

Schiff's bases complexation and solid phase extraction for improved trace element analysis

Vom Fachbereich Chemie
der Universität Duisburg-Essen
(Standort Duisburg)

zur Erlangung des akademischen Grades des
Doktors der Naturwissenschaften

genehmigte Dissertation

Von
Nabil Ramadan Bader
aus Libyen, Benghazi

Referent: Prof. Dr. Heinz-Martin Kuss
Koreferent: PD. Dr. Ursula Telgheder

Datum der Einreichung 11.12.2008
Datum der mündlichen Prüfung 24.03.2009

I dedicate this work to

my Friends for their efforts and assistance.

my Family for their unconditional support over the years.

my wife Nadia for her love, support, patience and being always there for me.

ACKNOWLEDGEMENTS

I am grateful to my supervisor, Prof. Heinz-Martin Kuss, for guiding me into the field of analytical chemistry, for his support and encouragement. I am also grateful to Dr. Ursula Telgheder for her co-operation and many conducive discussions

My colleagues, which are in and out of the Laboratory made a valuable contribution through their help and wide ranging conversation. This includes all the staff members and co-workers in IAC.

I thank my friends especially the ones I made here in Germany: Barbara Zimmermann, Kirstin Clausen, Amila Bilic, Stefan Meisen, Pauline Rutarova, Nonhlangabezo Mabho, Bülend Bayrakter, Yuan Qian, Myint Sein, and Roman Rodriguez.

I thank Rajab El Kailany, Salam Asweisi, Khaled Elsherif, Younis Ben Amir, Ashraf Elhashani, Hassan El Ghol, Rafiq Elgargory, Mohamed Bognash, Mahmoud sadaawia, Sami Elabbar, Saleh Edby, Sami Bugaigis, Abdulkarim Bukatwa, Mohamed Atia, Mohamed Altous, Luai Shrouf, Anneliese Fischer, and all my friends both in Germany and Libya for their support and encouragement.

Finally, I thank my family for their continuous support and their faith in me during these long and hard years of work. My sincerest appreciation goes to my beloved wife Nadia for her endless love, patience, and having the fortitude to stay by my side.

Duisburg 2008

Nabil Bader

Abstract

An analytical method based on the separation/pre-concentration of metal ions using solid sorbents coated with Schiff's bases has been proposed. Four Schiff's bases have been used in the study of the three environmental related and important elements Cd, Cu, and Zn. Different factors, including the pH of the sample solution, the sample volume, and the amount of the Schiff's base were examined to optimize the conditions for the pre-concentration of these metal ions in aqueous solutions. A pre-concentration factor of 100 was obtained. The interference effects of the additional ions in the solution have been studied, metal ions of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Ag^+ , Pd^{2+} , V^{4+} , and V^{5+} and anions, Cl^- , CO_3^{2-} , SO_4^{2-} , and acetate. The improved LODs are 0.007, 0.362, and 0.102 ng/ml for Zn, Cd, and Cu respectively. The improved LODs are much lower than the LODs of FAAS and approaching the LODs of GFAAS. All sorbents undergo Langmuir isotherm. The system has been used for ultra trace element analysis of Cd using GFAAS as a detection system. Pre-concentration factor of 1334 has been achieved which means a LOD of 0.005ng/L. The effect of the structure of the solid support on the adsorption of Zn ion has been also studied. Aminopropyl silica and C18 polar plus were taken as solid supports. The capacity sequence was as following Aminopropyl silica > Silica gel > C18 polar plus silica. The improved detection limits of SPE-FAAS for Zn using those sorbents have been found to be 0.007, 0.016, and 0.0237 ng/ml for silica gel, C18 polar plus silica, and aminopropyl silica respectively. The newly developed procedure SPE-FAAS has been found to be successful in separating the complex matrices (hair, vitamin tablets, and water) and pre-concentrating Zn and Cd ions from relatively large sample volume. The developed method for solid – liquid extraction is convenient, simple, sensitive and of low costs.

List of Contents

1	INTRODUCTION	1
2	FUNDAMENTALS	2
2.1	SEPARATION AND PRE-CONCENTRATION	2
2.1.1	<i>Solvent extraction</i>	2
2.1.2	<i>Flotation</i>	3
2.1.3	<i>Precipitation</i>	3
2.1.4	<i>Volatilization</i>	4
2.2	SOLID PHASE EXTRACTION	4
2.2.1	<i>Steps in solid phase extraction protocols</i>	4
2.2.2	<i>Advantages of SPE</i>	5
2.2.3	<i>Column and batch extraction</i>	5
2.2.4	<i>Solid phase (Sorbent)</i>	6
2.2.4.1	Silica gel.....	7
2.2.4.2	C18-bonded silica gel.....	8
2.2.4.3	Aminopropyl silica.....	9
2.2.5	<i>Pre-concentration of metal ions</i>	9
2.2.6	<i>Selection of SPE sorbent</i>	9
2.2.7	<i>Optimal pH</i>	10
2.2.8	<i>Exchange capacity</i>	10
2.2.8.1	Sample matrix	11
2.2.8.2	Analyte structure	11
2.2.8.3	The presence of co-retained compounds.....	11
2.2.9	<i>SPE and LLE</i>	11
2.3	ADSORPTION ISOTHERMS	13
2.3.1	<i>Classification of adsorption isotherms</i>	13
2.3.2	<i>The Langmuir isotherm</i>	16
2.3.3	<i>Freundlich isotherm</i>	20
2.4	IMMOBILIZED LIGANDS IN THE SOLID PHASE.....	22
2.4.1	<i>Chelation</i>	22
2.4.2	<i>Schiff's bases</i>	22
2.4.3	<i>Salen</i>	23

