone”, “1,2-alternate” and “1,3-alternate” which are generally accepted today, to these basis conformations. They differ with respect to the position of the phenolic OH groups (and the *p*-positions) wih respect to the molecular plane (here easily defined by C atoms of the methylene bridges).

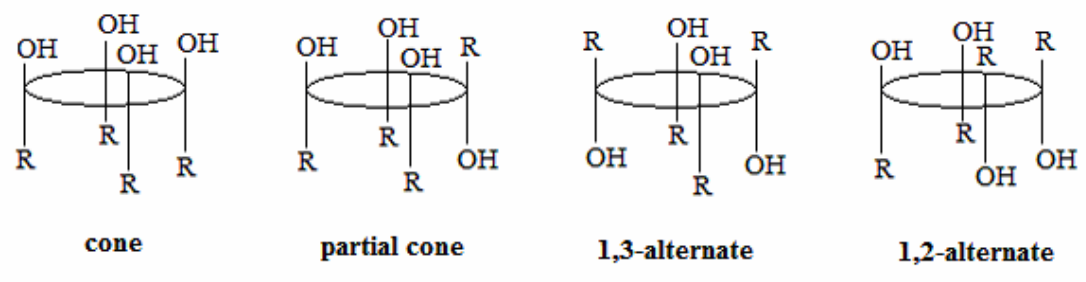


Figure 1.7 The four basic conformations of a calix[4]arene

Calixarenes because of their well defined frame and chemically stable intermolecular cavity of tunable size and co-ordination selectivity are frequently selected as host molecules in sensor research (Leyton, Domingo, Sanchez-Cortes, Campos-Vallette, Díaz & Garcia-Ramos; 2007) or in separation sciences (Li & Chen; 2003).

In particular the easy accessibility of *p-tert*-butylcalix[4]arene has made this member of the series increasingly popular as a building block or platform for assembling more elaborate structures with ligating side arms or pod ands for the reception of guest species, notably ions and neutral molecules. The complexes with alkali metals (Rozhenko, Schoeller, Letzel, Decker, Agena & Mattay; 2005), especially cesium (Araki,Watanabe, Oda, Hayashida, Yasutake & Shinmyozu; 2001) are promising in the nuclear waste management, with heavy metal ions (Benounis, Jaffrezic-Renault, Halouani, Lamartine & Dumazet-Bonnamour; 2006) are useful in view of environmental protection and with lanthanides and actinides (Coupez & Wipff, 2004; Souane, Hubscher, Asfari, Arnaud & Vicens, 2003) are used for their removal from industrial and nuclear waste.

1.5.3 Calixcrowns

Calix[4]arene-crown ethers or calix[4]crowns (Figure 1.8) are one of the most widely investigated class of cation ligands based on calixarenes. They refer to the family of macropolycycles constructed from calix[4]arene elements and polyethylene glycolic units. They show binding properties towards alkali metal, alkaline earth metal and ammonium cations which can be tuned by selecting the most appropriate conformation of the calix[4]arene and the crown ether size. The first member, *p-tert*-butylcalix[4]crown-6 was reported as early as 1983 and in this connection various calix[4]crowns have been reported with their host-guest properties in metal complexation, metal extraction, metal transportation, molecular switches and in ion sensing de vices. By Agrawal and Sharma, functionalized calix[6]crown hydroxamic acid has been reported for speciation, liquid-liquid extraction, sequential extraction and trace determination of Cr(III), Mo(VI) and W(VI) (Agrawal & Sharma; 2005).

Choi et al. have prepared calix[4]arene diaza-crown ethers which have high selectivity for transport of mercury(II) ion.

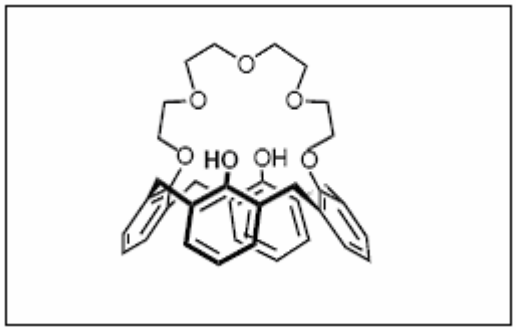


Figure 1.8 The structure of calix[4]arene-crown

Organosilicon resins with p-tert-butylcalix[6]-1,4-crown-4 derivatives have been used in adsorption of alkali metal ions, transition metal and heavy metal ions. By Kim et al., benzothiazolyl functionalized ionophore based on the calix[4]arene-crown-5-ether has been used as selective fluoroionophore toward physiologically important calcium ion (Kim, Cha & Chang; 2002).

In recent years, the interesting properties shown by these cyclic oligomeric compounds stimulated the exploitation of useful methods towards their incorporation into polymeric matrices, given its interest for the development of ion selective electrodes and membranes, chemical and biochemical sensors and selective extraction of ions and neutral molecules. Two main strategies have generally been followed with that purpose. One relies on the immobilization of the calixarene unity on an existing polymeric matrix (Barata, Costa, Granja & Prata; 2004) and the other is based on the copolymerization of an appropriate calixarene monomer with some other suitable monomers, either through linear step-growth polymerization (Costa, Barata & Prata; 2006) or radical addition reactions. This latter method potentially provides more control over several physical, chemical and mechanical properties of the final polymer.

1.5.3.1 Application of Calixarenes as Complex-Forming Reagent in Modified Sorbents for Preconcentration

The calixarenes and calixcrowns offer novel possibilities as selective reagents for metal ions because of their recognition based upon the cavity size.

By Hutchinson et.al., the synthetic macrocycles, calixarenes, have been shown to possess high ionophoric selectivity and form inclusion complexes with many ions. A tetrameric calixarene bearing hydroxamic acid functional groups was synthesised and supported on octadecylsilica and XAD-4 resin. When loaded with Fe(III), V(V) and Cu(II), the materials display the characteristic visible absorption spectra associated with hydroxamate coordination. The chemical bonding of the calixarene tetrahydroxamic acid to silica particles was also carried out using the triethoxysilane derivative of the p-allylcalix[4]arene hydroxamic acid (Hutchinson, Kearney, Horne, Lynch, Glennon, Mckervey & Harris; 1994).

By Jain et. al; "upper new" functionalized 11,23-disemicarbazono-26,28-n-dipropoxy-25,27-dihydroxy calix[4]arene was synthesized. This calix[4]arene-semicarbazone was then covalently linked with Merrifield's peptide resin and its analytical properties were investigated. The resin was used for the separation and preconcentration of La(III), Ce(III), Th(IV) and U(VI) (Jain, Handa, Pandya, Shrivastav & Agrawal; 2002).

By Li et.al; two organosilicon resins from *p-tert*-butylcalix[6]-1,4-crown-4 was prepared. The adsorption capacity of these resins toward alkali metal ions (Li^+, Na^+ and K^+), transition metal and heavy metal ions (Ni^{2+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Ag^+ and Hg^{2+}) were determined (Li & Chen; 2003).

By Matsumiya et. al., a rapid column-adsorption method has been developed for concentrating traces of copper, cadmium, and lead in water prior to their determinations by graphite-furnace atomic-absorption spectrometry. The adsorbent

used was prepared by loading a strongly basic anion-exchanger QAE-Sephadex A-25 with thiacalix[4]arenetetrasulfonate (Matsumiya, Iki, Miyano & Hiraide; 2004).

By Jain et.al., a very stable calixarene-based polymeric chelating resin has been synthesized by covalently linking calix[4]arene-o-vanillinthiosemicarbazone through its 'lower rim' to Merrifield resin. It was characterized by FT IR, H-1 NMR and elemental analysis. The resin was efficiently employed to separate and preconcentrate toxic metal ions such as Cu(II), Cd(II) and Pb(II) in a column prior to their determination by UV-Vis spectrophotometry and atomic absorption spectrophotometry (GFAAS) (Jain, Pandya, Pillai, Agrawal & Shrivastav; 2004).

By Jain et.al; Merrifield chloromethylated resin was anchored with calix[4]arene-*o*-vanillinsemicarbazone for simultaneous separation and solid phase extractive preconcentration of U(VI) and Th(IV). The validity of the proposed method was checked by analyzing these metal ions in natural water samples, monazite sand and standard geological materials (Jain, Pandya, Pillai & Shrivastav; 2006).

By Memon et. al., new calix[4]arene based polymeric resins have been synthesized via nucleophilic substitution reactions involving 5,11,17,23-tetrakis-[(N,N-dimethylamino)methyl]-25,26,27,28-tetrahydroxycalix[4]arene, and 5,11,17,23-tetracyanomethyl-25,26,27,28-tetrahydroxycalix[4]arene as precursors with a Merrifield resin. The extraction studies were made by using liquid-liquid extraction, solid-liquid batchwise and packed-column sorption procedures, and effect of different anion to extraction of dichromate anion was examined (Memon, Tabakci, Roundhill & Yılmaz; 2006).

1.6 Sol-Gel Processing

The sol-gel process is a versatile solution process for making ceramic and glass materials. In general, the sol-gel process involves the transition of a system from a liquid “sol” (mostly colloidal) into a solid “gel” phase. Applying the sol-gel process, it is possible to fabricate ceramic or glass materials in a wide variety of forms: ultra-

fine or spherical shaped powders, thin film coating, ceramic fibers, microporous inorganic membranes, monolithic ceramics and glasses or extremely porous aerogel materials.

The starting materials used in the preparation of the "sol" are usually inorganic metal salts or metal organic compounds such as metal alkoxides. In a typical sol-gel process, the precursor is subjected to a series of hydrolysis and polymerization reactions (Figure 1.9) to form a colloidal suspension, or a "sol". Further processing of the "sol" enables one to make ceramic materials in different forms. Thin films can be produced on a piece of substrate by spin-coating or dip-coating. When the "sol" is cast into a mold, a wet "gel" will form. Will further drying and heat treatment, the "gel" is converted into dense ceramic or glass articles. If the liquid in a wet "gel" is removed under a supercritical condition, a highly porous and extremely low density material called "aerogel" is obtained. As the viscosity of a "sol" is adjusted into a proper viscosity range, ceramic fibers can be drawn from the "sol". Ultra fine and uniform ceramic powders are powders by precipitation, spray pyrolysis or emulsion techniques.

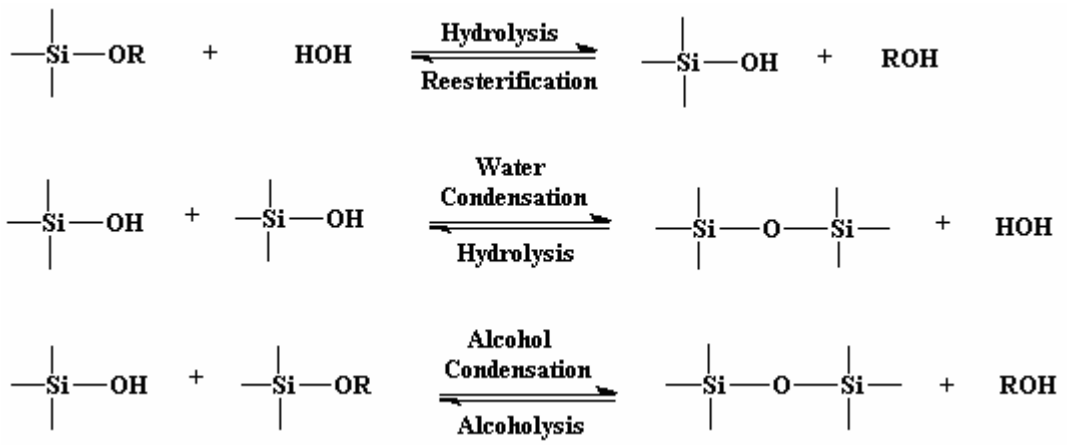


Figure 1.9 Example of a general sol-gel reaction

In recent years sol-gel techniques have been extensively used for incorporation of organic dyes into inorganic host matrices and this area are becoming increasingly important for optical devices. The sol-gel matrix provides two different advantages

for spectroscopic studies thus it acts as a diluted and optically transparent medium like a solution, and also acts a rigid medium like a solid phase.

In favourable cases, the immobilization of fluorescent molecules in the solid matrix may reduce intramolecular motions and rearrangements, thus leading to enhanced photostability and fluoresence capability. Entrapment of fluorescent molecules in sol-gel networks allows the investigation of both photophysical and photochemical properties and sensor applications.

1.6.1 Application of Porphyrins in Sol-Gel Matrix

The sol-gel immobilization of porphyrins in suitable matrices has been reported in literature due to the importance of these systems from many points of interest, such as: chemical and biochemical sensing, pH sensing, and optical limiting. The spectroscopic properties of such materials have been intensively investigated by different method, mostly by absorption and fluorescence spectrocopy, in as much sol-gel materials are characterized by good transparency in the uv-vis region.

Delmarre et.al; the influence of different solvents and of the pH on the spectroscopic properties of Sn(IV)tetrapyridyl and tetramethylpyridinium porphyrins was studied. Then these porphyrins were immobilized in sol-gel matrices. The spectroscopic properties of the immobilized porphyrins are dependent on the composition of the matrix and on its polarity (Delmarre, Lemarinier & Bied-Charreton; 1999).

Dargiewicz-Nowicka et.al; methyl-pyridyl porphyrin and its europium complex was prepared in the monolith gels by sol-gel method. The samples doped with the porphyrins were prepared by tetraethoxysilane hydrolysis and condensation. Their absorption and emission spectroscopic properties in comparison with the spectra of the same compounds in various solvents were investigated. It was seen that fluorescent properties of the europium in sol-gel matrices maked this complex useful

for some special applications as sensing of molecular oxygen or biomolecules (Dargiewicz-Nowicka, Makarska, Villegas, Legendziewicz & Radzki; 2004).

Guo et.al; a new optical sensor membrane was developed for sensing Hg^{2+} in aqueous solution by using 5,10,15,20-tetraphenylporphyrin as a lipophilized indicator, which was dissolved in an organically modified sol-gel (Guo, Zhang, Xie, Lin & Chen; 2006).

1.7 The Metal Ions Determined

Many elements occur in a sample in such minute amounts that, at the beginning of the development of instrumental analytical methods in the 19^{th} century, it was impossible with the existing techniques to determine their concentration quantitatively, although their presence could be dedected qualitatively. To indicate such low and barely detectable concentrations the term 'trace' was used and the elements were referred to as trace elements (World Health Organization ;1996).

The number of determinations of metallic elements in environmental samples is already very high and is rapidly increasing. The samples being analysed include:

- water: industrial waste water, leachate from landfills, river water, sea and ocean water, rain water, drinking water etc.,
- aerosols (dust) collected from the atmosphere (both outdoors and indoors, e.g. in work places), dry deposition, fly ash from electrical power plants etc.,
- soil, sewage sludge and so on.

During the past decades, increasing attention has been focused on pollution of the natural environment. The abatement of pollution of the air, of surface, drinking and sea water and of the soil is an important and very complex task, requiring the cooperation of specialists from various disciplines including analytical chemistry.

For geological research, the determination of trace elements in both rocks and water samples is of great importance, both from an academic and an applied point of view.

These analyses are usually rather difficult owing to the complex nature of the samples and to the low concentrations involved. In addition, it is usually not safe to limit the scope of the analyses to one or a few elements; one should rather determine as many elements as possible using a multi-element analytical technique in order not to overlook important buy unexpected elements.

Zinc, cadmium, nickel, manganese, copper and cobalt are released into the aquatic environmental from dissolution of rocks and soil, atmosphere fall out and biological cycles. Uranium and thorium find extensive application as nuclear fuel in power plants and their main sources are soil, rocks, plants, sand and water. Uranium and thorium are known to cause acute toxicological effects for human and their compounds are potential occupational carcinogens. Zirconium is also used in the nuclear industry as a shielding material, in metal alloys, as catalyst in organic reactions in manufacture of water repellent textiles, in dyes pigment and ceramics.The determination of these metals are of great importance and significance for environment science and life science to separate and determine trace amounts.

Although very sensitive analytical techniques such as FAAS, ICP-MS, ICP-AES and X-ray fluorescence are used for the determination of these metals, it is impossible to directly determine the amount of these metals various samples owing to the low concentration of these metals. In order to achieve accurate and reliable results, preconcentration step is necessary when the concentration of metal ions is too low to be directly determined. SPE is one of the most effective preconcentration methods because of simplicity, rapidity, high preconcentration factor and high recovery (Kumar, Rathore & Singh; 2000).

1.8 Objectives of This Work

Despite the selectivity and sensitivity of analytical techniques such as atomic absorption spectrometry or atomic emission spectrometry there is a crucial need for the preconcentration of trace elements before their analysis due to their frequent low concentrations in numerous samples.

Solid phase extraction (SPE) is of importance due to the advantages such as the shorter analysis time, the less solvent usage. SPE also allows the achievement of high recoveries for metal ions. SPE can be interfaced on-line with analytical techniques, such as liquid chromatography or atomic absorption spectrometry. Thus, its application for preconcentration of trace metals from different samples is very convenient.

Because of the importance of the selection of the sorbent in SPE technique, one of the aims of this study is firstly to develop new chemically bound sorbents and then to examine the use of these sorbents for the preconcentration and separation by SPE of some metal ions in several analytical matrix. For this:

Firstly, 5,10,15,20 tetrakis (phenoxy acetic acid) porphyrin will be synthesized from hydroxyphenyl porphyrin and the porphyrin derivated will be chemically bound to Merrifield polymer resin. The modified resin is characterized by FTIR and ^{1}H NMR and elemental analysis and used to preconcentrate *d*-electron metals such as Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) and *f*- electron metals such as U(VI) and Th(IV). Several parameters, such as sorption capacity of the chelating resin, pH for retention of metal ions, the recovery values and eluent will be investigated. The validity of the proposed method will be checked by analyzing these metal ions in standard samples.