2.4.4	<i>Synthesis</i>	24
2.4.5	<i>Metal salen complexes</i>	25
2.4.6	<i>Analytical application of Schiff's bases</i>	27
2.4.6.1	Applications of Schiff's bases chelates in quantitative analysis.....	27
2.5	METAL IONS USED IN THE STUDY.....	31
2.5.1	<i>Cadmium</i>	31
2.5.2	<i>Zinc</i>	31
2.5.3	<i>Copper</i>	32
2.6	DETECTION SYSTEM	32
2.6.1	<i>Atomic absorption spectroscopy (AAS)</i>	32
2.6.1.1	Interferences in AAS.....	34
2.6.1.2	Calibration and standard curves	34
2.6.1.3	Sample preparation for AAS measurements.....	34
2.6.1.4	Limit of detection (LOD) and limit of quantification (LOQ)	35
3	OBJECTIVE	36
3.1	GENERAL OBJECTIVE.....	36
3.2	SPECIFIC OBJECTIVES	36
4	EXPERIMENTAL	37
4.1	INSTRUMENTATION AND EQUIPMENTS	37
4.2	MATERIALS	37
4.3	CHEMICAL REAGENTS, SOLVENTS AND WATER	38
4.4	PROCEDURE.....	39
4.4.1	<i>Preparation of the column</i>	39
4.4.2	<i>General procedure for metal determination</i>	39
4.5	PREPARATION OF THE LIGANDS	39
4.6	EFFECT OF PH ON METAL ADSORPTION.....	40
4.7	EFFECT OF LIGAND AMOUNT	40
4.8	EFFECT OF SAMPLE VOLUME	40
4.9	CAPACITY OF THE SORBENTS.....	40
4.10	INTERFERENCE EFFECT	40
4.11	EXPERIMENTAL PART OF GFAAS MEASUREMENTS.....	41
4.11.1	<i>The Temperature Programme</i>	41
4.11.2	<i>Effect of Schiff's base volume</i>	41

4.11.3	<i>Effect of sample concentration</i>	41
4.11.4	<i>Effect of sample volume</i>	42
4.11.5	<i>Effect of eluent volume</i>	42
5	RESULTS AND DISCUSSION	43
5.1	PRE-CONCENTRATION/SEPARATION OF ZN, CD, AND CU ON DIFFERENT SCHIFF'S BASES LOADED ON SILICA GEL.....	43
5.1.1	<i>Structure of the Schiff's bases</i>	43
5.1.2	<i>Interaction between metal ions and sorbents</i>	45
5.1.3	<i>Effect of pH on metal adsorption</i>	46
5.1.4	<i>Effect of ligand amount and eluent volume and concentration</i>	50
5.1.5	<i>Effect of sample volume</i>	52
5.1.6	<i>Capacity of the sorbents</i>	54
5.1.7	<i>Interference effect</i>	56
5.1.7.1	<i>Interference effect on zinc ion</i>	57
5.1.7.2	<i>Interference effect on Cd ion</i>	60
5.1.7.3	<i>Interference effect on Cu ion</i>	63
5.1.8	<i>Selectivity</i>	66
5.1.9	<i>Adsorption isotherms</i>	72
5.1.10	<i>Analytical performance</i>	78
5.2	SPE-GFAAS WITH SCHIFF'S BASES FOR ULTRA TRACE ANALYSIS.....	82
5.2.1	<i>Effect of Schiff's base volume</i>	82
5.2.2	<i>Effect of sample concentration</i>	83
5.2.3	<i>Effect of eluent volume</i>	83
5.2.4	<i>Effect of sample volume</i>	84
5.3	COMPARISON BETWEEN DIFFERENT SILICA SUPPORTS LOADED WITH SCHIFF'S BASE ON PRE-CONCENTRATION/SEPARATION.	87
5.3.1	<i>Effect of pH on adsorption of Zn(II) ion</i>	87
5.3.2	<i>Effect of sample volume</i>	90
5.3.3	<i>Selectivity</i>	92
5.3.4	<i>Capacity of the sorbents</i>	97
5.3.5	<i>Analytical performance</i>	97
5.4	ANALYSIS OF TRACE ELEMENTS IN REAL SAMPLES	101
5.4.1	<i>Determination of zinc in drinking water</i>	101
5.4.2	<i>Zn in Zinc and multivitamins tablets</i>	102

5.4.3	<i>Analysis of Hair sample</i>	102
5.4.4	<i>Determination of Cadmium in drinking water</i>	103
6	SUMMARY	104
7	REFERENCES	106

List of figures

Figure 2-1 Schematic diagram for the chemical structure of silica gel surface.	7
Figure 2-2 Langmuir adsorption isotherm	18
Figure 2-3 Langmuir isotherm	19
Figure 2-4 Freundlich adsorption isotherm	21
Figure 2-5 Freundlich isotherm	21
Figure 2-6 preparation of Schiff's bases.	25
Figure 2-7 Schematic presentations of planar, umbrella, and stepped molecular confirmation of salen complexes.	26
Figure 5-1 Structure of Schiff's bases used in the study.	44
Figure 5-2 Influence of sample pH on the recovery of Zn(II) from silica gel coated with Schiff's bases ligands.	47
Figure 5-3 Influence of sample pH on the recovery of Cd(II) from silica gel coated with Schiff's bases ligands.	47
Figure 5-4 Influence of sample pH on the recovery of Cu(II) from silica gel coated with Schiff's bases ligands.	48
Figure 5-5 Influence of Schiff's base volume on the recovery of Zn(II) ion.	51
Figure 5-6 Influence of Schiff's base volume on the recovery of Cd(II) ion.	51
Figure 5-7 Influence of Schiff's base volume on the recovery of Cu(II) ion.	52
Figure 5-8 Effect of sample volume on the recovery of Zn(II) ion.	53
Figure 5-9 Effect of sample volume on the recovery of Cd(II) ion.	53
Figure 5-10 Effect of sample volume on the recovery of Cu(II) ion.	54
Figure 5-11 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with ES Schiff's base.	58
Figure 5-12 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with ES Schiff's base.	58
Figure 5-13 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with ES Schiff's base.	59
Figure 5-14 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with ES Schiff's base.	59
Figure 5-15 Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with ES Schiff's base.	61

Figure 5-16 <i>Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with PS Schiff's base.</i>	61
Figure 5-17 <i>Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with DMPS Schiff's base.</i>	62
Figure 5-18 <i>Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with SBTD Schiff's base.</i>	62
Figure 5-19 <i>Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with ES Schiff's base.</i>	63
Figure 5-20 <i>Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with PS Schiff's base.</i>	64
Figure 5-21 <i>Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with DMPS Schiff's base.</i>	64
Figure 5-22 <i>Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with SBTD Schiff's base.</i>	65
Figure 5-23 <i>Kurbatov plot for Zn adsorption on silica gel coated with Schiff's bases.</i>	69
Figure 5-24 <i>Kurbatov plot for Cd adsorption on silica gel coated with Schiff's bases.</i>	70
Figure 5-25 <i>Kurbatov plot for Cu adsorption on silica gel coated with Schiff's bases.</i>	71
Figure 5-26 <i>Adsorption isotherm of Zn ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.</i>	75
Figure 5-27 <i>Langmuir isotherm of Zn ion (pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.</i>	75
Figure 5-28 <i>Adsorption isotherm of Cd ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.</i>	76
Figure 5-29 <i>Langmuir isotherm of Cd ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.</i>	76
Figure 5-30 <i>Adsorption isotherm of Cu ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.</i>	77
Figure 5-31 <i>Langmuir isotherm of Cu ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.</i>	77
Figure 5-32 <i>Calibration curves of Zn(II) ion.</i>	79
Figure 5-33 <i>Calibration curves of Cd(II) ion.</i>	79
Figure 5-34 <i>Calibration curves of Cu(II) ion.</i>	80
Figure 5-35 <i>Effect of Schiff's base volume on Cd adsorption.</i>	82
Figure 5-36 <i>Effect of sample concentration.</i>	83

Figure 5-37 <i>Effect of eluent volume.</i>	84
Figure 5-38 <i>Effect of sample volume.</i>	85
Figure 5-39 <i>Comparison between The detection limit ranges of SPE combinations with some major atomic spectroscopy techniques.</i>	86
Figure 5-40 <i>Influence of sample pH on the recovery of Zn(II) from silica gel coated with Schiff's bases ligands</i>	88
Figure 5-41 <i>Influence of sample pH on the recovery of Zn(II) from C18 polar plus silica gel coated with Schiff's bases ligands.</i>	88
Figure 5-42 <i>Influence of sample pH on the recovery of Zn(II) from Aminopropyl silica gel coated with Schiff's bases ligands.</i>	89
Figure 5-43 <i>Effect of sample volume on the recovery of Zn(II) ion from silica gel coated with Schiff's bases ligands.</i>	90
Figure 5-44 <i>Effect of sample volume on the recovery of Zn(II) ion from C18 polar plus silica gel coated with Schiff's bases ligands.</i>	91
Figure 5-45 <i>Effect of sample volume on the recovery of Zn(II) ion from Aminopropyl silica gel coated with Schiff's bases ligands.</i>	91
Figure 5-46 <i>Kurbatov plot for Zn adsorption on silica gel coated with Schiff's bases.</i>	93
Figure 5-47 <i>Kurbatov plot for Zn adsorption on C18 polar plus silica gel coated with Schiff's bases.</i>	94
Figure 5-48 <i>Kurbatov plot for Zn adsorption on Aminopropyl silica gel coated with Schiff's bases.</i>	95
Figure 5-49 <i>Calibration curves of Zn(II) ion using silica gel coated by Schiff's bases.</i>	98
Figure 5-50 <i>Calibration curves of Zn(II) ion using C18 polar plus silica gel coated by Schiff's bases</i>	98
Figure 5-51 <i>Calibration curves of Zn(II) ion using aminopropyl silica gel coated by Schiff's bases.</i>	99

List of tables

Table 2-1 Comparison between Liquid-Liquid Extraction (LLE), SPE and Liquid Chromatography (LC).....	6
Table 4-1 <i>List of the chemicals used.</i>	38
Table 4-2 <i>Temperature programme of Cd determination by GFAAS</i>	41
Table 5-1 <i>Molecular weights and pKa values of Schiff's bases.</i>	44
Table 5-2 <i>Sorbents capacities towards Zn, Cd and Cu ions ($\mu\text{g/g}$ sorbent).</i>	55
Table 5-3 <i>Sorbents capacities towards Zn, Cd and Cu ions ($\mu\text{mol/g}$ sorbent).</i>	55
Table 5-4 <i>Selectivity sequence for the adsorption or co-precipitation of a variety of divalent transition and heavy metal cations on several sorbents.</i> ⁶⁹	68
Table 5-5 <i>Selectivity of silica gel coated with Schiff's bases toward metal ions.</i>	72
Table 5-6 <i>Analytical limits of the metal ions.</i>	80
Table 5-7 <i>Comparison between LODs of SPE-FAAS and FAAS.</i>	81
Table 5-8 <i>Selectivity of the sorbents toward Zn(II) ion.</i>	96
Table 5-9 <i>Sorbents capacities toward Zn ion ($\mu\text{g/g}$ sorbent).</i>	97
Table 5-10 <i>Analytical limits of the Zn(II) ion.</i>	99
Table 5-11 <i>Analysis results of water samples.</i>	101
Table 5-12 <i>Analysis results of Zinc in Multivitamin tablets.</i>	102
Table 5-13 <i>Analysis results of drinking water spiked with Cd.</i>	103

1 INTRODUCTION

There is a strong need to provide chemical analyses more quickly and at lower cost, this means more effort should be given for sample preparation portion of the analysis, which is asked to provide more reproducible results, decrease the use of organic solvents, provide cleaner extracts for instrumental measurements, saving time, and less cost.

The reason behind the need of such improvement is that the majority of the sample analysis time is spent in sample preparation which is more than 60% of analysis time compared to only 7% for the actual measurement of the sample constituents.¹

Also, it is necessary to determine the analyte in low level concentration, for this purpose there are of course a lot of highly sophisticated techniques, they are fast, sensitive but expensive and complicated.

Most of the authors in the field of analytical chemistry are talking about some techniques as very powerful techniques, like GC-MS, GC-MS-MS or ICP-MS, this is true but in fact these techniques are combination between two techniques, for example on case of GC- MS, the GC part is a separation tool or we can say “further sample preparation” for MS part which is the detection tool. We can do the same thing if we combine SPE with FAAS to reach the limit of detection of GFAAS or with GFAAS to reach the limit of detection of ICP-MS. This gives the opportunity to the small laboratories to increase their efficiency since SPE is cheap and has a lot of advantages instead of having high sophisticated instrument which are always expensive and need a lot service and accessories.

In the present study we will try to find a low cost alternative method for chemical analysis of metals to overcome the difficulties accompanied to use of highly sophisticated instruments especially in the developing countries.

2 FUNDAMENTALS

2.1 Separation and pre-concentration

Under ideal conditions the analysis of trace elements would not require any initial concentration or separation stages. However even with recent developments in selectivity and sensitivity direct instrumental analysis is often not possible because the concentrations of the analytes are below the limits of determination of the method or the matrix provides an interference which must be removed. Both problems may be encountered simultaneously in a sample. Separation and concentration procedures are therefore frequently components of an overall analytical scheme either separately or in combination. These manipulations must provide a final sample that is optimized for the determination step.²

In the following we will discuss some methods which are currently used for separation and pre-concentration.

2.1.1 Solvent extraction

Solvent extraction separation is based on the differences in the solubility of elements and their compounds in two immiscible liquid phases. Usually, the initial phase is an aqueous solution and the second phase is an organic solvent immiscible with water. Extraction is usually fast and simple process; it demands only very simple equipments. Stripping (re-extraction, back-extraction or scrubbing) involves bringing the element from the organic extract back to the aqueous phase. The extraction efficiency, %E, i.e. the degree of transfer of the species from the aqueous to the organic phase, is defined as a function in distribution (extraction) coefficient (D), which is the ratio of the total concentration of the element in the organic phase (C_o) to the total concentration in the aqueous phase (C_{aq}) at equilibrium.