In second stage, p-tert-butylcalix [4] arene-crown ether will be synthesized. Then this ligand will be chemically bounded to Merrifield resin and the structure is characterized by FTIR and ^{1}H NMR and elemental analysis. The resin modified with

calixarene-crown ether will be used to preconcentrate Cu(II), Cd(II), Co(II), Ni(II) and Zn(II). Effective extraction conditions will be optimized. The method will be employed for the analysis of standard materials.

The other aim of this study is to carry out the determination of zirconium by fluorescence quenching of porphyrin using in aqueous medium and sol-gel matrix. For this:

The determination of zirconium using 5,10,15,20 tetrakis (phenoxy acetic acid) porphyrin will be carried out spectrofluorimetrically. The fluorescent property of porphyrin in sol-gel matrix and aqueous solution will be compared. The optimum pH range and linear range for zirconium will be studied and the proposed method will be validated by the analysis of real samples.

CHAPTER TWO

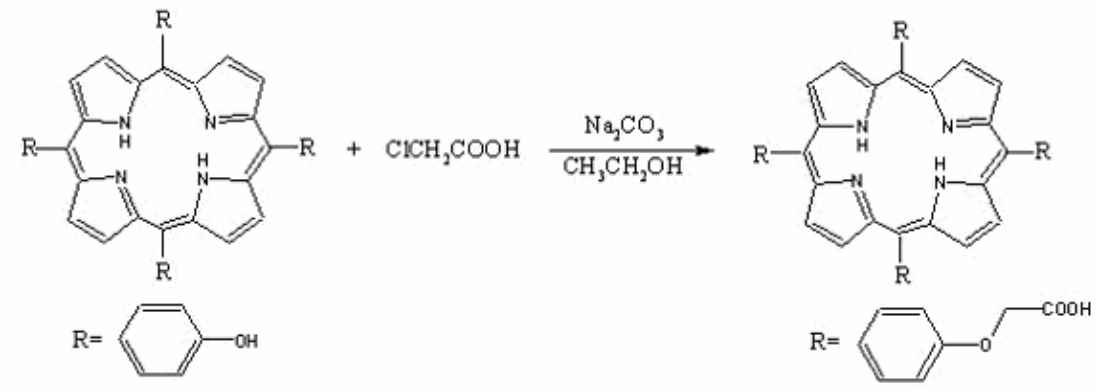
MATERIAL AND METHOD

2.1 Synthesis of Chelating Agents and Modified Resins

5,10,15,20-tetrakis(phenoxy acetic acid) porphyrin (PAAP) and calix[4]crown-4 was used as chelating agent in the determination and preconcentration of metal ions. The synthesis of PAAP was carried out using 5,10,15,20-tetrakis(hydroxyl phenyl) porphyrin (THPP) and *p-tert*-butylcalix[4]arene was used as starting material in the synthesis of calix[4]crown-4. Then the chelating agents synthesized were chemically bound to Merrifield polymeric resin.

2.1.1 Preparation of PAAP

0.050 g THPP (Aldrich, USA) mixed with 0.042 g chloro acetic acid (Merck) and 0.156 g sodium carbonate (Fluka) and 150 mL of ethanol (Merck, 99%, absolute) in a round-bottomed flask and refluxed for 12 h at 70^0 C. The reaction mixture was left to cool, filtered off and evaporated ethanol. Then, 10 mL of doubly distilled water was added and pH ~2.0 was adjusted by 0.1 mol L^{-1} HCl using Mettler Toledo pH meter. The mixture was filtered off and finally dried under vacuum (Scheme 2.1).

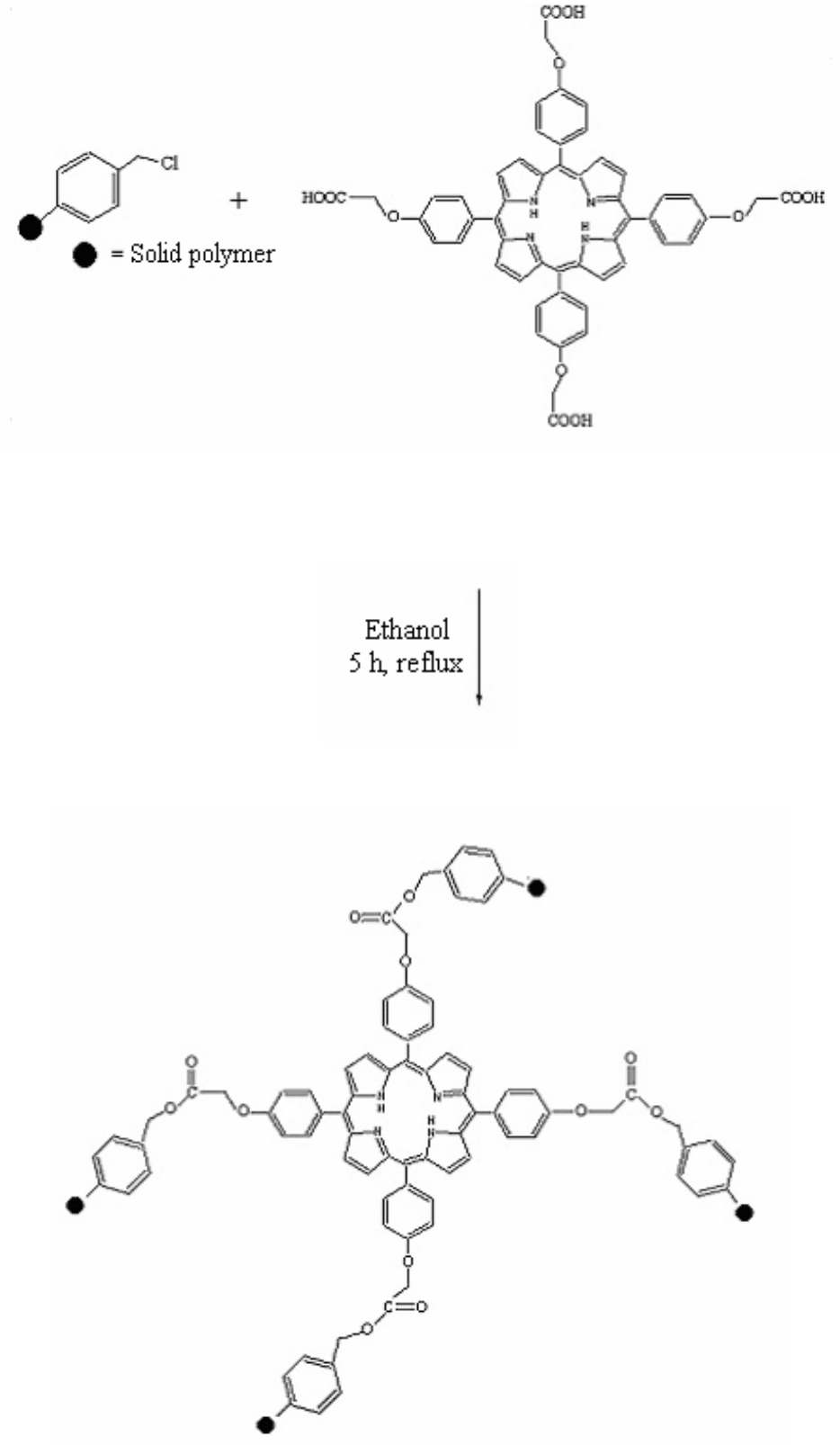


Scheme 2.1 Preparation of PAAP

2.1.2 Preparation of PAAP Modified Resin

Merrifield polymer having 200-400 mesh size (chloromethylated styrene cross-linked with 2.0 % divinyl benzene containing 4.3 mequiv. Cl^- g^{-1}) was obtained from Fluka and it was washed with 1.0 mol L^{-1} HNO_3 and 0.1 mol L^{-1} NaOH successively. It was made free from alkali by washing with double distilled water and washed finally with ethanol and air-dried. Then, it was stored in a polyethylene bottle.

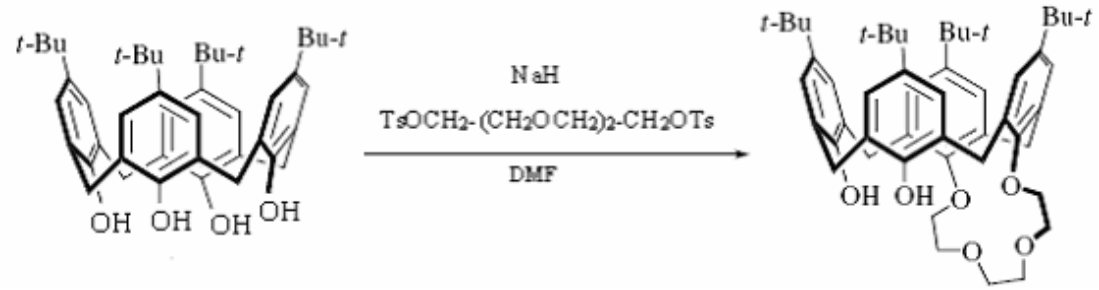
The pH of 0.200 g PAAP in 50 mL of ethanol was adjusted to pH 9.5 by 0.5 mol L^{-1} NaOH and porphyrin solution was refluxed with 0.500 g Merrifield resin for 5 h at 65^0C until the supernatant solution became colorless. It was then filtered out, washed with distilled water and ethanol and finally dried under vacuum and 0.640 g sorbent was obtained. The amount of attached PAAP to the polymer resin was found to be 0.242 mmol g^{-1} resin (Scheme 2.2.)



Scheme 2.2 Preparation of PAAP modified resin

2.1.3 Synthesis of 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26-dihydroxy-27,28-crown-4-calix[4]arene

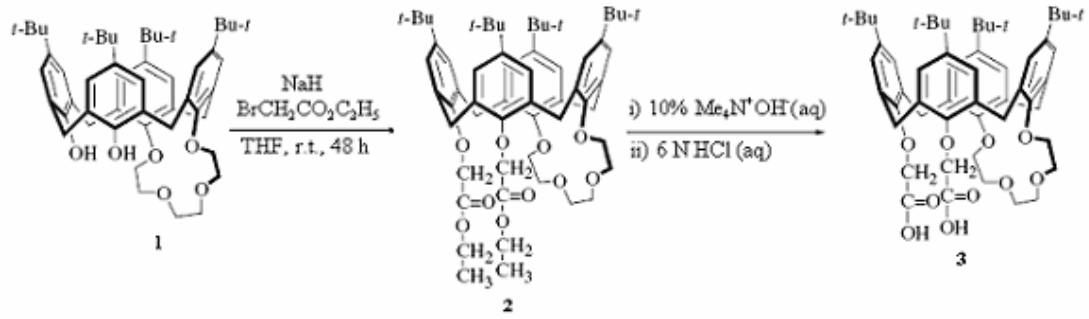
To a suspension of *p-tert*-butylcalix[4]arene (Fluka) (1.00 g, 1.54 mmol) in DMF (100 mL) was added NaH (Fluka) (0.19 g, 7.70 mmol) and the reaction mixture was stirred at room temperature for 1 h. Then triethylene glycol ditosylate (Fluka) (0.35 g, 0.77 mmol) dissolved in dimethylformamide (DMF) (10 mL) was added and the mixture was reacted at 50^0 C. When the starting ditosylate had disappeared methanol (Merck) (10 mL) was added drop wise to remove the excess of NaH. The resulting solution was evaporated to dryness and the residue taken up with aqueous HCI (10% w/v, 100 mL) filtered off and the filtrate evaporated to give a residue which was purified by column chromatography (hexane/ethyl acetate=65/35) to give 0.437 g (75% yield) as a white solid in the cone conformation (Arduini, Domiano, Pochini, Secchi, Ungaro, Ugozzoli, Struck, Verboom & Reinhoudt; 1997) (Scheme 2.3).



Scheme 2.3 Synthesis of 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26-dihydroxy-27,28-crown-4-calix[4]arene

2.1.4 Synthesis of p-tert-butylcalix[4]arene-1,2-crown-4

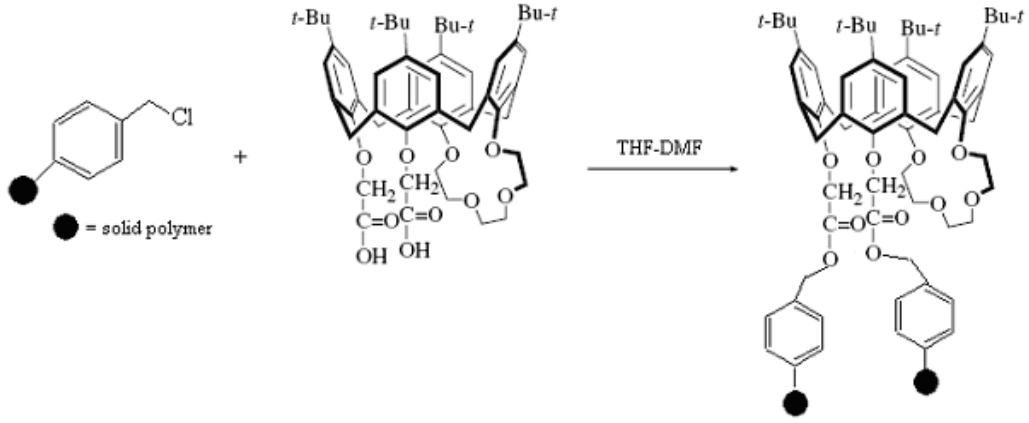
5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26-dihydroxy-27,28-crown-4-calix[4]arene (1) (0.55 g, 0.7 mmol) synthesized was reacted with ethyl bromoacetate (Fluka) (1.4 mmol) and NaH (0.086 g, 3.6 mmol) in tetrahydrofuran (THF) for 48 h to give diester (2) in 70% yield. Hydrolysis of diester with tetramethylammonium hydroxide (Merck) in aqueous THF gave diacid (3) in nearly quantitative yield (Tu, Surowiec & Bartsch; 2006) (Scheme 2.4).



Scheme 2.4 Synthesis of p-tert-butylcalix[4]arene-1,2-crown-4 compound

2.1.5 Synthesis of p-tert-butylcalix[4]arene-1,2-crown-4 Modified Resin

The 2.50 g (2.85 mmol) of *p-tert*-butylcalix[4]arene-1,2-crown-4 compound was refluxed with 1.00 g of Merrifield resin in tetrahydrofuran-dimethylformamide medium (THF-DMF, 50 mL, 7:3) for 24 h in a nitrogen atmosphere. The functionalized resin beads were separated through a porous filter on a water pump. Repeated washing with THF was done to ensure removal of unreacted calix[4]crown-4 and modified resin was dried under vacuum and 1.43 g sorbent was obtained. The amount of attached calix[4]-crown-4 to the polymer resin was found to be 0.489 mmol g^{-1} resin (Scheme 2.5).



Scheme 2.5 Synthesis of p-tert-butylcalix[4]arene-1,2-crown-4 anchored polymeric support

2.2 Characterization of Synthesized Chelating Agents and Modified Resins

To determine chemical properties of the synthesized chelating agents and modified resins, FTIR, ^{1}H NMR and elemental analysis have been used.

2.2.1 FTIR Measurements

FTIR spectra were recorded with Midac-1700 Fourier Transform infrared (FTIR) spectrometer using KBr discs in the range 4000-700 cm^{-1}, 30 co-added interferograms were scanned at 2.0 cm^{-1} resolution. 1 mg of sample was intimately mixed with about 100 mg of dried potassium bromide powder (Merck) using an agate mortar. The mixture was then pressed into pellets under pressure. The pellet was investigated by FTIR.

2.2.2 ^{1}H NMR Measurements

^{1}H NMR spectra were recorded on Bruker instruments operating at 400 MHz using TMS as internal standard. ~ 0.1 g sample was dissolved in chloroform with deuterium and this solution was taken in NMR tube and then was located in NMR device. ^{1}H NMR spectra obtained was evaluated.

2.2.3 Elemental Analysis

The elemental analysis was carried out on a Fisons Carlo Erba (Milan, Italy) model EA-1108 elemental analyzer. The elemental analysis of nitrogen in resin modified with PAAP and the elemental analysis of carbon, hydrogen and oxygen in resin modified with calix[4]crown-4 were carried out by this elemental analyzer.

2.3 Metal Ions Preconcentrated

PAAP chemically bound to Merrifield resin was used for preconcentration of *d*-electron metals such as Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) and *f*- electron

metals such as U(VI) and Th(IV). Calix[4]crown-4 chemically bound to Merrifield resin was used for preconcentration of Cu(II), Cd(II), Co(II), Ni(II) and Zn(II).

Standard metal solutions (100 mg L^{-1}) were prepared by dissolving the required amount of $MnCl_2$, $CoCI_2$, $NiSO_4$, $Cu(NO_3)_2$, $Zn(NO_3)_2$, $CdCl_2$, $UO_2(NO_3)_2.6H_2O$ and $Th(NO_3)_4.6H_2O$ in double deionized water and working solutions were prepared by dilution of the stock solutions.

2.4 Recommended Procedure for Preconcentration and Determination of Metal Ions

Both column and batch methods were used to preconcentrate metal ions from aqueous solutions. The determinations of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were carried out by flame atomic absorption spectrophotometer (FAAS) and the determination of U(VI) and Th(IV) was carried out by visible spectrophotometry.

2.4.1 Column Procedure

A Varian cartridge (polyethylene, 1.0 cm x 6.0 cm) equipped with 20 μm polypropylene frits was used for solid phase experiments.

0.1 g of PAAP on Merrifield resin or 0.25 g of calix[4]crown-4 on Merrifield resin was firstly wetted in 5 mL methanol and 5 mL of doubly deionized water. The mixture was transferred to the polyethylene column. Later, 25 mL of methanol:water (10:90) was passed through the column. Before passing metal solution, column was washed with 20 mL of suitable pH solution.

A sample solution (50 mL) containing 50 μg of metal ion was taken and the pH was adjusted to ~ optimum pH for each metal and passed the column at optimum flow rate by applying vacuum. After this step, stripping was carried out by suitable eluting agent. The stripping solution was diluted when required and analyzed by

FAAS or visible spectrophotometry. All runs were carried out at ambient temperature (23-25 ^{0}C).

2.4.2 Batch Procedure

The batch procedure was used for distribution coefficients of metal ions. A sample solution containing metal ion was taken and pH was adjusted to ~ optimum pH for metal ion. Modified resin (0.1 g of with PAAP or 0.25 g of with calix[4]crown-4) was added to above solution and stirred for sorption of metal ion for 2 h. The metal ion adsorbed chelating resin was filtered and metal ion on resin was eluted and the determination of metal ion in eluate was carried out by FAAS or visible spectrophotometry after diluting to necessary ratio.

2.5 Optimum Conditions Investigated

2.5.1 Effect of pH

For the resin modified with PAAP, the metal ion sorption behaviour as a function of pH was studied between 3.0 and 9.0; for the resin modified with calix[4]crown-4, was studied between pH 3.0 and 8.0 according to column procedure. The optimum pH for each metal ion was determined from sorption values obtained. The sorption experiments were done in triplicate.

2.5.2 Effect of Flow Rate

In the column procedure, the effect of flow rate on sorption and desorption of the metal ions was investigated for each modified resin. At a flow rate of 1.0-2.5 mL min^{-1} was studied for resin modified with PAAP and at a flow rate of 1.0-4.0 mL min^{-1} was studied for resin modified with calix[4]crown-4. The optimum flow rate for each metal ion was determined from sorption and desorption values obtained. The sorption experiments were done in triplicate.

2.5.3 Stripping of Metal Ions

The effects of eluents were studied using HCl and HNO_3 for the modified resin in the column procedure. 0.05-2.5 mol L^{-1} HCl and HNO_3 were used in preconcentration carried out using the resin modified PAAP and 0.25-2.0 mol L^{-1} HCl and HNO_3 were used in preconcentration carried out the resin modified calix[4]crown-4. The recovery was calculated for each acid. Then optimum eluting agent was determined for each metal ion.

2.5.4 Sorption Capacity

The sorption for each metal ion was investigated using column method. Suitable aliquot of metal ion solution at optimum pH and optimum flow rate was passed the column. The loading capacity for each metal ion on the resin was calculated from the difference between the metal ion concentrations before and after desorption.

2.5.5 Preconcentration Factor

The resin was studied for column concentration of metal ions in terms of their preconcentration factor (PF). Preconcentration factor was calculated in the equation given following.

$$PF = \frac{\text{Concentration of metal in the stripping solution}}{\text{Initial concentration of metal in the feed solution}}$$

Quantitative collection of metal ions as 100 ng mL^{-1} was studied for a 500 mL solution.

2.5.6 Distribution Coefficient

The distribution coefficients (K_D) of the metal ions, defined by the equation were determined by using the batch method. In the PAAP modified resin, 25 mL of solution containing 100 µg of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), U(VI) and

Th(IV) at the optimum pH was treated with 0.1 g of fresh resin. In the resin modified with calix[4]crown-4, 10 mL of solution containing not more than 150 μg of the metal ion in question was treated with 0.25 g of fresh resin at optimum pH and then stirred for 1 h at room temperature. The solution was filtered to remove the resin and the metal ion content of the filtrate was determined by FAAS or visible spectrophotometry. Distribution coefficient was calculated in the equation given following.

$$K_D = \frac{\text{Amount of metal ion taken by the resin}}{\text{Amount of metal ion remaining in the solution}} \times \frac{\text{Volume of solution}}{\text{Amount of resin taken}}$$

2.6 Determination of Metal Ions Preconcentrated

The determination of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) was carried out by Unicam (Cambridge, UK) model 929 atomic absorption spectrometer (FAAS) with deuterium background correction and the determination of U(VI) and Th(IV) was carried out by Shimadzu UV-1601 visible spectrophotometer.

2.6.1 Procedure for FAAS

All the metal ion solutions were of analytical reagent. Doubly distilled water was used in all solution preparation.

Air-acetylene flame was used for all metals. Concentration ranges for calibration solutions of each metal at working wavelengths (Table 2.1) were selected with respect to linear range of signal versus concentration of each metal. Calibration solutions containing from 1.0 to 4.0 μg mL^{-1} for Mn(II), from 0.25 to 1.0 μg mL^{-1} for Co(II), form 0.25 to 2.0 μg mL^{-1} for Ni(II), from 2.0 to 8.0 for Cu(II), from 0.25 to 2.0 μg mL^{-1} for Zn(II) and from 0.5 to 2.0 μg mL^{-1} for Cd(II) were prepared freshly before use. All of them were prepared in 10 mL volumetric flasks.

Table 2.1 Instrumental parameters for metal determination by AAS

Element	Wavelength (nm)	Slitwidth (nm)	Lamp current (mA)
Mn(II)	279.5	0.2	20
Co(II)	240.7	0.2	15
Ni(II)	232.0	0.7	15
Cu(II)	324.8	0.5	15
Cd(II)	228.8	0.7	10
Zn(II)	307.6	0.7	15

2.6.2 Procedure for Spectrophotometric Measurement

Arsenazo III {3,6-bis[(2-arsonophenyl)-azo]-4,5-dihydroxy-2,7-naphthtalene disulphonic acid}reacts with Th(IV) in strongly acidic medium at 667 nm, giving grey-green water-soluble complex and U(VI) in pH 2.0 at 653.5 nm, giving a green-blue complex (Marczenko;1986) .

The metal ion bonds to the nitrogen atom of the azo group, the oxygen atom of the arsenic acid group, and the oxygen atom of the hydroxyl group. Arsenazo III reacts with these metal ions in acidic media.

KCl / HCl solution having pH 2 was prepared by mixing 8.1 mL of 0.2 mol L^{-1} HCl and 41.9 mL of 0.2 mol L^{-1} KCl solutions and diluting to 100 mL with doubly distilled water. Reagent blank solution containing Arsenazo III (1%(w/v)) and buffer solution was freshly prepared at each measurement.

Calibration solutions and sample solutions containing 2.0 mL of standard/sample solution + 0.2 mL of Arsenazo III solution + 1 mL of buffer solution were diluted to 5.0 mL with distilled water and measured at 653.5 nm for U(VI). For Th(IV) at 667 nm, instead of adding buffer, solution was completed by 1.0 mol L^{-1} HNO_3.

2.7 Preparation of Electrolyte Solutions

The effects of common electrolytes on the recovery of Mn(II), Co(II), Ni(II), Cu(II), Cd(II), Zn(II),U(VI) and Th(IV) were studied. For this purpose, NaCl, $NaNO_3$, Na_2SO_4, $CaSO_4$, KBr, Kl, $MgSO_4$ and Na_3PO_4 (0.05–2.5 mol L^{-1}, Merck/Riedel) were used to prepare their working solutions.

2.8 Fluorimetric Measurements of Zirconium in Aqueous Solution

2.8.1 PAAP in Aqueous Solution

A 1.0 mL of $2x10^{-5}$ mol L^{-1} PAAP in THF and 1.0 mL of 50 µg mL^{-1} zirconium aqueous solution ($ZrCl_2$, Fluka) were put into volumetric flask and diluted to 10 mL with THF and pH was adjusted (between pH 3.0-9.0) by HCl (0.01-0.1 mol L^{-1}) and NaOH (0.01-0.1 mol L^{-1}) and final solution was taken into a beaker and stirred for 60 min at room temperature. The fluorescent intensity of this solution was measured at studied pH at different times during the stirring. All measurements were carried out at room temperature.

2.8.2 PAAP in Sol-Gel Matrix

2.8.2.1 Sol-Gel Matrix and Film Preparation Process

PAAP doped glasses were prepared by first hydrolyzing tetraethyl orthosilicate (tetraethoxysilane, TEOS, Fluka). A 2.14 mL TEOS and 2.14 mL absolute ethanol were mixed and stirred for 5 min. This was followed by the addition of 0.6 mL acidic water (6.26 mL doubly deionized water and 0.96 mL HCl). This solution was mixed for 2 h. Then 0.6 mL TritonX-100 (Fluka) was added into this solution to improve the homogeneity of the sol-gel. Afterwards 3.0 mL of this mixture prepared and 1.0 mL PAAP solution ($2x10^{-6}$ mol L^{-1}) prepared in THF were mixed and the final mixture was fixed on glass slides. Then the glass slides doped with PAAP were dried at room temperature (Figure 2.1).

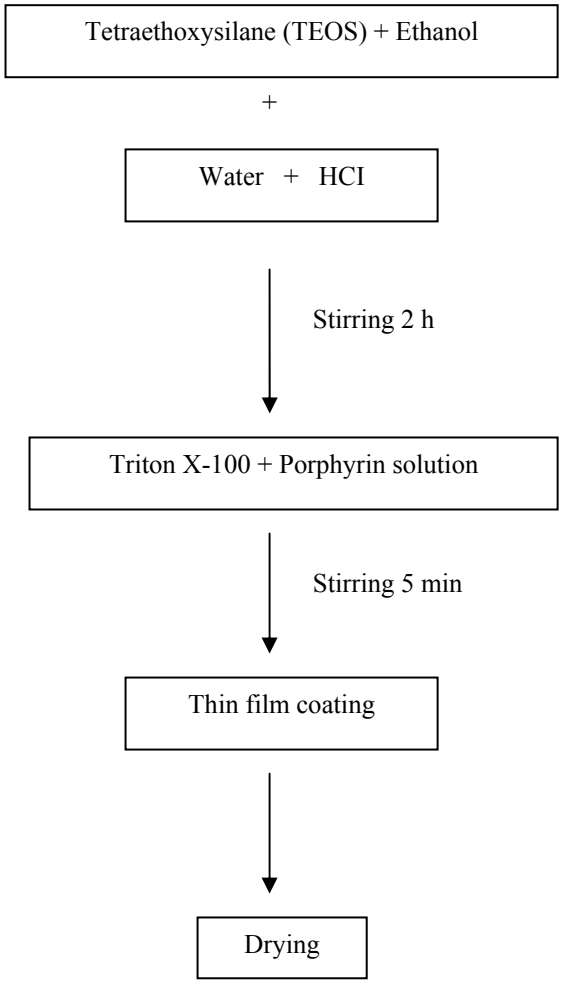


Figure 2.1 Sol-gel preparation process

Glass slides (11mm x 40mm x 1mm) were used as solid support onto which the sol-gel was cast by manual dip-coating technique. Prior to casting, the glass surface was activated by treating with concentrated HNO_3 for 24 h and then washed with distilled water, and then ethanol or methanol. After evaporation of the solvents in a vacuum dessicator, the glass slides were fixed diagonally in a quartz sample cuvette. The advantage of this kind of placement was to improve the reproducibility of the measurements.

The glass slides prepared was fixed diagonally in a quartz sample cuvette which contains zirconium solution at different concentrations. Then fluorescent intensity was measured by Fluoresence Spectrophotometer.

2.8.3 Calibration Graph

A calibration curve of the fluorescence intensity of PAAP in water/sol-gel matrix versus Zr(IV) concentration was plotted. All solutions were prepared freshly in 10 mL volumetric flasks before use. Then fluorimetric measurements were carried out and the calibration graph was obtained from the fluorescent intensities of zirconium solutions.

Fluorescence measurements were recorded on a Varian Cary Eclipse Fluoresence Spectrophotometer, equipped with a xenon lamp source and 1.0 cm quartz cells and excitation and fluorescence emission wavelengths of 570 and 660 nm (658 nm in sol-gel matrix) respectively were employed. Slit widths of both monochromators were set at 10 nm.

2.9 Analysis of Soil Samples

Various soil samples both standard reference and collected from a place nearby Izmir were used to check the applicability of the proposed methods for preconcentration and determination of , Mn(II), Co(II), Ni(II), Cu(II), Cd(II), Zn(II), U(VI) and Th(IV) using the newly modified resins and also for fluorimetric determination of Zr(IV) using PAAP in water.

2.9.1 Standard Reference Materials (NIST SRM 2709, San Joaquin Soil and NIST SRM 2711, Montana Soil)

0.200 g of the sample was treated in a PTFE beaker with 15 mL of 48 % hydrofluoric acid, and the mixture was allowed to stay for about 1 h at room temperature. After heating the mixture in a sand bath (150^0C) until dryness and an aliquot of (15 mL) of the mixture of concentrated nitric and perchloric acids 3:1 (v/v) was added and heated again until dryness. The residue was then treated with 2.5 mL of concentrated hydrochloric acid, water (2 mL) and four drops of 30% hydrogen peroxide under gentle warming to achieve complete solubility (Boaventura, Hirson &

Santelli; 1995). The volume was made up to 50 mL with distilled water and this solution was used in the preconcentration step by applying column procedure given in Section 2.4.1 and was also used in the fluorimetric determination of zirconium by applying procedure in Section 2.8.1. The analysis of SRM samples was repeated as triplicate.

2.9.2 Soil Sample from Kula Region

0.25 g of the soil sample from Kula region a place nearby Izmir, was treated with 5.0 mL of HF and 1.0 mL of conc. H_2SO_4 at 150 ^{0}C on a hot plate. The process was repeated thrice. The residue is cooled and fused with 2.0 g of $KHSO_4$ at 800 ^{0}C in an electric Bunsen for 45 min. The melt is then cooled, dissolved in 50 mL of deionized water and diluted to 100 mL. The determination of zirconium was carried out using procedure described in Section 2.8.1.

CHAPTER THREE

RESULTS

3.1 Characterization Studies for Chelating Agents and Modified Resins

3.1.1. Infrared Spectrum

3.1.1.1 For PAAP and PAAP Modified Resin

The infrared spectrum of PAAP (Figure 3.1) was compared with that of THPP. PAAP has more absorption bands that belong to the carbonyl (C=O) stretching vibration at 1755 cm^{-1} and the C-O-C groups stretching vibration at 2875 cm^{-1} in the PAAP was absent in the THPP. The OH stretching vibration at 3315 cm^{-1} and 3280 cm^{-1} was observed sharply in the PAAP and THPP, respectively. The yield of PAAP was 81%.

The infrared spectrum of the modified resin (Figure 3.2) was compared with that of PAAP. The carbonyl (C=O) stretching vibration at 1755 cm^{-1} and 1783 cm^{-1} were observed sharply in the PAAP and PAAP modified resin, respectively. The OH stretching vibration at 3315 cm^{-1} in the PAAP was absent in the modified resin with PAAP. The stretching vibration belonging to C-O-C groups were at 2860 and 2875 cm^{-1} and the bending vibration belonging to C-O-C groups were at 1171, 1122 and 1160 cm^{-1} in the PAAP modified resin and PAAP. The C-Cl bond peak in the Merrifield resin at 750 cm^{-1} was absent in the modified resin. The above points evidenced the functionalization of resin with PAAP.

3.1.1.2 For calix[4]crown-4 Modified Resin

The infrared spectrum of the calix[4]crown-4 modified resin was compared with that of Merrifield resin. Comparing the IR spectra of Merrifield chloromethylated resin (Figure 3.3) and calix[4]crown-4 modified resin (Figure 3.4), the latter one

show additional peaks at 1755 cm^{-1} belong to carbonyl (C=O) stretching vibration and 1130 cm^{-1} corresponding to C-O-C groups bending vibration. The C-Cl bond peak in the Merrifield chloromethylated resin at 751 cm^{-1} was absent in the calix[4]crown-4 anchored resin. The above points evidenced the functionalization of resin with *p-tert*-butylcalix[4]arene-1,2-crown-4.