Where

$$D = \frac{C_o}{C_{aq}}$$

and the extraction efficiency is given by

$$\%E = \frac{100D}{D + (V_{aq}/V_o)}$$

Where V_o and V_{aq} are the volumes of organic and aqueous phases respectively. Shaking the two phases in a separating funnel during extraction or re-extraction must continue till equilibrium is attained. Extraction is equally useful in pre-concentration and separation of small amounts of elements. Extraction systems are divided into two classes: uncharged covalent species (simple molecules and chelates), and ion-association complexes (uncharged electrovalent species).

Simple molecules (e.g. I_2 , $HgCl_2$, BiI_3 , $GeCl_4$) are extracted with non-polar (inert) solvents such as benzene, chloroform and carbontetrachloride and the extraction is comparatively selective.^{3,4}

Uncharged chelates are formed when metal ions react with bifunctional ligands such as dithizone, oxine, dithiocarbamates, ethylxanthates, cupferron, acetylacetone and thenoyltrifluoroacetone.^{5,6}

2.1.2 Flotation

Ions of precipitated particles are adsorbed or attached at the surface of bubbles rising through a liquid, and thereby separated. A substance not surface – active itself can be made so through union with or adherence to a surface–active agent (surfactant). Froth flotation involves separation (pre-concentration) by frothing. If an insoluble product results from interaction between the ion to be separated and a surfactant, the process is called ion flotation. If the ion is firstly precipitated and the precipitate then floated with or without the addition of a surfactant, the process is called precipitate flotation

Ion flotation has been used, for example, as preconcentration technique for heavy metal ions in water. Anionic complexes of these elements are formed by adding complexing agents, e.g. cyanide, oxalates, thiosulfates and then floated by using a cationic surfactant and nitrogen gas bubbles. The quantity of surfactant must be larger than the stoichiometric amount, but a large excess may decrease trace recoveries.⁷

2.1.3 Precipitation

Precipitation methods for separation of elements are based on the difference in solubility of their compounds in aqueous solutions. Trace elements are separated quantitatively from the solution by using collectors (scavengers or carriers). Elements which are coprecipitated as hydroxides can also be isolated by organic reagents of the R–OH type, such as 8-Hydroxyquinoline, cupferron, β -diketones. Metal ions giving sparingly soluble sulfides may be coprecipitated by organic thiocompounds (R–SH) such as dithiocarbamates or thionalide.⁸ Activated charcoal can serve

as collector in isolation of many trace elements precipitated in various forms.⁹ Highly dispersed silicic acid has been used as collector for multielement pre-concentration.¹⁰

2.1.4 Volatilization

These methods for separating elements are based on differences in the vapor pressure of individual elements and their compounds. Covalent compounds are fairly volatile whereas ionic compounds are not, though polymeric covalent species (e.g. diamond) are non – volatile. Covalently bonded compounds are more soluble in non – polar solvents. Therefore, volatile compounds bear certain resemblances to those soluble in non – polar solvents. Examples are AsCl₃, GeCl₄, OsO₄ and certain volatile chelates.¹¹ Gas chromatography may be regarded as a special case of volatilization separation.

2.2 Solid phase extraction

Solid phase extraction (SPE) is a method for rapid sample preparation in which a solid stationary phase is typically packed into a syringe barrel, cartridge or column and used to selectively extract, concentrate, and purify target analytes prior to analysis.

Solid phase extraction is not limited to the use of solid particles to extract solutes from liquid sample. Air or other gaseous samples can also be passed through a packed tube to extract organic vapours or other substance present in the sample.

Compared to solvent extraction, solid phase extraction is a relatively new technique, but it has rapidly become established as the prime means of sample pre-treatment or the clean up of dirty samples, i.e. those containing high levels of matrix components such as salts, proteins, and organic compounds.

2.2.1 Steps in solid phase extraction protocols

There are four steps involved in SPE process.

1- Conditioning of the sorbent

The cartridge or the column is flushed through with the sample solvent to wet the surface of the sorbent and to create the same pH and solvent composition as those of the sample, thus avoiding undesirable chemical changes when the sample is applied.

2- Sample loading or retention

The sample solution is passed through the sorbent with the object of either retaining the analytes of interest while the matrix components pass through or retaining the matrix components while the analyte pass through.

3- Washing

This is necessary to remove components not retained by the sorbent during the retention step which may remain trapped in the interstitial solvent.

4- Elution

This final step is to recover retained analytes. The solubility rule of “like dissolve like” hold special significance in terms of which solvent systems can be successfully used for sample dilution and analyte elution, in SPE „like retains like“ and „like elutes like“. ¹²

2.2.2 Advantages of SPE

The popularity and use of solid phase extraction is growing up at a fast rate. SPE is easily automated, faster, and in general more efficient than liquid-liquid extraction. The particles used in SPE are non-polluting and the consumption of the solvents used is much smaller liquid- liquid extraction. Comparison between solid phase extraction, liquid-liquid extraction, and liquid chromatography is illustrated in Table 2-1.

2.2.3 Column and batch extraction

Although SPE can be done in a batch equilibration similar to that used in liquid-liquid extraction, its more common to use a small tube (minicolumn) or cartridge packed with the solid particles. The liquid sample is passed through the column, thus coming into intimate contact with the solid particles. With modern solid extractants equilibrium is rapidly attained and the analytes tend to be extracted in a zone near to the top of the SPE column. Unlike batch extraction, being only a single equilibration of solutes between the two phases, there are in effect several equilibrations when SPE is performed with a packed minicolumn. This is because the solutes continuously encounter fresh particles (containing little, if any, extracted solutes) as they pass down the column. A higher percentage of extraction is thus expected in column SPE compared to batch type extractions.