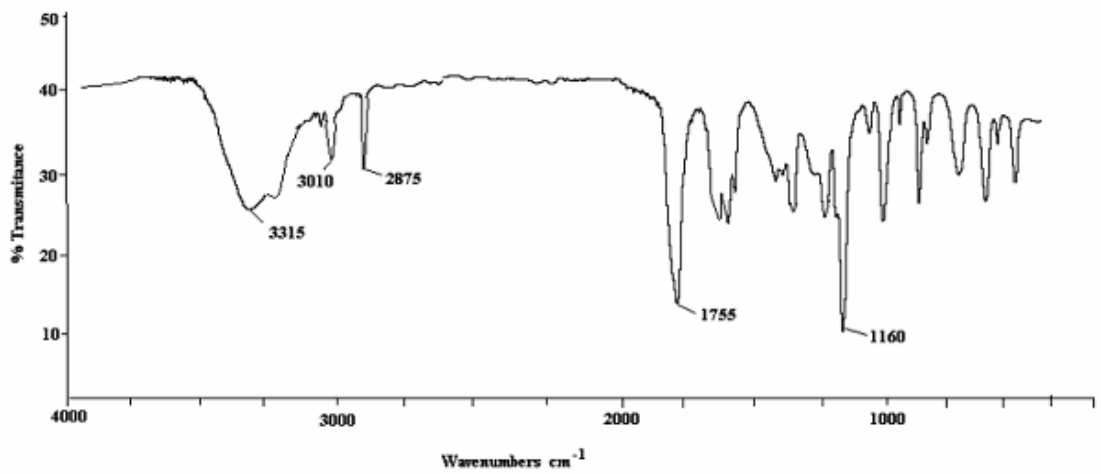


Figure 3.1 The infrared spectrum of PAAP

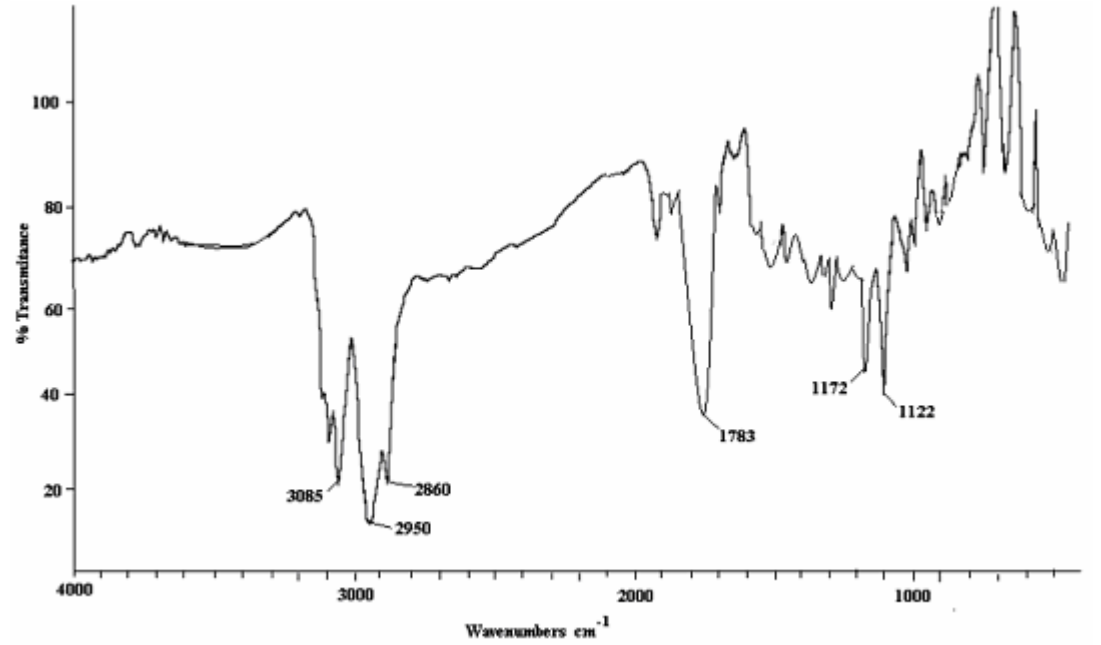


Figure 3.2 The infrared spectrum of PAAP modified resin

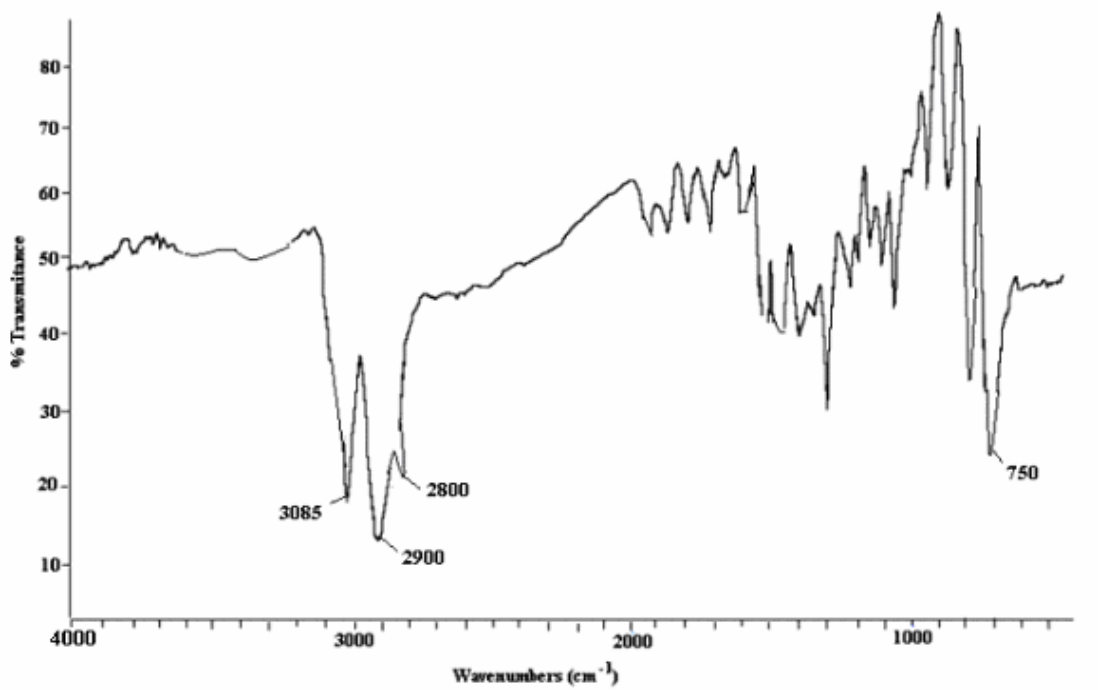


Figure 3.3 The infrared spectrum of Merrifield resin

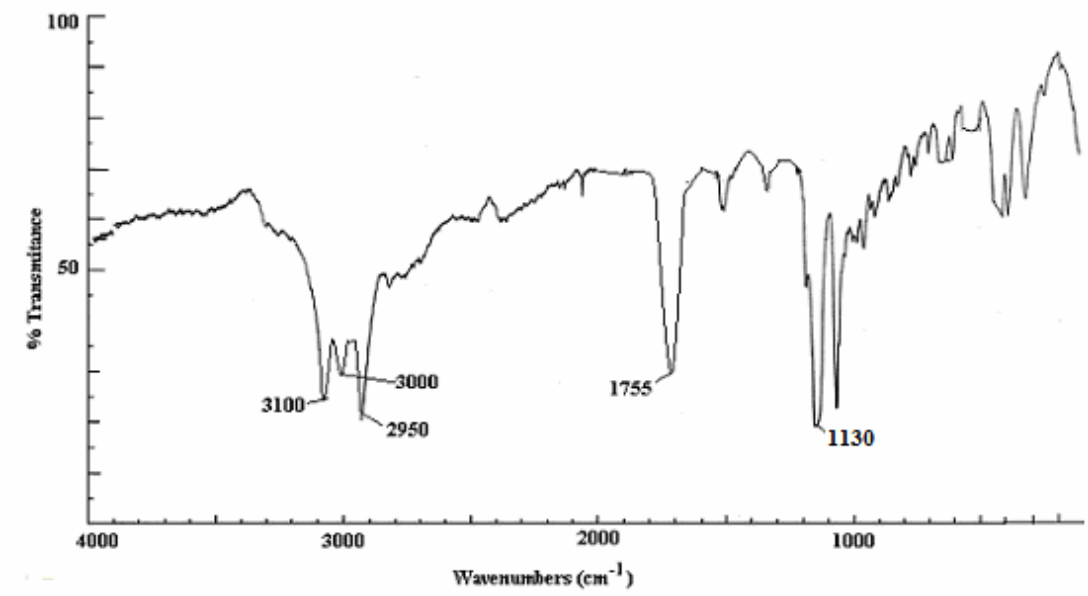


Figure 3.4 The infrared spectrum of the *p-tert*-butylcalix[4]arene-1,2-crown-4 modified resin

3.1.2 ^{1}H NMR Spectrum

3.1.2.1 For PAAP and PAAP Modified Resin

For the ^{1}H NMR spectrum of PAAP: (NMR) (DMSO, 400 MHz): 10.25 ppm (s, 4H), 7.23 ppm (s, 2H), 7.01-6.38 ppm (m, 24H), 4.55 ppm (s, 8H) (Figure 3.5). The ^{1}H NMR spectrum of THPP was given in Figure 3.6 to compare with ^{1}H NMR spectrum of PAAP.

For the ^{1}H NMR spectrum of the resin immobilized with PAAP: (NMR) ($CDCI_3$, 400 MHz): 8.33-8.01 ppm (m, bound porphyrin signals), 7.01, 6.50 ppm (m, polymer signals), 4.51 ppm (s, (bound polymer signals), 1.75-1.48 ppm (m, polymer signals) (Figure 3.7). The ^{1}H NMR spectrum of Merrifield resin was given in Figure 3.8 to compare with ^{1}H NMR spectrum of the modified resin.

In the ^{1}H NMR spectrum in the resin-PAAP, it was seen that the porphyrin signals were broadened due to the large molecular size of the polymer and that the intensities of porphyrin signals were lower than the intensities of polymer signals because of the lower amount of porphyrin in the resin.

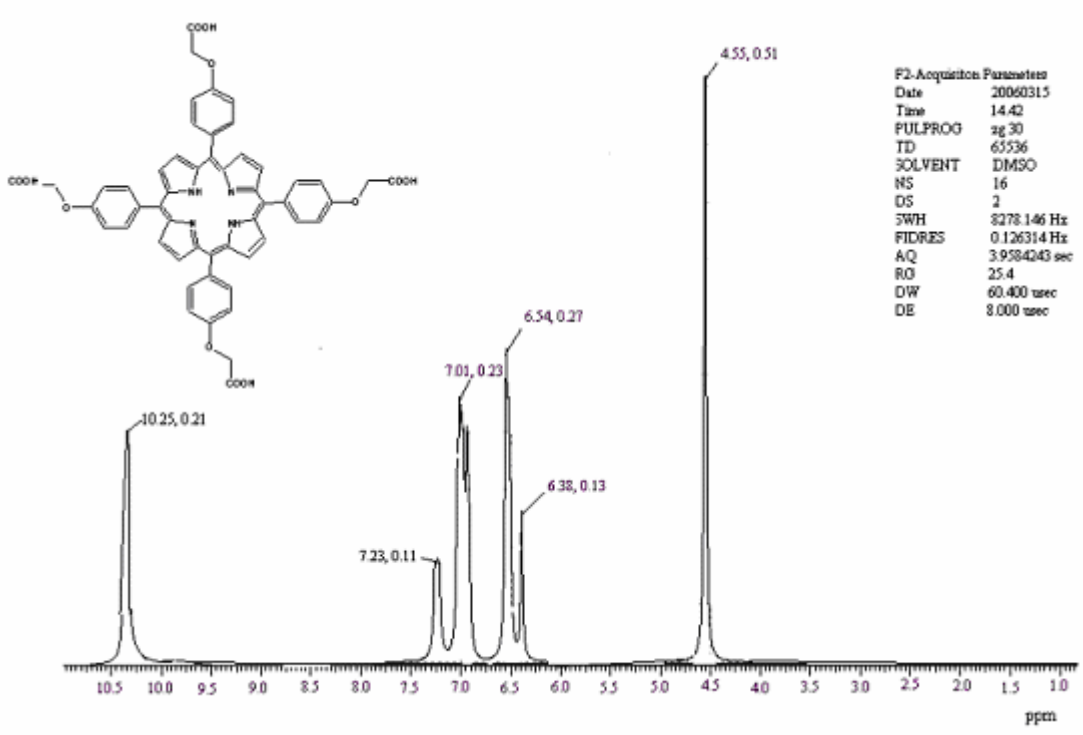


Figure 3.5 The ^{1}H NMR Spectrum of the PAAP

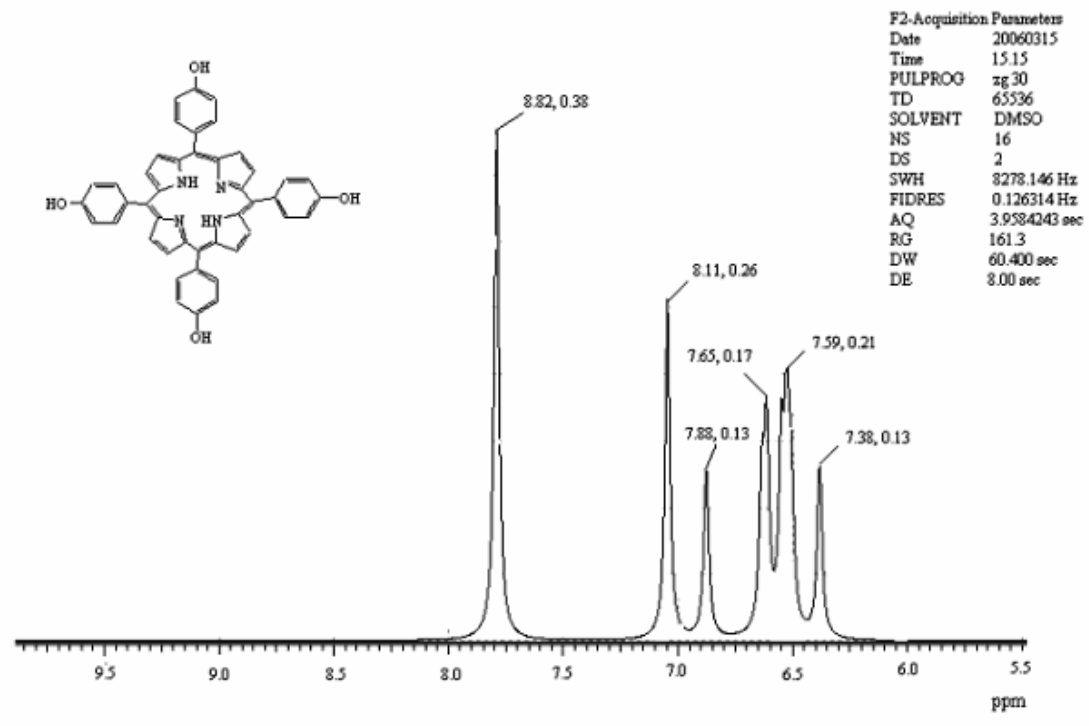


Figure 3.6 The ^{1}H NMR spectrum of the THPP

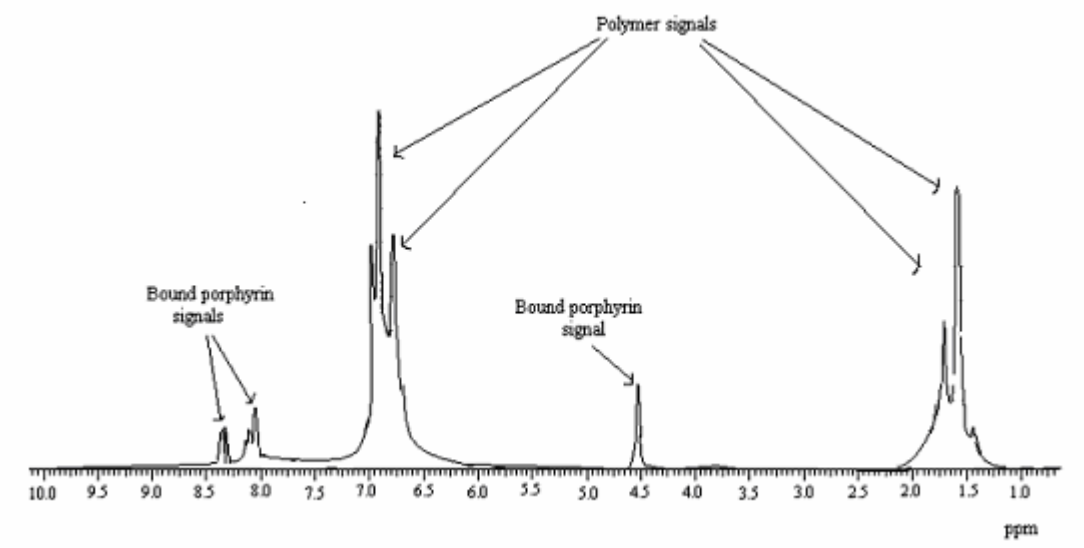


Figure 3.7 The ^{1}H NMR spectrum of the modified resin

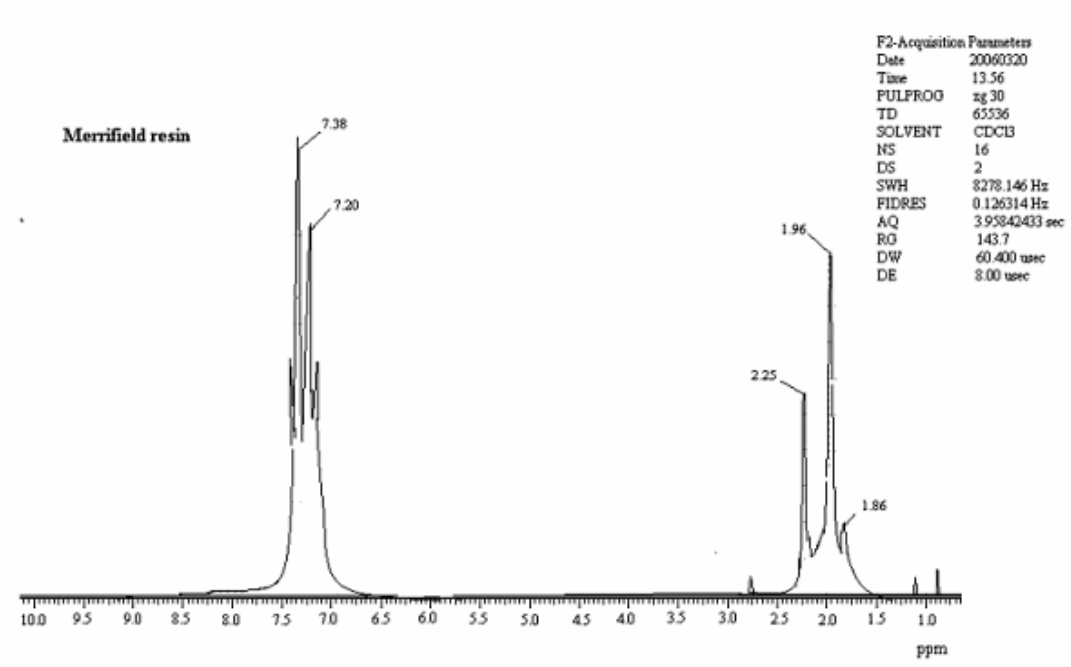


Figure 3.8 The ^{1}H NMR spectrum of the Merrifield resin

3.1.2.2 For calix[4]crown-4 and Modified Resin

The structure of 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26-dihydroxy-27,28-crown-4-calix[4]arene and *p-tert*-butylcalix[4]arene-1,2-crown-4 compound and the conformation of these compounds were characterized by ^{1}H NMR.

In spectrum of 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26-dihydroxy-27,28-crown-4-calix[4]arene: ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.81 (s, 2H, OH), 7.08 and 6.95 (2d, 4H, J= 1.9 Hz, ArH), 7.02 and 6.98 (2d, 4H, J= 1.7 Hz, ArH), 4.68 (d, 1H, J= 12.3 Hz, $ArCH_2Ar$ axial), 4.32 (d, 3H, J= 12.6 Hz, $ArCH_2Ar$ axial), 4.1-3.7 (m, 12H, CH_2-CH_2), 3.4-3.3 (m, 4H, $ArCH_2Ar$ equatorial), 1.21 and 1.14 (2s, 36H, $C(CH_3)_3$) (Arduini et al; 1997).

In addition to ^{1}HNMR spectrum of 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26-dihydroxy-27,28-crown-4-calix[4]arene, in the ^{1}HNMR spectrum of *p-tert*-butylcalix[4]arene-1,2-crown-4 compound was seen δ= 4.80 and 4.82 (s, 4H, O-CH_2-C=O) and δ= 10.50 (s, 2H, COOH).

Arduni et al. reported that 1,2-bridged calix[4]arenes can be obtained directly from calix[4]arenes in the cone or in the 1,2-alternate conformations depending on the base/solvent systems (NaH/DMF, tBuOK/toluene, tBuORb/tolune, tBuOCs/toluene). These authors concluded that the small and "hard" sodium ion in DMF probably directs the reaction to the cone isomer and on the contrary, the larger and "softer" potassium in low polarity solvent directs the synthesis to the 1,2-alternate conformation (Arduini et al; 1997). It was seen these compounds were in the cone conformation.

When ^{1}HNMR spectrum of Merrifield chloromethylated resin was compared to calix[4]crown-4 modified resin it was seen that the *p-tert*-butylcalix[4]arene-1,2-crown-4 compound signals were broadened due to the large molecular size of the polymer and that the intensities of this calix[4]crown-4 signals were lower than the

intensities of polymer signals because the amount of calix[4]crown-4 in the resin was lower.

3.1.3 Elemental Analysis

3.1.3.1 For PAAP Modified Resin

The elemental analysis of nitrogen in the modified resin was found to be 1.31 %. The calculated value of nitrogen in the modified resin was 1.35%, which shows the amount of PAAP bound on the resin was 27-28% by mass (0.242 mmol g^{-1}).

3.1.3.2 For calix[4]-crown-4 Modified Resin

The elemental analysis of the modified resin found to be C: 78.75%, H: 7.97%, O: 13.58% (calculated values: C: 77.27%, H: 7.58%, O: 15.15%), which shows the amount of calix[4]crown bound on the resin was 43% (0.489 mmol g^{-1}).

3.2 Preconcentration and Determination of Metal Ions with PAAP Modified Resin

3.2.1 Effect of pH

pH is the main parameter for the metal ion sorption onto the complexation-ligand modified resin. Mainly at low pH values, the metal ion-ligand complexes are unstable because of the protonation of some functional groups onto the complexation ligands. Also, at low pHs, removal of toxic metal ions from some aqueous medium is not feasible consuming high amount of acidic reagents.

A polypropylene column was packed with 0.100 g of the synthesized resin. A 50 mL solution containing 1.0 μg mL^{-1} of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), U(VI) or Th(IV) was passed with a flow rate of 1.0 mL min^{-1} in the pH interval of 3.0-9.0. The amount of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) left in the solution after sorption

were determined by FAAS and the amount of U(VI), Th(IV) left in the solution after sorption were determined by visible spectrophotometry.

The optimum pH for maximum sorption was found as 6.0 for Mn(II) by a sorption of 95%, as 6.0-7.0 for Co(II) by a sorption of 96%, as 8.0 for Ni(II) by a sorption of 100%, as 7.0-8.0 for Cu(II) by a sorption of 99%, as 6.0-7.0 for Zn(II) by a sorption of 100%, as 4.0-5.0 for U(VI) and Th(IV) by sorption of 85% and 80% respectively (Figure 3.9). The sorption experiments for each metal ion were done in triplicate.

It was seen that the maximum sorption of *d*-electron metals were carried at higher pH's compared to *f*-electron metals.

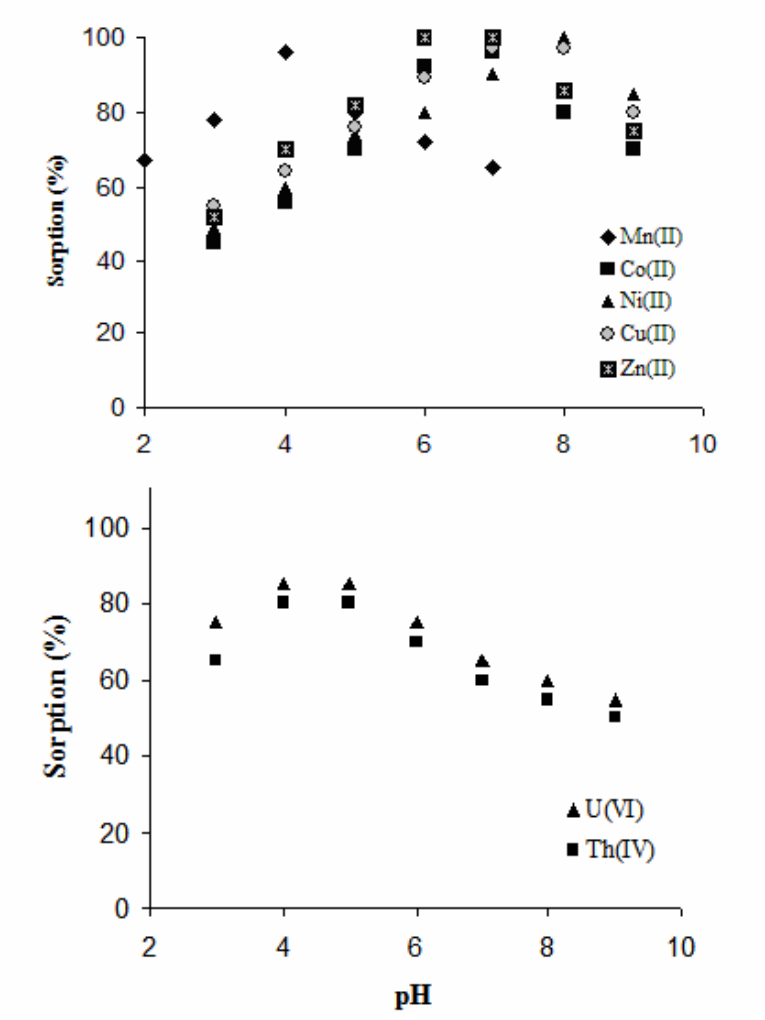


Figure 3.9 Metal sorption as a function of pH

3.2.2 *Effect of Flow Rate*

The dependence of the metal ion sorption and desorption at various flow rates was studied in the column procedure. A 25 mL solution containing 1.0 μg mL^{-1} of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), U(VI) or Th(IV) prepared at pH 6.0 for Mn(II), pH 7.0 for Co(II), pH 8.0 for Ni(II), pH 7.0 for Cu(II), pH 6.0 for Zn(II), pH 5.0 for U(VI) and Th(IV) were passed through the column. The optimal sorption flow rate was found to be 1.5 mL min^{-1} for Mn(II), Co(II) and Ni(II) and 1.0 mL min^{-1} for Cu(II) and Zn(II) and 2.0 mL min^{-1} for U(VI) and Th(IV) with the recoveries of 97-99% (Table 3.1).

It was observed that the flow rates required to elute *d*-electron metals was lower than flow rate of *f*-electron metals. It was found that the optimal desorption flow rate was same that of the optimal sorption flow rate.

3.2.3 Stripping of Metal Ions

In order to determine the stripping conditions of metal ions from the modified resin, a 50 mL sample solution containing 50 μg of metal ion was taken and the pH was adjusted to 6.0 for Mn(II), 7.0 for Co(II), 8.0 for Ni(II), 7.0 for Cu(II), 6.0 for Zn(II), 5.0 for U(VI) and Th(IV) and passed through the column at 1.5 mL min^{-1} flow rate for Mn(II), Co(II), Ni(II) and 1.0 mL min^{-1} flow rate for Cu(II), Zn(II) and 2.0 mL min^{-1} flow rate for U(VI), Th(IV). Then the metal ions were desorbed using 25 mL of 0.05-2.0 mol L^{-1} HCl and HNO_3 and determined by FAAS (Mn(II), Co(II), Cu(II), Ni(II) and Zn(II)) or visible spectrophotometry (U(VI) and Th(IV)).

The retained metal ions were desorbed quantitatively with 2.0 mL of 2.0 mol L^{-1} HNO_3 for *d*-electron metals and 2.0 mL of 0.1 mol L^{-1} HCl for Th(IV) and 2.0 mL of 0.25 mol L^{-1} HCl for U(VI) (Table 3.2).

Table 3.1 Effect of flow rate on the metal sorption on PAAP modified resin

Metal ion	Sorption (%)			
	Flow rate (mL min^{-1})			
	1.0	1.5	2.0	2.5
Mn(II)	80	94	85	70
Co(II)	85	93	87	68
Ni(II)	88	96	88	71
Cu(II)	98	90	78	70
Zn(II)	100	93	85	75
U(VI)	70	75	85	70
Th(IV)	65	75	80	70

Experimental conditions: 0.1 g modified resin, 1.0 μg mL^{-1} of 25 mL metal solution, optimum pH for each metal ion

Table 3.2 Results of stripping; HNO_3 and HCl as stripping solution for metal ions on the PAAP modified resin

Metal ion	Recovery (%)													
	with HNO_3 (mol L^{-1})							with HCl (mol L^{-1})						
	0.05	0.1	0.25	0.5	1.0	2.0	2.5	0.05	0.1	0.25	0.5	1.0	2.0	2.5
Mn(II)	75	78	81	83	90	99	88	60	65	70	75	82	85	80
Co(II)	70	73	75	78	89	98	85	63	65	68	70	74	80	78
Ni(II)	65	70	74	78	85	99	89	60	64	67	68	73	78	70
Cu(II)	75	78	83	85	92	99	90	65	68	70	72	78	86	77
Zn(II)	78	83	85	89	93	98	92	58	63	65	68	75	84	72
U(VI)	60	64	73	68	55	50	42	75	90	98	90	80	72	55
Th(IV)	65	75	70	62	52	45	40	85	99	90	80	73	65	50

Experimental conditions: $c_{metal\ ion}$ = 1.0 μg mL^{-1} at optimum pH; 50 mL; 0.1 g PAAP modified resin

3.2.4 Sorption Capacity

The sorption for each metal ion was investigated using column method: first 0.1 g of PAAP modified resin was packed in a column, then 25 mL of solution containing metal ion at concentrations in a range of 5.0-45 mg L^{-1} at pH 6.0 for Mn(II), pH 7.0 for Co(II), pH 8.0 for Ni(II), pH 7.0 for Cu(II), pH 6.0 for Zn(II), pH 5.0 for U(VI) and Th(IV) was passed through the column at 1.5 mL min^{-1} flow rate for Mn(II), Co(II) and Ni(II) and 1.0 mL min^{-1} flow rate for Cu(II) and Zn(II) and 2.0 mL min^{-1} flow rate for U(VI) and Th(IV). The retained metals were desorbed quantitatively with 2.0 mol L^{-1} HNO_3 for Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) and 0.1 mol L^{-1} HCI for Th(IV) and 0.25 mol L^{-1} HCI for U(VI). The Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) desorbed were determined by FAAS and U(VI) and Th(IV) desorbed were determined visible spectrophotometry.

The sorption capacity for each metal ion on the resin was calculated from the difference between the metal ion concentrations before and after desorption. The results were given in Table 3.3.

Table 3.3 Sorption capacity of the metal ions on the PAAP modified resin

Metal ion	Sorption Capacity	
	mg g^{-1} resin	mmol g^{-1} resin
Mn(II)	115	0.209
Co(II)	126	0.214
Ni(II)	130	0.222
Cu(II)	146	0.229
Zn(II)	152	0.233
U(VI)	257	0.108
Th(IV)	234	0.101

In aqueous solutions, the reaction of modified resin and metal ions is a kind of ionic exchange system. The sorption of metal ions from aqueous solutions onto resin and its complexation with the PAAP was occurred with the loss of two protons from the nitrogen atoms.

As seen from the Table 3.3., the sorption capacities of *d*-electron metals are higher than that of *f*-electron metals. In the *d*-electron metals, the dynamic capacity increase Mn(II), Co(II), Ni(II), Cu(II) and Zn(II), respectively because the atomic radii of metal ions decrease Mn(II), Co(II), Ni(II), Cu(II) and Zn(II), respectively. Consequently, when the atom radii of the metal ions decrease, the nitrogen atoms in porphyrin ring are bound more strongly with the metal ions.

3.2.5 Preconcentration Factor

The resin was studied for column concentration of the metal ions in terms of their preconcentration factor. Quantitative collection of metal ions was possible at volume not more than 500 mL (100 ng mL^{-1}) with recovery up to 96-99%.

The preconcentration factors were found to be 150 for Mn(II), 180 for Co(II), 200 for Ni(II), 125 for Cu(II), 215 for Zn(II), 115 for U(VI) and 114 for Th(IV) (Table 3.4).The preconcentration factors of *d*-electron metals are higher than *f*-electron metals and there is little difference between preconcentration factors of *f*-electron metals.

3.2.6 Distribution Coefficient

The distribution coefficients (K_D) of the metal ions were determined by using the batch equilibration method. In the equilibration experiment, 25 mL of solution containing 100 μg of Mn(II), Co(II), Ni(II),Cu(II), Zn(II), U(VI) and Th(IV) at their optimum pH was treated with 0.1 g of fresh resin. The solution was stirred for 1 h at room temperature, filtered to remove the resin, and the Mn(II), Ni(II), Co(II), Cu(II) and Zn(II) content in the filtrate was determined by FAAS and U(VI), Th(IV)

content in the filtrate was determined by visible spectrophotometry. The results were given in Table 3.5.

Table 3.4 Preconcentration factor for the metal ions

Metal ion	Concentration (ng mL^{-1})	Stripping solution (mL)	Recovery (%)	Preconcentration factor
Mn(II)	100	2.0	99	150
Co(II)	100	2.0	98	180
Ni(II)	100	2.0	98	200
Cu(II)	100	2.0	99	125
Zn(II)	100	2.0	98	215
U(VI)	100	2.0	97	115
Th(IV)	100	2.0	97	114

Exp. conditions: at optimum pH; at optimum flow rate; 0.1 g PAAP modified resin; stripping solution: 2.0 mol L^{-1} HNO_3 (*d*-electron metals), 0.25 mol L^{-1} HCI (U(VI)), 0.1 mol L^{-1} HCl (Th(IV))

Table 3.5 Distribution coefficients for the metal ions

Metal ion	Distribution coefficient
Mn(II)	3088
Co(II)	3781
Ni(II)	4308
Cu(II)	2684
Zn(II)	4557
U(VI)	2336
Th(IV)	2312

3.2.7 Effect of Electrolytes

The effect of various electrolytes like NaCI, KBr, $NaNO_3$, Na_2SO_4, Na_3PO_4, $CaSO_4$ and $MgSO_4$ on the sorption of metal ions on the modified resin was studied by column procedure. It was found that NaCl, KBr and $NaNO_3$ were tolerable in the 1.0-2.5 mol L^{-1} concentrations and Na_2SO_4, Na_3PO_4, $CaSO_4$ and $MgSO_4$ were tolerable 0.10-1.0 mol L^{-1} concentrations. The reported tolerance limit was defined as the ion concentration a relative error < ± 3% in the recovery of the metal ions. The results are given in Table 3.6.

Table 3.6 Tolerance limits of electrolytes on the sorption of metal ions on the modified resin

Metal ion	Electrolyte (mol L^{-1})						
(1μg mL^{-1})	NaCl	KBr	$NaNO_3$	Na_2SO_4	Na_3PO_4	$CaSO_4$	$MgSO_4$
Mn(II)	2.0	1.5	1.5	0.5	0.25	0.25	0.15
Co(II)	2.0	1.5	1.5	0.5	0.25	0.25	0.20
Ni(II)	2.5	2.0	2.0	1.0	0.5	0.5	0.25
Cu(II)	2.5	2.0	2.0	1.0	0.5	0.25	0.25
Zn(II)	2.5	2.5	2.0	1.0	0.5	0.25	0.20
U(VI)	1.5	1.0	1.0	0.25	0.10	0.15	0.10
Th(IV)	1.5	1.0	1.0	0.25	0.10	0.10	0.10

Experimental conditions; modified resin 0.1 g; volume of passed 50 mL, optimum pH for metal ions

3.2.8 Resin Stability

When the resin was used repeatedly as described before for enrichment and determination of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), U(VI) and Th(IV) ions (after these ions were desorbed from the resin each time with 2.0 mol L^{-1} HNO_3 or 0.25 mol L^{-1} HCl, the resin column was washed for neutrality with distilled water), their sorption efficiencies were still found higher than 94% (up to 15 times).