Table 2-1 Comparison between Liquid-Liquid Extraction (LLE), SPE and Liquid Chromatography (LC)¹

	LLE	SPE	LC
Extractive phase	Liquid emulsion	Porous solid	Porous solid
Typical samples	H ₂ O, ≥ 10- 100 ml	H ₂ O; ≥ 10-100 ml	H ₂ O-organic liquid or organic 5-50μl
Partition equilibrium	Single	Multiple	Multiple
Separation of individual analytes	NO	NO	YES
Easily automated	NO	YES	YES
Elution from extractive phase	Seldom needed; H ₂ O, pH control	Organic liquid	H ₂ O-organic liquid mixture
Concentration factor achieved	Moderate	High	Slight

2.2.4 Solid phase (Sorbent)

The solid phase typically consists of small, porous particles of silica with a bonded organic phase or of an organic polymer, such as crosslinked polystyrene. The extraction can take place in a batch mode in which the solid extractant is intimately mixed with liquid sample solution. In chemical analysis it is more common to pack the solid extractant into a small tube and pass the liquid sample through the tube.¹

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 analytes over a wide pH range (along with selectivity towards major ions); (2) the fast and quantitative sorption and elution; (3) a high capacity; (4) regenerability; and (5) accessibility. In particular, sorbents that allow fast reaction rates are preferred to achieve faster extraction as well as higher loading capacities.¹³

The particles of the ideal support should be of uniform and/or spherical shape with a narrow size distribution, mechanically stable, and porous to allow free passage of molecules, thus allowing rapid diffusion and rapid equilibration. Equally distributed reactive sites of high specific area

should also be present. Nearly all of the mentioned properties are typical for different kinds of silica.¹⁴

2.2.4.1 Silica gel

Silica gel is one of the most effective solid phase materials for use in SPE separation. It can be obtained in pure form, is hydrophilic, has a high concentration of active sites for chemical bonding and is easy to convert to SPE material by chemical synthesis or physical coating.¹⁵

The surface of silica gel is characterized by the presence of silanol groups which are responsible for either weak or strong adsorption with the analyte of interest. Modifications of the silica gel surface with certain functional groups are of great interest when it comes improving the physical and chemical properties of silica gel. Silica gel phases modified with chelating compounds are found to exhibit a strongly for metal ion removal, extraction, separation and pre-concentration from different matrices.¹⁶ The silanol groups could be isolated (free silanol groups), where the surface silicon atom has three bonds into the bulk structure and the fourth to OH group and the vicinal or bridged silanols, where two isolated silanol groups attached to two different silicon atoms are bridged by O atom. A third type of silanols called geminal silanols consists of two hydroxyl groups attached to one silicon atom (Figure 2-1). There are also three silanol group where three OH groups are attached to the same silicon atom.¹⁷

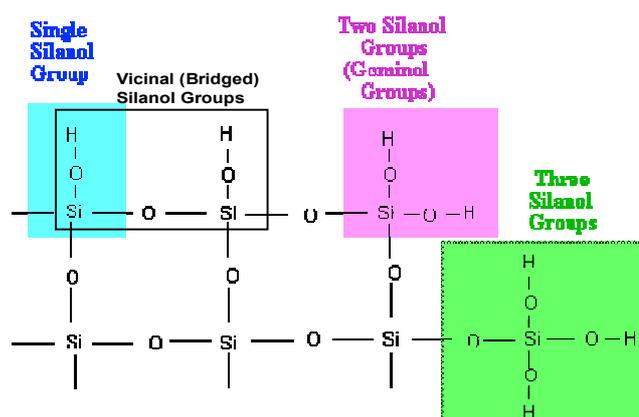


Figure 2-1 Schematic diagram for the chemical structure of silica gel surface.

On adsorption of polar substance hydrogen bonds are formed between the hydroxyl groups of the silica gel and adsorbate groups with active hydrogen (e.g. water, alcohols, ether, ketones, phenols and amine). The adsorption of aromatic hydrocarbons (benzene, etc.) and unsaturated hydrocarbons also depends to some extent on the presence of the hydroxyl groups in silica gel with which the aromatic hydrocarbons form π -complexes where the adsorption proceeds by the hydrogen bonding between the benzene ring and the surface hydroxyl groups. In case of phenols, a hydrogen bonding is also suggested between the oxygen atom of the phenol and the hydrogen atom of a surface hydroxyl group, but the interaction does not exclude the possibility of hydrogen bonding between π -electrons of the phenols as contributing to the overall adsorption.⁸⁵ On the other hand, aliphatic and alicyclic hydrocarbons are adsorbed by means of Van der Waals forces and they are sensitive to dehydration of the silica gel surface.¹⁸

Two approaches are used for loading the surface with specific organic compounds, chemical immobilization and physical adsorption. In the first case, a chemical bond is formed between the silica gel surface groups and those of the organic compound (*functionalized* sorbent). In the second approach, the organic compound is directly adsorbed on the silanol groups of the silica gel surface (*impregnated* or *loaded* sorbent), either by passing the reagent solution through a column packed with the adsorbent, or by soaking the adsorbent in the reagent solution.

It must be kept in mind that despite chemical bonding of functional groups on the silica gel surface, free silanol groups still remain. Their number can be minimised by end-capping the sorbent, but some will still be present. As a consequence, they will participate in the retention of trace elements somewhat, especially at pHs above their pK_a (ionized form).¹³

2.2.4.2 C18-bonded silica gel

Despite the large variety of bonded phases available, octadecyl bonded silica has currently become the most popular phase used. Bare C18-silica can also retain a fraction of inorganic trace elements, probably due to the presence of silanol groups on its surface. However, in practice, due to its hydrophobic character, C18-silica is not well suited for retention of trace element species, as the latter are often polar or ionic. Retention on C18-silica may be improved by addition of a ligand reagent to the sample before its percolation through the sorbent. The hydrophobic part of the ligand will thus have hydrophobic interaction with the C18 – silica and be retained on the sorbent, while the functional group of the ligand will ensure chelation of the trace element.^{19,20}

An alternative approach is to form the complex by passing the sample through a C18 -silica containing the immobilized reagent. Octadecyl bonded silica, modified by suitable ligands has been successfully used for the separation and sensitive determination of metal ions.^{21,22,23}

Despite their broad application to trace element preconcentration, bonded silica phases (either C18- silica or functionalized-silica gel) present the drawback of a limited range of pH that can be used, as in acidic (below 2 to 4) and basic (above 8) pHs hydrolysis may occur, which changes the interactions that occur between the sorbent and the trace elements.¹³

2.2.4.3 Aminopropyl silica

The support material is silica end capped by amino propyl group for normal phase extraction of polar compounds, weak anion exchange for carbohydrates, weak anions, and organic acids.

2.2.5 Pre-concentration of metal ions

Several mechanisms exist in pre-concentration of metal ions by SPE. Firstly, ion exchange mechanism, including uptake of metal cations or anions by simple ion exchange, uptake of ionic metal complexes on an ion exchange resin, is followed by selective elution of metal ions from an ion exchanger by inorganic or organic complexing reagents.