3.2.9 Analysis of Standard Reference Materials

The accuracy of the proposed method was checked by analyzing a NIST soil reference material (SRM 2709, San Joaquin Soil). The sample was mineralized by using the dissolution procedure described in Section 2.9.1 and was adjusted to preconcentration and determination by adopting the recommended procedure.

The results in Table 3.7 revealed that the present method agrees well with certified values. Thus, the SPE preconcentration method developed in the present work enables to use simple and low cost instruments to analyze soil and sediment samples containing trace and ultra race amounts of these metal ions.

Table 3.7 Analysis of the soil sample SRM 2709 by column preconcentration

Metal	Metal Amounts ($\mu g\ g^{-1}$)	
	Found[a]	Certified
Mn(II)	537 ± 14	538 ± 17[b]
Co(II)	12.6 ± 0.7	13.4 ± 0.7
Ni(II)	87 ± 5	88 ± 5[b]
Cu(II)	33.8 ± 0.7	34.6 ± 0.7[b]
Zn(II)	105 ± 3	106 ± 3[b]
U(VI)	2.4 ± 0.1	3[c]
Th(IV)	10.0 ± 0. 5	11[c]

[a] Average of three determinations, [b] Certified values are weighed means of result from two or more independent analytical methods, [c] Noncertified values are weighed means of result one analytical method

3.2.10 Analytical Figures of Merits

The precision (relative standard deviation, RSD%) of the method was at a concentration of 1.0 μg mL^{-1} for a series of ten replicates; 2.12% for Mn(II), 2.04% for Co(II), 1.99% for Ni(II), 2.00% for Cu(II), 2.13% for Zn(II), 2.15% for U(VI) and 2.18% for Th(IV) (Table 3.8).

The detection limit based on three times the standard deviation of the blank using ten replicates was found to be 1.78 μg L^{-1} for Mn(II), 1.91 μg L^{-1} for Co(II), 2.18 μg L^{-1} for Ni(II), 1.60 μg L^{-1} for Cu(II), 2.32 μg L^{-1} Zn(II), 1.33 μg L^{-1} for U(VI), 1.19 μg L^{-1} Th(IV) (Table 3.8).

Table 3.8 RSD%, LOD and LOQ for metal ions

Metal ion	RSD (%)	LOD (μg L^{-1})	LOQ (μg L^{-1})
Mn(II)	2.12	1.78	4.93
Co(II)	2.04	1.91	5.36
Ni(II)	1.99	2.18	6.25
Cu(II)	2.00	1.60	4.78
Zn(II)	2.13	2.32	6.73
U(VI)	2.15	1.33	4.45
Th(IV)	2.18	1.19	3.87

3.3 Preconcentration and Determination of Metal Ions with calix[4]crown-4 Modified Resin

3.3.1 Effect of pH

The optimum pH of metal ion uptake was determined using the column procedure. 0.25 g calix[4]crown-4 modified resin (due to that low sorption amounts were obtained with 0.1 g modified resin) was packed in a polypropylene column. A 50 mL solution containing 1.0 μg mL^{-1} of Cu(II), Ni(II), Co(II), Cd(II) or Zn(II) was passed with an 1.0 mL min^{-1} flow rate at pHs varied from 3.0 to 8.0 (Figure 3.10). The metal amount left in the solution after sorption was determined by FAAS.

The optimum pH for maximum sorption was found to be between pH 6.0-7.0 for Cu(II) by a sorption of 100%, pH 6.0 for Cd(II) by a sorption of 100% , pH 5.0 for Co(II) by a sorption of 100%, pH 4.0-4.5 for Ni(II) by a sorption of 100% and pH 4.5 for Zn(II) by sorption of a 100%. The sorption experiments were done in triplicate.

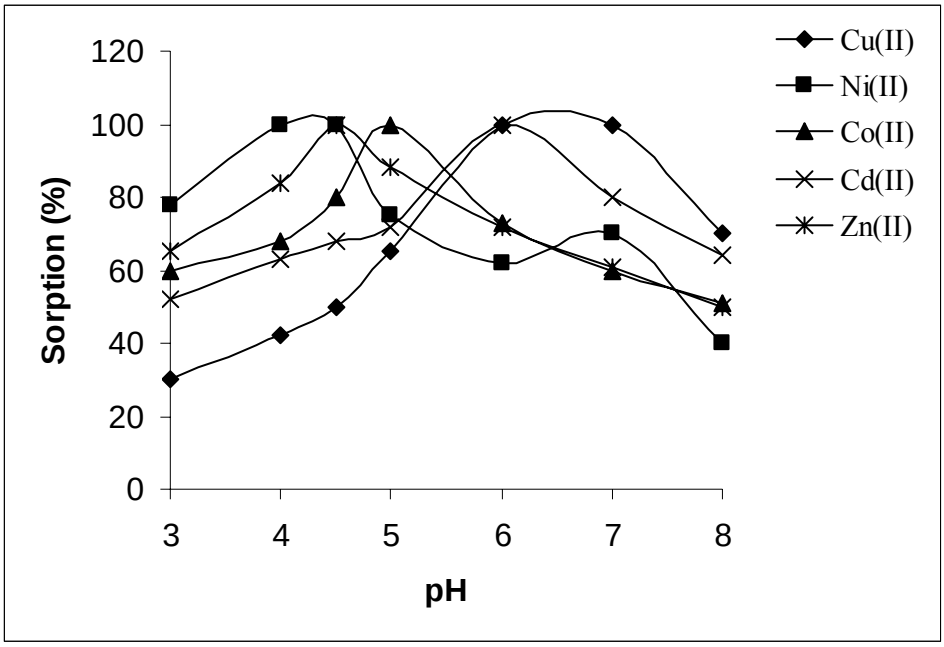


Figure 3.10 Effect of pH on the sorption of Cu(II), Co(II), Cd(II), Ni(II) and Zn(II)

3.3.2 Effect of Flow Rate

In the column procedure, the effect of flow rate on sorption and desorption of the metal ions was investigated. A 50 mL solution containing 1.0 μg mL^{-1} of Cu(II), Ni(II), Co(II), Cd(II) or Zn(II) was passed at pH 6.0 for Cu(II), pH 6.0 for Cd(II), pH for 5.0 for Co(II), pH 4.0 for Ni(II), pH for 4.5 for Zn(II).

The studies show that the flow rate influences the sorption and desorption of metal ions. The metal ions can be sorbed quantitatively by the resin at a flow rate of 1.0-3.0 mL min^{-1}. The sorption of metal ions above 3.0 mL min^{-1} was ≤ 95 % (Table 3.9). The reason might be insufficient time for the binding of metal ion onto resin. For desorption, these metal ions can be desorbed quantitatively by the resin at a flow rate 1.5-2.5 mL min^{-1}. Above 2.5 mL min^{-1}, the recovery for metal ions was < 95 % (Table 3.9).

3.3.3 Stripping of Metal Ions

The effect of eluents was studied using HCl and HNO_3 (0.25-2.0 mol L^{-1}). A 50 mL sample solution containing 50 μg of metal ion was taken and the pH was adjusted to 6.0 for Cu(II) and Cd(II), pH 5.0 for Co(II), pH 4.0 for Ni(II), pH 4.5 for Zn(II) and passed through the column at 1.0 mL min^{-1} flow rate for each metal ion. Then the metal ions were desorbed with HCl and HNO_3 in 0.25-2.0 mol L^{-1} concentrations at 1.5 mL min^{-1} flow rate and determined by FAAS.

The recovery percentages with nitric acid solutions were found around 90% (Table 3.10). However, complete recovery was accomplished using HCl solutions (4 mL of 0.5 mol L^{-1} HCI for Cu(II), Co(II) and Ni(II), 4 mL of 1.0 mol L^{-1} HCI for Cd(II) and Zn(II) (Table 3.10).

Table 3.9 Effect of flow rate on the metal sorption on calix[4]crown-4 modified resin

Metal ion	Sorption (%)				Desorption (%)				
	Flow rate (mL min^{-1})				Flow rate (mL min^{-1})				
	1.0	2.0	3.0	4.0	1.0	1.5	2.0	2.5	3.0
Cu(II)	100	100	100	95	90	100	100	100	94
Ni(II)	100	100	100	90	95	100	100	100	94
Co(II)	100	100	100	95	90	100	100	100	92
Cd(II)	100	100	100	93	92	100	100	100	90
Zn(II)	100	100	100	92	92	100	100	100	90

Exp. conditions: 0.25 g modified resin, 1.0 µg mL^{-1} 50 mL metal solution, optimum pH for each metal ion

Table 3.10 Results of stripping; HNO_3 and HCl as stripping solution for metal ions on the calix[4]crown-4 modified resin

Metal ion	Recovery (%)													
	with HNO_3 (mol L^{-1})							with HCl (mol L^{-1})						
	0.25	0.5	0.75	1.0	1.25	1.5	2.0	0.25	0.5	0.75	1.0	1.25	1.5	2.0
Cu(II)	82	90	85	76	70	65	58	92	100	96	92	88	84	76
Ni(II)	80	89	83	75	68	62	55	94	100	95	90	87	83	80
Co(II)	82	88	85	79	71	66	60	85	100	90	84	79	75	71
Cd(II)	72	78	85	90	84	78	72	78	85	92	100	95	89	80
Zn(II)	68	75	82	91	85	78	73	81	89	93	100	94	88	75

Experimental conditions: 0.25 g modified resin, 1.0 µg mL^{-1} 50 mL metal solution, optimum pH for each metal ion

3.3.4 Sorption Capacity

In order to show the applicability of calix[4]crown-4 chemically bound on the resin for preconcentration of trace metal ion, the sorption for each metal ion was investigated using column method: first 0.25 g of calix[4]crown-4 anchored resin was packed in a column, then 10 mL of solution containing metal ion at concentrations in a range of 10-65 mg L^{-1} at pH 6.0 for Cu(II), pH 6.0 for Cd(II), pH 5.0 for Co(II), pH 4.0 for Ni(II) and pH 4.5 for Zn(II) was passed through at 1.0 mL min^{-1} flow rate for each metal ion. Then Cu(II), Co(II) and Ni(II) were desorbed with 0.5 mol L^{-1} HCI and Cd(II) and Zn(II) were desorbed with 1.0 mol L^{-1} HCI at 1.5 mL min^{-1} flow rate and determined by FAAS.

The sorption capacity for each metal ion on the resin was calculated from the difference between the metal ion concentrations before and after desorption. The results were shown in Table 3.11.

Table 3.11 Total sorption capacity of the metal ions on the calix[4]crown-4 modified resin

Metal ion	Sorption Capacity	
	mg g^{-1} resin	mmol g^{-1} resin
Cu(II)	137	0.215
Cd(II)	230	0.205
Co(II)	110	0.186
Ni(II)	102	0.173
Zn(II)	97	0.149

3.3.5 Preconcentration Factor

The resin was studied for column concentration of metal ions in terms of their preconcentration factor. Quantitative collection of metal ions was possible at volume not more than 500 mL (100 ng mL^{-1}) with recovery up to 97-99%.

The preconcentration factors for Cu(II), Cd(II), Co(II), Ni(II) and Zn(II) were to be 208, 199, 168, 125 and 119 respectively (Table 3.12).

Table 3.12 Preconcentration factor for the metal ions

Metal ion	Concentration (ng mL^{-1})	Stripping solution (mL)	Recovery (%)	Preconcentration factor
Cu(II)	100	4.0	97	208
Cd(II)	100	4.0	99	199
Co(II)	100	4.0	98	168
Ni(II)	100	4.0	98	125
Zn(II)	100	4.0	98	119

Exp. conditions: at optimum pH; at optimum flow rate; 0.1 g modified resin; stripping solution: 0.5 mol L^{-1} HCI for Cu(II), Co(II) and Ni(II) and 1.0 mol L^{-1} HCI for Cd(II) and Zn(II).

3.3.6 Distribution Coefficient

The distribution coefficients (K_D) of the metal ions were determined by using the batch equilibration method. In the equilibration experiment, 10 mL of solution containing not more than 150 μg of the metal ion in question was treated with 0.25 g of fresh resin at pH 6.0 for Cu(II), pH 6.0 for Cd(II), pH 5.0 for Co(II), pH 4.0 for Ni(II) and pH 4.5 for Zn(II) and then stirred for 1 h at room temperature. The solution was filtered by 0.5 mol L^{-1} HCI for Cu(II), Co(II) and Ni(II) and 1.0 mol L^{-1} HCI for Cd(II) and Zn(II) to remove the resin and the metal ion content of the filtrate was determined by FAAS. The results were given in Table 3.13.

Table 3.13 The disribution coefficients for the metal ions

Metal ion	Distribution coefficient
Cu(II)	4287
Cd(II)	4102
Co(II)	3255
Ni(II)	2545
Zn(II)	2425

3.3.7 Effect of Electrolytes

The effect of NaCl, KBr, Kl, $NaNO_3$, Na_3PO_4, Na_2SO_4, $CaSO_4$ and $MgSO_4$ on the sorption of all the metal ions was studied by column procedure. The tolerance limits for the investigated electrolytes were given in Table 3.14. The reported tolerance limit is defined as the electrolyte ion concentration causing an error of ± 3-4% in the recovery of metal ions.

Table 3.14 Tolerance limits of electrolytes on the sorption of metal ions on the resin

Metal ion	Electrolytes or metal ions (mol L^{-1})							
	NaCl	KBr	Kl	$NaNO_3$	Na_3PO_4	Na_2SO_4	$CaSO_4$	$MgSO_4$
Cu(II)	0.25	0.25	0.15	0.25	0.10	0.25	0.25	0.25
Cd(II)	0.25	0.25	0.15	0.15	0.10	0.15	0.25	0.15
Co(II)	0.15	0.25	0.25	0.05	0.05	0.15	0.15	0.25
Ni(II)	0.05	0.15	0.25	0.15	0.05	0.05	0.15	0.15
Zn(II)	0.15	0.15	0.15	0.15	0.05	0.05	0.15	0.15

Experimental conditions: resin, 0.25 g; volume of solution passed, 50 mL, 1.0 μg mL^{-1}, optimum pH for metal ions

3.3.8 Resin Stability

To test the resin stability, it was subjected to several loading and elution batch operations. The conditions employed for the study were: 0.25 g of resin beads were stirred with 50 mL metal solution containing 2 μg mL^{-1} of metal ion for 24 h at room temperature. The elution operations were carried out by shaking the resin with 25 mL of suitable eluent for 4 h to ensure complete equilibration. The resin shows good stability up to 12 repeated cycles, after which it shows 2-3% decrease in efficiency.

3.3.9 Analysis of Standard Reference Materials

The accuracy of the developed preconcentration procedure was tested by analyzing standard reference materials (NIST SRM 2709, San Joaquin Soil and NIST SRM 2711, Montana Soil). The samples were mineralized by using the dissolution procedure described in Section 2.9.1 and was subjected to preconcentration and determination by adopting the recommended procedure. The metal contents established by the present procedure agree well with certified values (Table 3.15).

Table 3.15 Metal determination in reference material (SRM 2709, San Joaquin Soil and SRM 2711, Montana Soil) using proposed method

Metal	Metal Amounts (μg g^{-1})			
	SRM 2709		SRM 2711	
	Found[a]	Certified	Found[a]	Certified
Cu(II)	33.3 ± 0.6	34.6 ± 0.7	112 ± 2	114 ± 2
Cd(II)	0.37 ± 0.01	0.38 ± 0.01	40.72 ± 0.30	41.70 ± 0.25
Co(II)	12.9 ± 0.7	13.4 ± 0.7	9.6 ± 0.5	10[b]
Ni(II)	87 ± 4	88 ± 5	19.8 ± 1.2	20.3 ± 1.1
Zn(II)	104 ± 3	106 ± 3	348.2 ± 4.6	350.4 ± 4.8

[a] Average of three determinations

[b] Noncertified values (are weighed means of result one analytical method)

3.3.10 Analytical Figures of Merit

The precision (relative standard deviation, RSD%) of the method was at a concentration of 1.0 μg mL^{-1} for a series of ten replicates; 1.95% for Cu(II), 1.98% for Cd(II), 1.98% for Co(II), 1.94% for Ni(II) and 2.05% for Zn(II).

The limit of detection (LOD) was defined as that analyte concentration giving a signal equal to three times the standard deviation of blank signal. LOD was found to be 1.10, 1.25, 1.83, 1.68, 2.01 μg L^{-1} for Cu(II), Cd(II), Co(II), Ni(II) and Zn(II) in the given order (Table 3.16).

Table 3.16 RSD%, LOD and LOQ for metal ions

Metal ion	RSD (%)	LOD (μg L^{-1})	LOQ (μg L^{-1})
Cu(II)	1.95	1.10	3.34
Cd(II)	1.98	1.25	4.06
Co(II)	1.98	1.83	5.78
Ni(II)	1.94	1.68	5.16
Zn(II)	2.05	2.01	5.79

3.4 Fluorimetric Determination for Zirconium

3.4.1 PAAP in Aqueous Solution

3.4.1.1 Excitation and Emission Spectra of PAAP

In order to determine the optimum wavelength, the excitation and the emission spectra of PAAP in THF were taken and were shown in Figure 3.11 and Figure 3.12. Its maximum absorption is observed at 579 nm. The PAAP emits a strong fluorescence with a maximum at 660 nm for an excitation at 570 nm.