Secondly, addition of reagents to form an ion-association complex or a neutral metal-organic complex followed by SPE of the complex by a non-ionic solid extractant. Finally, Retention of selected metal ions by use of a chelating resin¹

2.2.6 Selection of SPE sorbent

Several parameters determine the type of SPE sorbent that can be applied.

a. Characterization of the sample

Factors such as the analyte's polarity relative to the matrix, the presence of charged functional groups, solubility, molecular weight, and how strongly the analyte is retained by the packed bed have pronounced influence on the selection of the sorbent.

b. Selection of retention strategy

There are two basic methods for sample treatment. The first is the selection of the packing bed to retain the desired analyte. The contaminants are washed out and the desired analyte is then eluted for analysis. The second is selection of the packing bed to retain the contaminants and the desired analyte passes directly through.

c. Selection of the proper packing type

As mentioned above there are three categories in SPE depending on the analyte, sorbent, and the matrix. Reversed-phase packings are hydrophobic, silica-based materials that retain moderately polar to nonpolar compounds from a polar matrix while washing out polar interferences. Or to retain non-polar contaminants while the polar compounds pass through unretained.

Normal-phase packings are hydrophilic, silica-based materials that retain polar compounds from a non-polar matrix while washing out non-polar interferences. Or to retain polar contaminants while non-polar compounds pass through unretained, and ion-exchange resins retain charged compounds or remove ionic interferences.

d. Optimization of SPE conditions

Selection of the proper bed size and suitable conditioning, washing and elution solvents. Poor sample recovery often occurs when the packed bed dimensions are not optimized. Too large bed results in incomplete elution while too small bed results in incomplete retention.

Considering the solvent strength relative to the packing material, the final conditioning solvent should be weak so that it does not act as an eluting solvent. Buffers should be used to control ionization of potentially charged compounds. Moreover washing solvents should remove weakly retained interferences without being strong enough to elute the analyte, On the other hand, elution solvents should be strong enough to completely elute an analyte into a small volume.

2.2.7 Optimal pH

Acids and bases in the samples need to be in their ionized form to develop interactions with the sorbent. To maintain reproducible and repeatable recovery rates, sample and solvent need to be buffered at an optimised pH.

2.2.8 Exchange capacity

SPE capacity is the amount of analyte that a packing bed will retain from a sample matrix.

This is expressed as “breakthrough volume” which means the amount of analyte that can be passed through a packing bed before the analyte is no longer retained and recovery starts to decrease. There are three main variables that affect capacity.

2.2.8.1 Sample matrix

The polarity of the sample matrix relative to the packing material will affect capacity. Reversed phases (C18, C8 and Phenyl) have higher capacity in polar matrices. As the matrix becomes less polar reversed phase capacity decreases. Likewise normal phases such as (Silica, NH₂, and CN) have the highest capacity in nonpolar matrices.

2.2.8.2 Analyte structure

The polarity of the analyte relative to the packing material will affect capacity. Reversed phase sorbents have the highest capacity for nonpolar compounds. They also have more capacity for the neutral form of the compound as opposed to its charged form. Whenever possible, adjustment of sample pH so that the analyte of interest is neutral to increase the capacity of reversed phases. Normal phase sorbents have the highest capacity for polar compounds or compounds with polar functional groups.

2.2.8.3 The presence of co-retained compounds

The presence of complex matrices in the sample will reduce the capacity of the SPE sorbent towards a given analyte due to the presence of co-retained compounds. For example, C18 SPE bed have less capacity for a drug in urine or plasma than in a buffer solution due to the presence of lipids, small proteins or other compounds that are readily retained by C18.

There is no single capacity for SPE sorbent. Capacity for certain analyte varies according to the structure of the analyte, composition of the sample matrix, and the presence of co-retained compounds in the sample. Finally capacity is best determined experimentally for specific analyte in a given sample matrix.

2.2.9 SPE and LLE

Liquid–liquid (solvent) extraction (LLE) is very widely applied to metal separation and enrichment. The technique is based on the formation of metal complexes and their distribution between an aqueous phase and an organic solvent. LLE depends on the stability and partition constants of metal complexes. One of the essential differences of the LLE methods from chromatographic ones, particularly from those based on the use of surface-modified adsorbents, consists in the possibility of the extraction of large metal amounts. This is why LLE is widely used in various technologies, but not only on a laboratory scale, and a huge amount of information on the chemistry of liquid–liquid extraction of various compounds has been accumulated.

Noticeable similarities of LLE and SPE allowed us to hope that a variety of different metal compounds used in LLE could be employed in SPE.

The extraction constants for any metal chelates and, therefore, the metal recovery and extraction selectivity are functions of stability and partition constants of the chelate compounds.^{24,25,26}

Because similar metal chelates are used in LLE and SPE, it seemed to be obvious that the behaviour of metal complexes, depending on their thermodynamic stability, should be common for both techniques, and that the HSAB principle could be applied to the prediction of metal behaviour in SPE.²⁶

The partition constants for coordinatively saturated hydrophobic chelates are mainly dependent on the energy of cavity formation and on the formation of hydrogen bonds between electronegative atoms of chelate molecule and water molecules at the cavity surface, which is the same in the LLE and SPE systems.^{27,28}

In a discussion of the experimental data, one can also assume that the models applicable to LLE can be used for describing the formation and distribution of metal chelates in SPE.

Solvent extraction is based on the formation of compounds soluble in organic solvents and on their distribution between the aqueous and organic phases, whereas in SPE not only a distribution but a filtration mechanism of extraction is realized as well, *i.e.*, both types of complexes, hydrophobic and hydrated, can be retained by the non-aqueous phase. The difference in their behaviour can occur during elution.

The pH dependences for SPE are generally consistent with the literature data on LLE with the same reagents, but show a small shift to higher pH values. This shift is probably due to the microenvironment on the solid-phase material, as compared with the bulk liquid-liquid environment, where the pH can easily be maintained.²⁹

2.3 Adsorption isotherms

Adsorption is the process by which a chemical species becomes attached to surface or is the process of trapping of chemical species (*adsorbate*) that are attached at or near a solid surface (*adsorbent*).