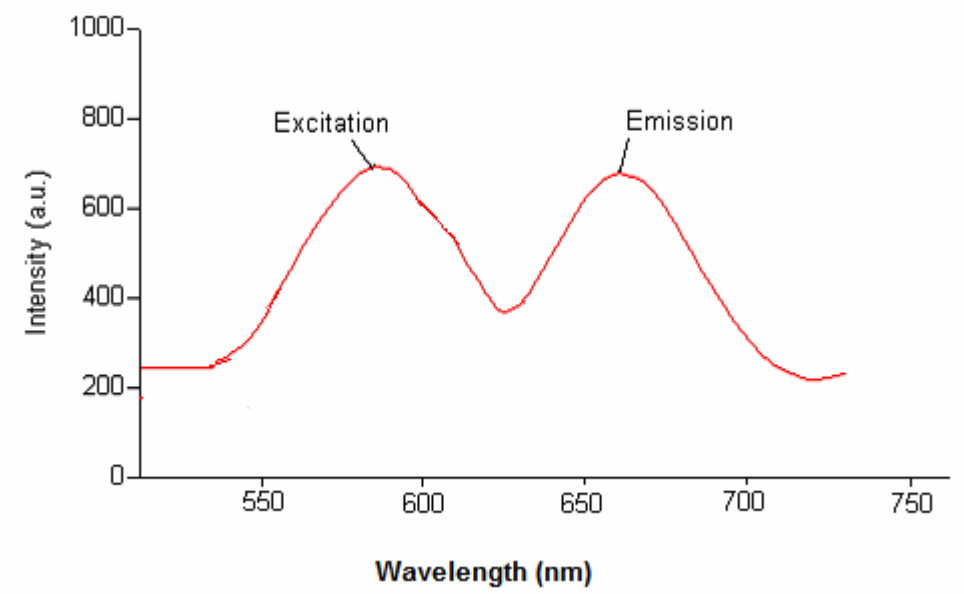


Figure 3.11 The excitation and emission spectra of $2x10^{-6}$ mol L^{-1} PAAP in THF. λ_{ex}=570 nm, λ_{em}=660 nm, slit width=10 nm

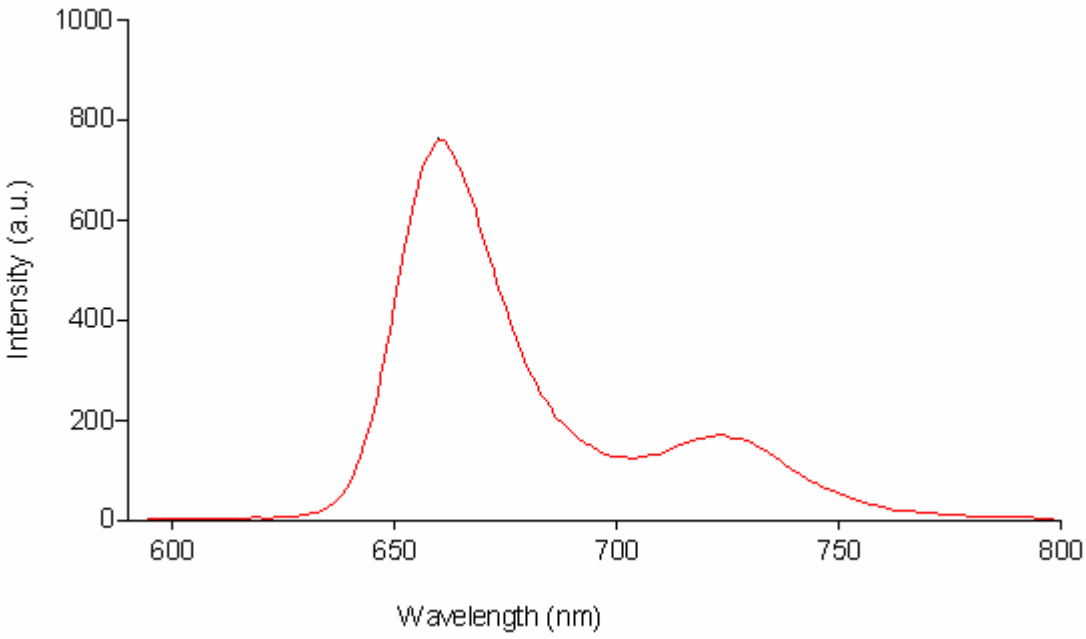


Figure 3.12 The emission spectra of $2x10^{-6}$ mol L^{-1} PAAP in THF. λ_{em}=660 nm, slit width =10 nm

3.4.1.2 Effect of pH

The fluorescence intensity of PAAP was obtained by adjusting the solution pH with hydrochloric acid (0.01-0.1 mol L^{-1}) and sodium hydroxide (0.01-0.1 mol L^{-1}) and fixing the Zr(IV) concentration at 5.0 µg mL^{-1}. In the alkaline condition, the high

concentrations of Zr(IV) might cause precipitation of ZrO_2. To find the best pH for Zr-PAAP complex formation was studied between pH 3.0-9.0. It can be seen that, in the lower pH values (3.0-4.0), the fluorescence intensity of PAAP decreased with decreasing pH (Figure 3.13). This phenomenon might be caused by the protonation of the nitrogen atom in PAAP in acidic medium which decreased the mobility of its π-electrons. From Figure 3.14 and 3.15 one can see that in the pH from 5.0 to 9.0, acidity does not affect the fluoresence intensity so determination of Zr(IV) with the proposed method. The formation of complex of PAAP with zirconium ion caused static fluorescence quenching in the pH range 5.0-9.0. It could be explained that, zirconium ion had effect of invertible charge transfer or electron spin-orbit coupling, which accelerated the singlet-triplet transition and internal conversion deactivation of excited PAAP. The fluorescence intensity of PAAP was changed from 695 to 425 a.u. at pH 5.0-7.0 within 20 min and at pH 8.0-9.0 within 55 min. In subsequent experiments, a pH 6.0 NaOAc-HOAc buffer solution was used.

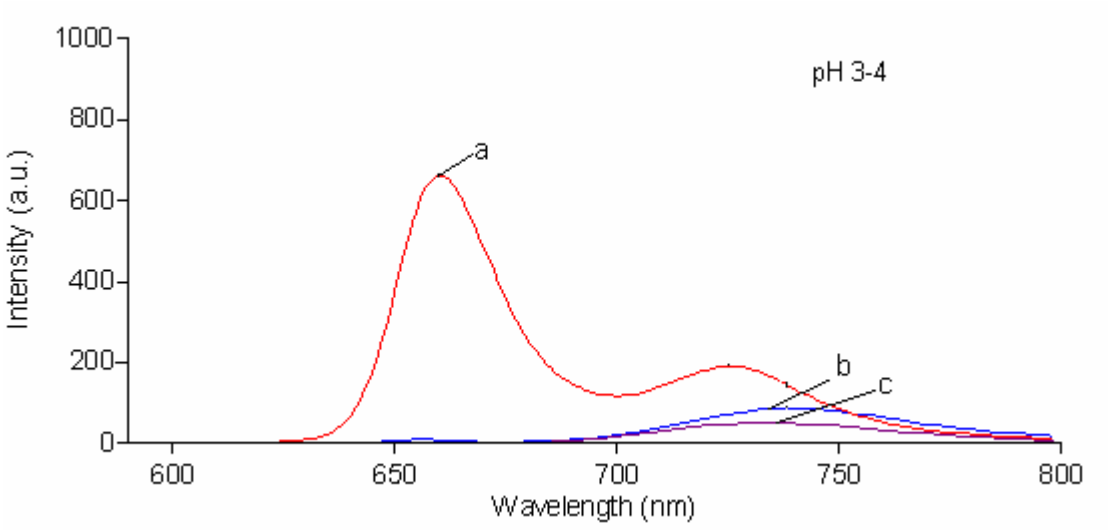


Figure 3.13 Fluorescence spectra of PAAP and its complex with 5.0 μg mL^{-1} zirconium at different times. λ_{ex}=570 nm, λ_{em}=660 nm. a) PAAP in THF, Zr-PAAP complex: b) 5.0 c) 10 min.

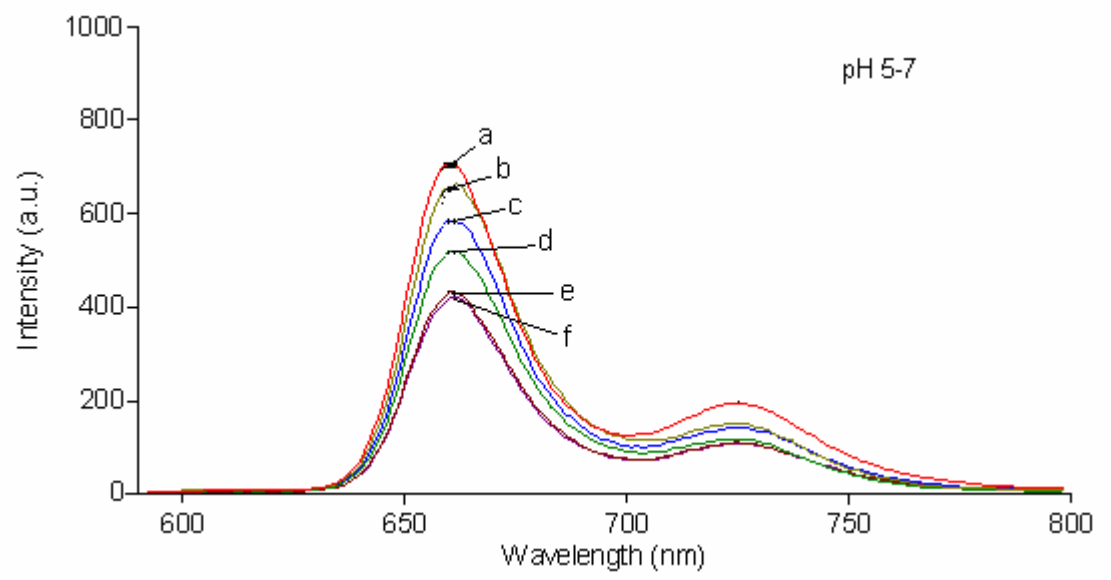


Figure 3.14 Fluorescence spectra of PAAP formed complex with 5.0 µg mL^{-1} zirconium at different times. λ_{ex}=570 nm, λ_{em}=660 nm. a) PAAP in THF, Zr-PAAP complex: b) 5.0, c) 10, d) 15, e) 20, f) 30 min.

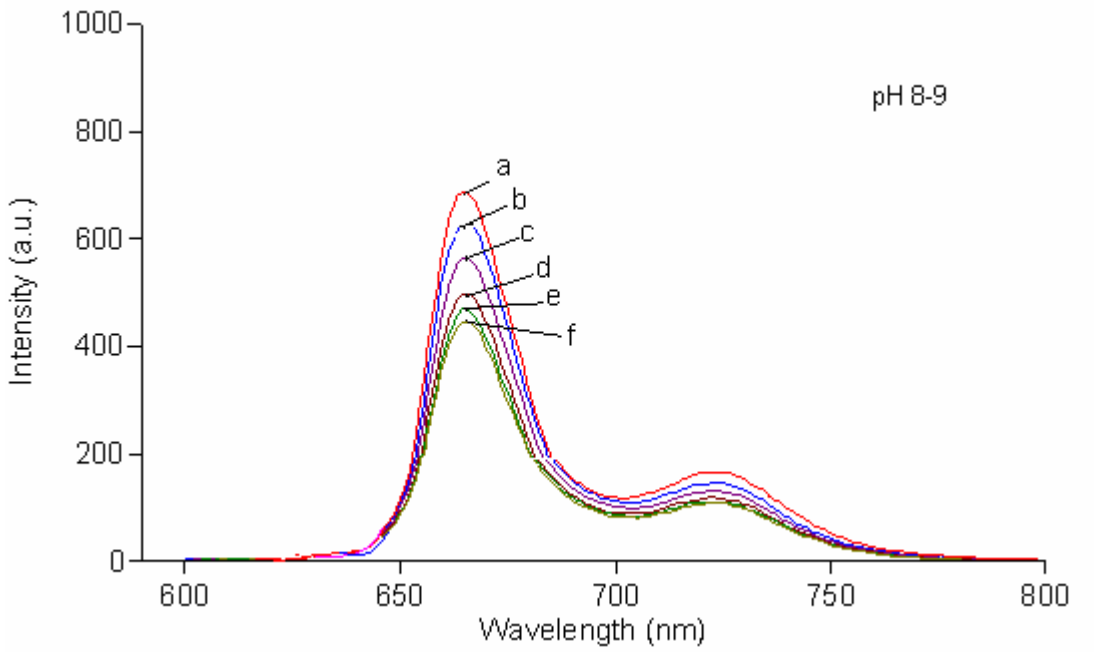


Figure 3.15 Fluorescence spectra of PAAP formed complex with 5.0 µg mL^{-1} zirconium at different times. λ_{ex}=570 nm, λ_{em}=660 nm. a) PAAP in THF, Zr-PAAP complex: b) 15, c) 30, d) 45, e) 50, f) 55 min.

3.4.1.3 Calibration Graph

Calibration graph was obtained by PAAP in aqueous solution using different zirconium concentrations (Figure 3.16). The fluorescence intensity was linear for 2.0-20 μg mL^{-1} of zirconium at 660 nm. The stability time of complex at zirconium concentrations lower than 5.0 μg mL^{-1} was 35 min., at the concentrations higher than 5.0 μg mL^{-1}, the stability time was 20 min. The calibration graph obtained was given in Figure 3.17. The regression equation for the system is y= -22.624x + 632.84 and correlation coefficient was obtained as R^2 = 0.9918.

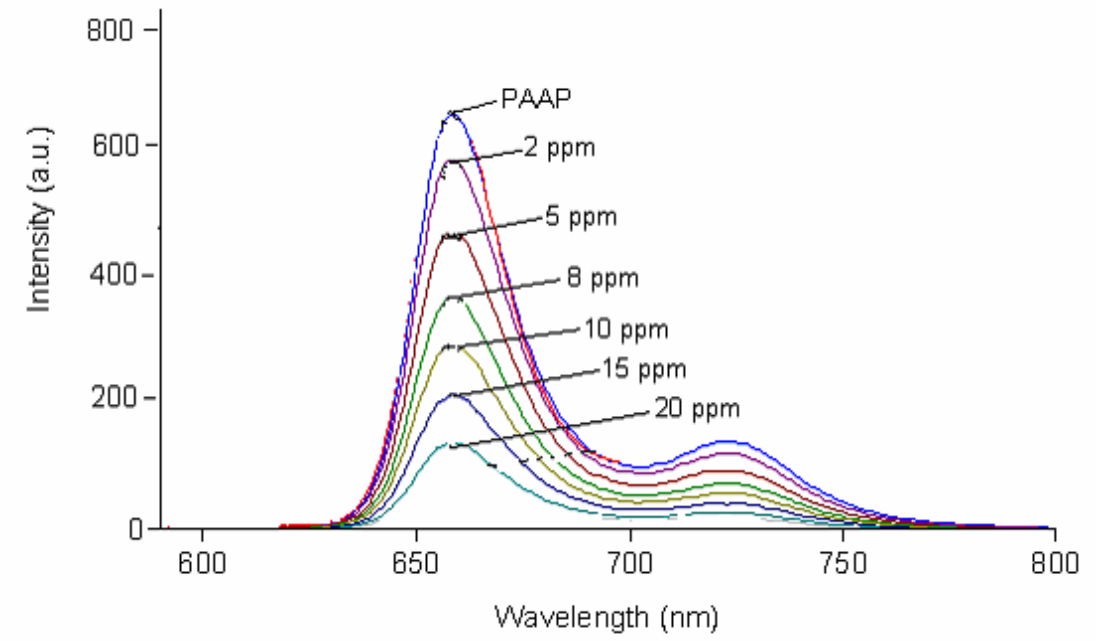


Figure 3.16 Fluorescence spectra of PAAP formed complex with zirconium at different concentrations. λ_{ex}=570 nm, λ_{em}=660 nm. , pH=6.0

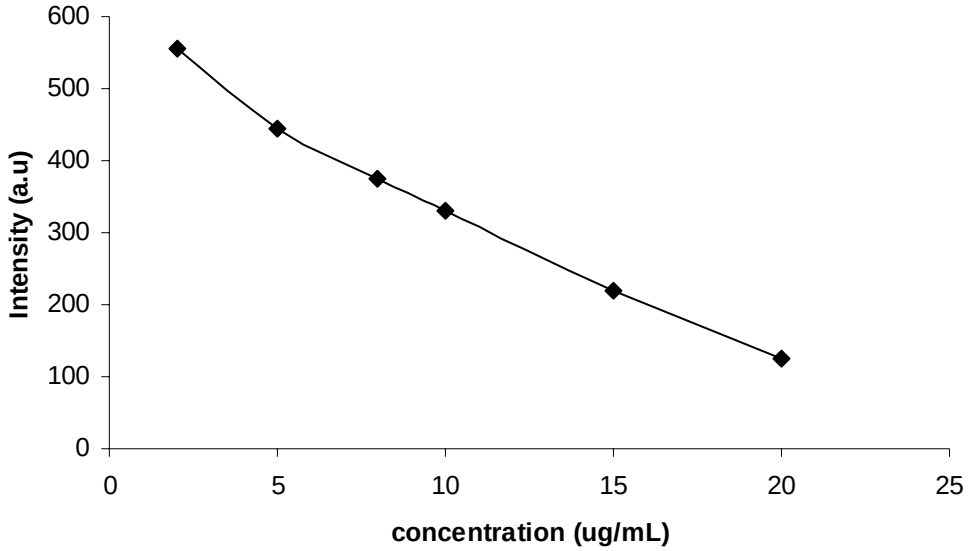


Figure 3.17 Calibration graph for the determination of zirconium (c_{Zr}: 2.0-20 μg mL^{-1})

3.4.2 PAAP in Sol-Gel Matrix

3.4.2.1 Emission Spectra of PAAP in Sol-Gel Matrix

Figure 3.18 shows the emission spectra of PAAP in sol-gel matrix. As can seen this reagent has also strong fluorescence with emission wavelengths of 658 nm in sol-gel matrix. But in sol-gel matrix, blue shift was observed in the maximum emission wavelength of PAAP (660 → 658 nm). When the fluorescence intensity of PAAP in aqueous solution and sol-gel matrix was compared it was seen that the fluorescence intensity of PAAP in sol-gel matrix was higher than of in aqueous solution.

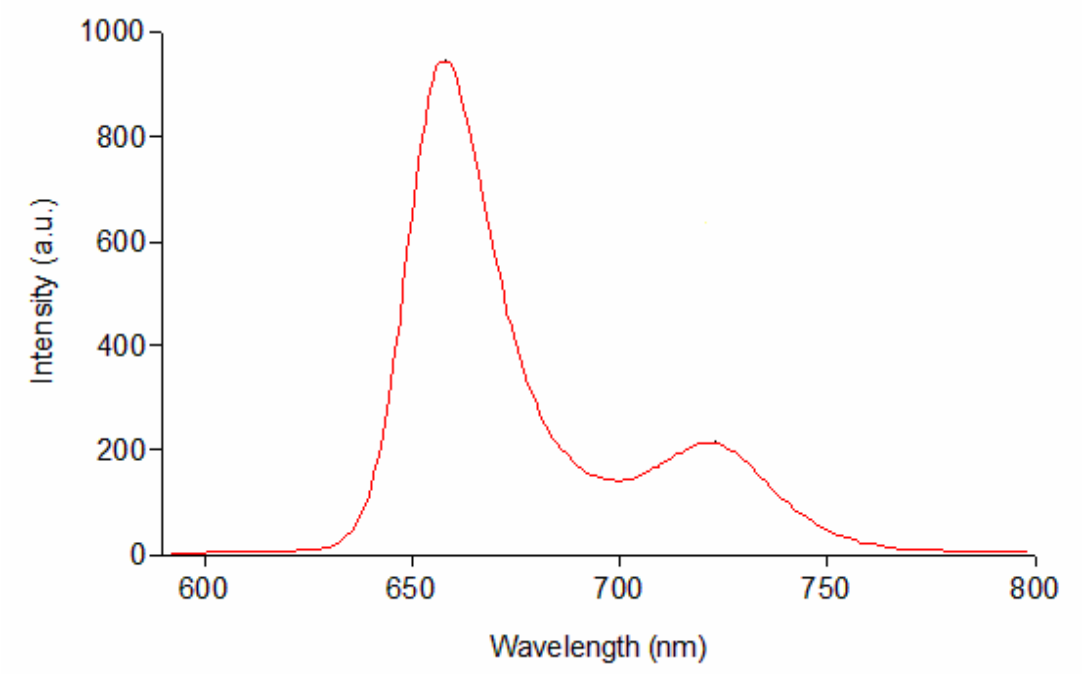


Figure 3.18 The emission spectra of $2x10^{-6}$ mol L^{-1} PAAP in sol-gel matrix. λ_{ex}=570 nm, λ_{em}=658 nm, slit width =10 nm

3.4.2.2 Effect of pH

It was also studied effect of pH within 3.0-9.0 in sol-gel matrix. The formation of complex of PAAP with zirconium ion caused static fluorescence quenching in PAAP in sol-gel matrix. The effects of pH for PAAP in sol-gel matrix were same with the PAAP in aqueous solution. Only the difference was seen in stability time of complex formation and intensity strength of PAAP. The immobilization of PAAP solution in the solid matrix may reduce intramoleculer motions and rearrangements fluorescence intensity was higher. The fluorescence intensity of PAAP was changed from 960 to 775 a.u at pH 5.0-7.0 within 40 min (Figure 3.19) and at pH 8.0-9.0 within 75 min.

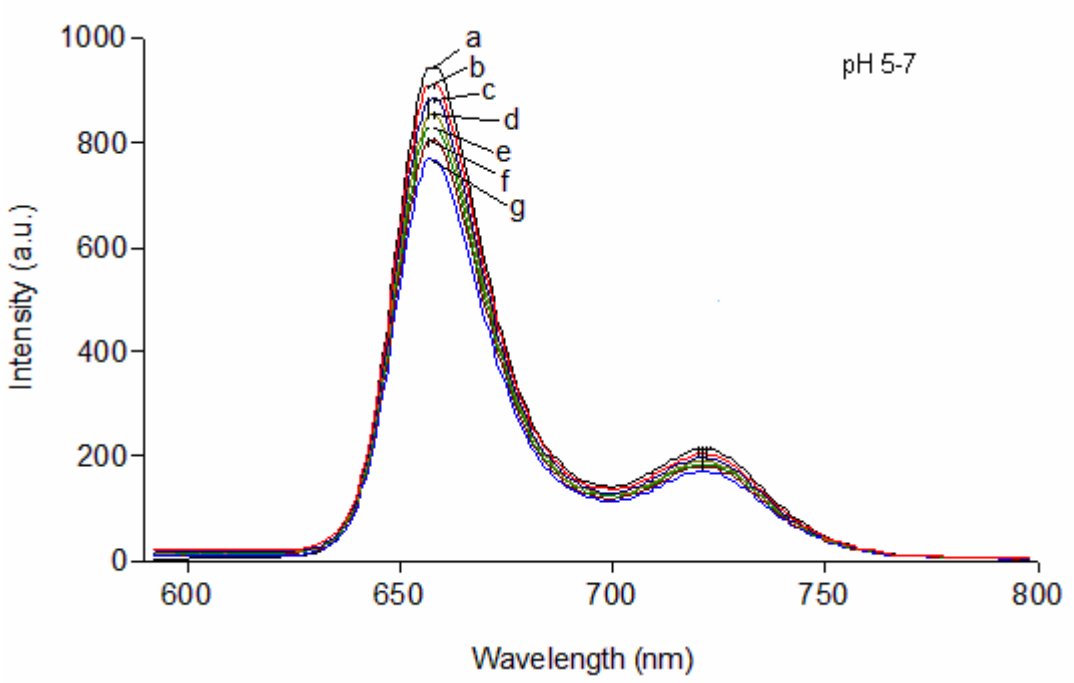


Figure 3.19 Fluorescence spectra of PAAP formed complex with 5.0 μg mL^{-1} zirconium at different times. λ_{ex}=570 nm, λ_{em}=658 nm. a) PAAP in sol-gel matrix, Zr-PAAP complex: b) 10, c) 15, d) 20, e) 25, f) 30, g) 40 min.

3.4.2.3 Calibration Graph

Calibration graph was obtained by PAAP also in sol-gel matrix using different zirconium concentrations. The same results were obtained in sol-gel matrix. The fluorescence intensity was linear for 2.0-20 μg mL^{-1} of zirconium at 658 nm. The stability time of complex at zirconium concentrations lower than 5.0 μg mL^{-1} is 60 min., at the concentrations higher than 5.0 μg mL^{-1}, the stability time is 40 min. Because of the increasing stability time in sol-gel matrix, the further studies have been carried out in medium which PAAP prepared in aqueous solution.

3.4.3 Selectivity

The interference effect of metal ions which may form complexes with PAAP was investigated. The experiments were carried out by fixing the concentration of Zr(IV) at 5.0 μg mL^{-1} and then recording the change of the fluorescence intensity before and after adding the interferent into Zr(IV) solution buffered with a NaOAc-HOAc

solution (pH 6.0). Experimental results showed that some alkali and alkaline earth metal cations and some transition metal cations such as Mn(II), Cd(II), Pb(II), Mo(VI), Zn(II), Cu(II) and Co(II) existing in concentration up to 2.0 μg mL^{-1}, did not have obvious interfering effect on the Zr(IV) assay.

3.4.4 Applications

NIST standard reference material 2709 (San Joaquin Soil) and 2711 (Montana Soil) and soil sample (Trakidasit and Andexit) from Kula region, Manisa nearby İzmir were subjected to the recommended procedure in Section 2.8.1 for fluorimetric determination of zirconium using PAAP. The zirconium content found by the present procedure was in agreement with certified values. The results were given in Table 3.17 and 3.18, which demonstrate the applicability and the accuracy of the present method in soil analysis.

Table 3.17 Zirconium determination in reference material (SRM 2709, SRM 2711) using proposed method

	Metal Amount (μg g^{-1})			
	SRM 2709		SRM 2711	
Zirconium	Found[a]	Certified	Found[a]	Certified
	165 ± 1	160[b]	227 ± 1	230[b]

[a] Average of three determinations

[b] Noncertified values (are weighed means of result one analytical method)

Table 3.18 Zirconium determination in soil sample using proposed method

	Metal Amount		(μg mL^{-1})	
	Trakidasit		Andexit	
Zirconium	Found[a]	ICP-MS*	Found[a]	ICP-MS
	170.63 ± 0.1	174.60	286.58 ± 0.3	284.40

[a] Average of three determinations

* ACME laboratory, Canada

3.4.5 *Analytical Characteristics*

Under the experimental conditions, there is a linear relationship between fluorescence intensity and metal ion concentration. The relative standard deviation, RSD% (n=5) was 1.78 % for 75 μg of zirconium in 25 mL, indicating that this method is highly precise and reproducible.

The limit of detection (LOD) was defined as that analyte concentration giving a signal equal to three times the standard deviation of blank signal. LOD for zirconium was found to be 0.006 μg mL^{-1}.

CHAPTER FOUR

CONCLUSIONS

4.1 Preconcentration and Determination of Metal Ions

One of the aim of this work was to develop chemically bound sorbents and these sorbents were to use for the preconcentration and separation of Mn(II), Co(II), Cd(II), Ni(II), Cu(II), Zn(II), U(VI) and Th(IV) ions by solid phase extraction.

For this; 5,10,15,20 tetrakis (phenoxy acetic acid) porphyrin and calix[4]crown-4 were synthesized and these ligands were chemically bound to Merrifield polymer resin. By FTIR, ^{1}H NMR and elemental analysis, the resulting resins were characterized. The amount of attached PAAP and calix[4]crown-4 to the polymer resin and were found to be 0.242 and 0.489 mmol g^{-1} resin, respectively.

The porphyrin modified resin was used for preconcentration and determination of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), U(VI) and Th(IV) and the calix[4]crown-4 modified resin was used for preconcentration and determination of Ni(II), Cu(II), Cd(II), Zn(II) and Co(II). Several parameters; flow rate for sorption and desorption, concentration of acid for desorption, sorption capacity of the chelating resin, optimum pH, preconcentration factor and distribution coefficient for metal ions were examined. In addition, the validity of the proposed method by these modifed resins was checked by analyzing these metal ions in NIST standard reference material 2709 and 2711 (San Joaquin Soil and Montana Soil). Parameters optimized for sorption and desorption of metal ions using modified resins were summarized in Table 4.1.

The sorption pH of copper, cobalt, nickel and zinc in calix[4]crown-4 modified resin was lower than in PAAP modified resin. The best stripping agent is HCI for calix[4]crown-4 modified resin and HNO_3 for PAAP modified resin. Sorption capacities and preconcentration factors of investigated metal ions in PAAP modified resin were similar or better than the other. Also, the newly modified resins were

compared with the other modified resins in literature with respect to the sorption capacity (Table 4.2).

The synthesized resins were also selective towards analyte extraction even in the high concentration salt solutions and has greater reusability nature even up to 15 (for porphyrin modified resin) and 12 (for calix[4]crown-4 modified resin) cycles of continuous usage.The results obtained from the analysis of soil reference standard materials proved the reliability of the proposed method.

Table 4.1 Parameters optimized for sorption and desorption of metal ions on modified resins

Parameters	PAAP Modified Resin							Calix[4]crown-4 Modified Resin				
	Cu(II)	Co(II)	Ni(II)	Zn(II)	Mn(II)	U(VI)	Th(IV)	Cu(II)	Co(II)	Ni(II)	Zn(II)	Cd(II)
pH range	7.0-8.0	6.0-7.0	8.0	6.0-7.0	6.0	4.0-5.0	4.0-5.0	6.0-7.0	5.0	4.0-4.5	4.5	6.0
Flow rate (sorption) (mL min^{-1})	1.0	1.5	1.5	1.0	1.5	2.0	2.0	1.0-3.0	1.0-3.0	1.0-3.0	1.0-3.0	1.0-3.0
Stripping solution (mol L^{-1})	2.0 HNO_3	2.0 HNO_3	2.0 HNO_3	2.0 HNO_3	2.0 HNO_3	0.25 HCI	0.1 HCI	0.5 HCI	0.5 HCI	0.5 HCI	1.0 HCI	1.0 HCI
Sorption capacity (mmol g^{-1} of resin)	0.229	0.214	0.222	0.233	0.209	0.108	0.101	0.215	0.186	0.173	0.149	0.205
Preconcentration factor	125	180	200	215	150	115	114	208	168	125	125	199
Distribution coefficient	2684	3781	4308	4557	3088	2336	2312	4287	3255	2545	2425	4102
Average Recovery (%)	98-99	99	99	98-99	98-99	98	97-98	99	99	98	99	98
RSD (%)*	2.00	2.04	1.99	2.13	2.12	2.15	2.18	1.95	1.98	1.94	2.05	1.98
Dedection limit (μg L^{-1})	1.60	1.91	2.18	2.32	1.78	1.33	1.19	1.10	1.83	1.68	2.01	1.25

*10 determinations of 1.0 μg mL^{-1} each metal ion

Table 4.2 Comparison of porphyrin modified resin and calix[4]crown-4 modified resin with other chelating resin matrices in terms of resin sorption capacity ($mmolg^{-1}$)

Sorbent	Mn(II)	Cd(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)	U(VI)	Th(IV)
Merrifield resin / PAAP (this work)	0.209		0.214	0.222	0.229	0.233	0.108	0.101
Merrifield resin / calix[4]crown-4 (this work)		0.205	0.186	0.173	0.215	0.149		
XAD-4 / Dithiocarbamates (Ramesh, Mohan & Seshaiah; 2002)	0.157	0.082		0.123	0.174	0.162		
XAD-4 / aminobenzoic acid (Çekiç, Filik & Apak; 2004)		0.080	0.091	0.121		0.116		
XAD-2 / pyrocatechol (Lemos et al; 2006)			0.028	0.024	0.031			
Merrifield resin / calix[4]arene-semicarbazone (Jain et al; 2002)							0.013	0.012
XAD-2 / tiron (Kumar, Rathore & Singh; 2000)		0.085	0.110	0.215	0.220	0.170	0.03	
XAD-4 / o-vanilinsemicarbazone (Jain, Handa, Sait, Shrivastav & Agrawal; 2001)							0.01	0.01

Sorbent	Cd(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
XAD-4 / p-tertbutylcalix[8]arene (Pathak & Rao; 1996)	0.70	0.36	0.44	0.78	0.72
XAD-2 / Pyrocatechol violet (Saxena & Singh; 1997)	0.011	0.056	0.011		0.022
XAD-2 / o-aminophenol (Tewari & Singh; 1999)	0.030	0.056	0.055	0.053	0.045
XAD-4/Ammoniumpyyrolidine dithiocarbamate (Ramesh, Mohan & Seshaiah; 2002)	0.084		0.123	0.169	0.162
XAD-2 / 1-(2-pyridylazo)-2-naphthol (Bermejo-Barrera, Nancy, Cristina & Adela; 2003)	0.023			0.108	
Merrifield resin / calix[4]arene-o-vanilinthiosemicarbazone (Jain, Pandya, Pillai, Agrawal & Shrivastav; 2004)	0.033			0.038	
XAD-2 / Camalgite (Ferreira, Ferreira, Dantas, Lemos, Araujo & Costa ; 2000)				$1.56x10^{-3}$	
XAD-2 / 2-(methylthio)aniline (Guo et al; 2004)		0.176	0.240	0.139	0.130

4.2 Fluorimetric Determination for Zirconium

The other aim of this study was to determine the zirconium by fluorescence quenching of PAAP in sol-gel matrix and aqueous medium. The linear range for the zirconium was 2.0-20 μg mL^{-1} and the proposed method was validated by the analysis of real samples.

PAAP has highly luminescent property with excitation and emission wavelengths of 570 nm and 660 nm, respectively in aqueous medium. In pH < 4.0, PAAP has decomposed because of protonation of nitrogen atoms. The formation of complex of PAAP with zirconium(IV) ion caused static fluorescence quenching in PAAP. The fluorescence intensity of PAAP after complexation became stable within 20 min at pH 5.0-7.0 and at 55 min at pH 8.0-9.0.

Porphyrin reagent has stronger fluorescence with emission wavelengths of 658 nm in sol-gel matrix than in aqueous solution. The complex formation time was longer in sol-gel matrix.

The photostability time of Zr-PAAP complex was 75 min in aqueous medium and 120 min in sol-gel matrix. That the photostability time is higher in sol-gel matrix is of great importance because of usability of sol-gel matrix. Because the complex formation time in aqueous solution is shorter than sol-gel matrix, the determination of zirconium in various soil samples was carried out by medium that porphyrin was in aqueous solution. The results obtained from the analysis of soil reference standard materials proved the reliability of the proposed method for possible application to various samples.

In future work three objectives can be suggested.

The new chelating agent that will be the combination of derivatized porphyrine and calixcrown chelating agents could be tried to synthesize and then its usage in separation and enrichment of some transition metals ions.

Secondly, the limit of quantitation for zirconium(IV) determination could be tried to get better by adding micelle.

Thirdly, these synthesized ligands PAAP and the other one could be tried to use in analysis of the some transition metals by HPLC and HPTLC using densitometry.

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