Adsorption processes are divided into two classes: physical adsorption and chemical adsorption (*chemisorption*). In physical adsorption, the binding forces are London dispersion forces, and dipole-dipole attractions. In chemisorption, covalent chemical bonds are formed between the atoms or molecules of the surface and the atoms or molecules of the adsorbed substance.³⁰

The amount of adsorbed substance is proportional to the surface of the adsorbent. Consequently to obtain a high level of adsorption, the adsorbent surface must be as big as possible. Therefore, good adsorptive potentials will be found only in materials with highly developed surfaces such as substances with a porous or spongy structure, or substances divided into fine particle size.^{31,32}

The relationship between the concentration of a given species in solution and the amount adsorbed can be expressed as an equation or as a plot at constant temperature. The line obtained is called the adsorption isotherm.^{32,33}

2.3.1 Classification of adsorption isotherms

The shape of all adsorption isotherms studied using nitrogen as adsorbate, fit into one of the five different types shown in the following diagrams. Each one of them reflects some unique condition and occurrence.³²

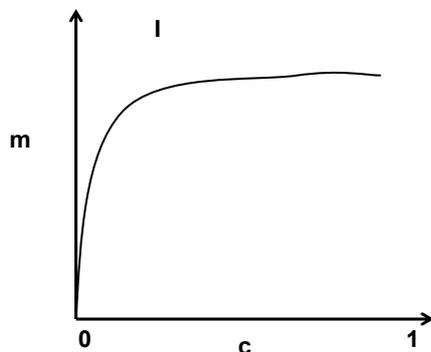
Where

m = adsorbed mass of nitrogen on the solid material.

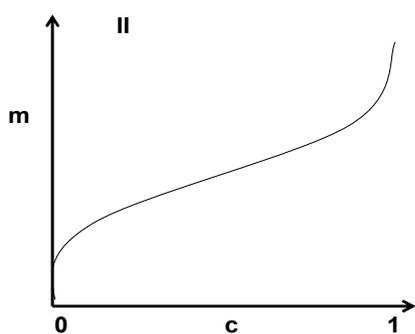
$c = P/P_0$ = relative pressure

P = pressure at equilibrium

P_0 = initial pressure

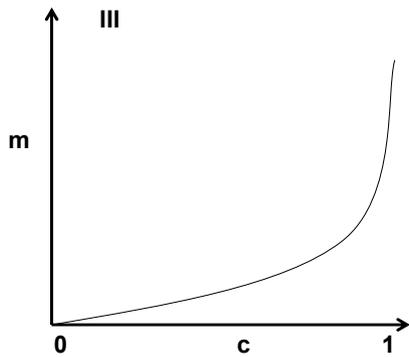


- I- Limited to only few molecular layers. It is encountered in chemisorption where the asymptotic approach to a limiting quantity indicates that all the surface sites are occupied. In physical adsorption it is typically encountered with microporous adsorbents, which once filled with the adsorbate have no external surface for additional adsorption. Characteristic of Langmuir isotherm.

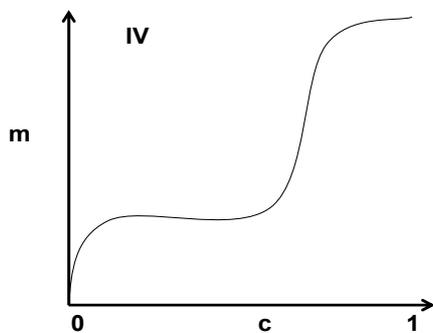


- II- Represent unrestricted multilayer adsorption on nonporous or macroporous adsorbents. The inflection point occurs near the completion of the first adsorbed monolayer. In this point, a second layer is formed and therefore, the amount adsorbed is increased. The process continues with the formation of higher layers. At saturation,

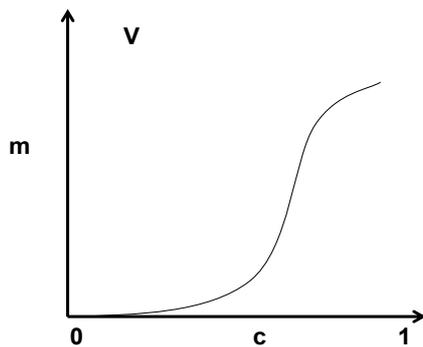
the number of adsorbed layers becomes infinite. Characteristic of Brunauer-Emmett-Teller isotherm (BET).



- III- It is associated with multi layer formation from the beginning of the adsorption process where the adsorbate interacts greater with the adsorbed layer than with the adsorbent surface. Similar as isotherm type II, the process continues with the formation of higher layers and at saturation, the number of adsorbed layers becomes infinite.



- IV- It is related with capillary condensation taking place in mesopores. Presents considerable interest in the study of catalysis because an increase in concentration to a certain level results in a different coverage when compared to the coverage formed by decreasing the concentration to the same level.



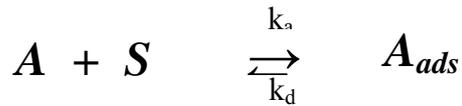
- V- Results from monolayer formation where the adsorbate and adsorbent present weak interaction.

IUPAC classification: Micropores have width not exceeding 2nm.
Mesopores have width between 2nm and 50nm.
Macropores have width exceeding 50nm.

There are basically two well established types of adsorption isotherm

2.3.2 The Langmuir isotherm

The study of adsorption was pioneered by Langmuir, who derived an expression for the equilibrium fraction of a solid surface covered by an adsorbed substance as a function of the concentration of the substance in the gas or liquid phase. Langmuir adsorption isotherm describes quantitatively the build up of a layer of molecules on an adsorbent surface as a function of the concentration of the adsorbed material in the liquid in which it is in contact. In a modified form it can also describe a bi-layer deposition. The Langmuir isotherm contains two assumptions. The assumptions are that the energy of adsorption is constant, and the numbers of binding sites are finite. In general way, an adsorption process can be presented by:



$$K = \frac{k_a}{k_d} = \frac{[A_{ads}]}{[A][S]}$$

Where

S = the surface sites

A = adsorbate molecule

[S] = molar concentration of surface site

[A] = concentration of A molecules in the liquid phase

K is equilibrium constant

k_a = reaction rate constant for adsorption

k_d = reaction rate constant for desorption

A_{ads} = the amount of A adsorbed

The surface contains a set of sites at which the molecules of A can be adsorbed, and that only a single layer of molecules can be adsorbed on the surface. The adsorption process is assumed to be an elementary process so that the rate of adsorption is first order in the concentration of A in the fluid phase and is also proportional to 1- Θ the fraction of the surface sites available for adsorption:

$$\text{Rate of adsorption} = k_a [A] (1 - \Theta)$$

Where Θ is fraction of occupied surface sites

The desorption is also assumed to be an elementary process, so that

$$\text{Rate of desorption} = k_d \Theta$$

At equilibrium

$$\text{Rate of desorption} = \text{Rate of adsorption}$$

$$k_d \Theta = k_a [A] (1 - \Theta)$$

Solving this equation for Θ to give the Langmuir isotherm

$$\Theta = \frac{k_a[A]}{(k_d + k_a[A])}$$

$$\Theta = \frac{K[A]}{(1 + K[A])}$$

Where K is the equilibrium constant, and has the units of reciprocal concentration (L/mol). The value of K can be determined from the Langmuir isotherm (Figure 2-2) by determining the value of the concentration A corresponding to $\Theta = 1/2$ where $[A] = 1/K$ and the surface adsorption sites are half occupied.

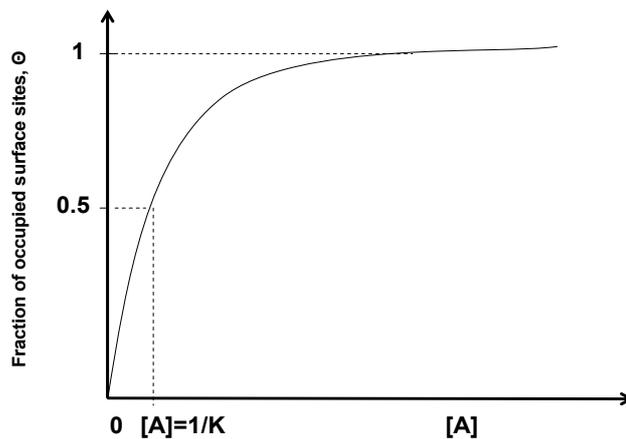


Figure 2-2 Langmuir adsorption isotherm

The Langmuir isotherm is shown here in the graph. The curvature at the end of the graph marks a point at which the receptor sites on the sorbent particle are full and there is no more room for additional adsorption.

Since the value of Θ is not directly measurable the mass adsorbed is proportional to Θ , and a graph of the mass adsorbed is used for plotting the isotherms. The location of the asymptote

corresponds to the value where $m_{\text{ads}} = 1$, but accurately locating the asymptote on the graph is difficult if the data suffer from experimental errors, therefore, it is desirable to make a linear plot using $1/m_{\text{ads}}$ vs. $1/[A]$, as shown in Figure 2-3 corresponding to the equation

$$\frac{1}{\Theta} = \frac{(1 + K [A])}{K [A]}$$

$$\frac{1}{\Theta} = \left(\frac{1}{K[A]}\right) + 1$$

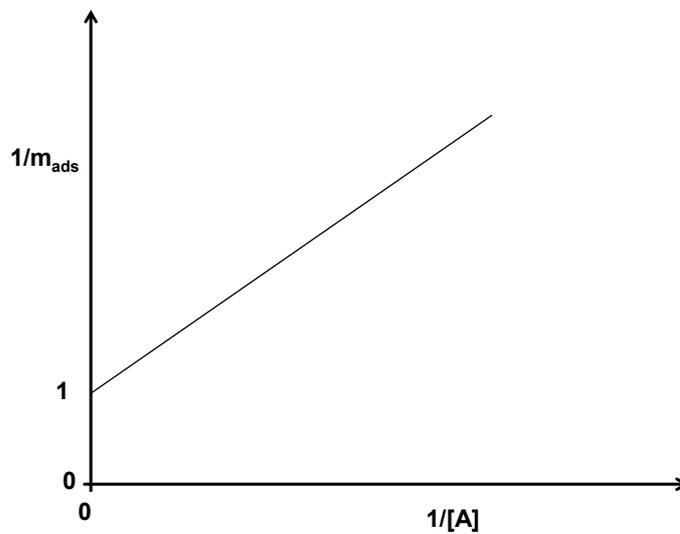


Figure 2-3 *Langmuir isotherm*

In practice, the equation used for calculating the Langmuir adsorption isotherm is expressed as indicated in the following equation:

$$\frac{1}{m_{ads.}} = \left(\frac{1}{K[A]V_m}\right) + \left(\frac{1}{V_m}\right)$$

Where

m_{ads} = adsorbed mass of A in the solid material

[A] = concentration of A in the solution

K = concentration equilibrium constant

V_m = limit sorption capacity

2.3.3 Freundlich isotherm

The adsorption of solutes on solids from liquids often follows the empirical Freundlich isotherm which states a logarithmic adsorption:

$$W_s = C_1 [A]^{1/c_2} \quad \text{(Figure 2-4)}$$

or

$$\ln W_s = \ln C_1 + \frac{1}{C_2} \cdot \ln [A] \quad \text{(Figure 2-5)}$$

Where:

W_s = amount of solute adsorbed per unit mass of adsorbent

[A] = solute concentration

C_1 and C_2 = adsorption constant

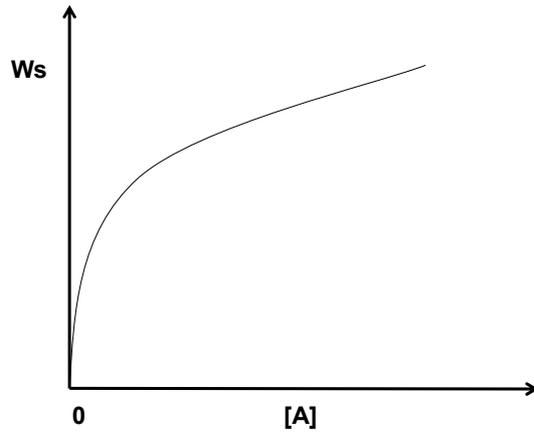


Figure 2-4 *Freundlich adsorption isotherm*

The adsorption process will follow the Freundlich isotherm if the plot $\ln [A]$ vs. $\ln W_s$ gives a straight line^{24,26}. (Figure 2-5)

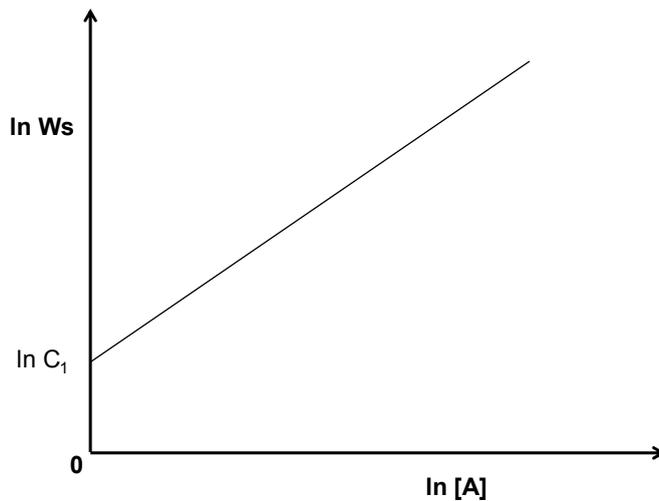


Figure 2-5 *Freundlich isotherm*

The Freundlich equation is most useful for diluted solutions over a small concentration range. In chromatography the Freundlich isotherm is not common, most adsorption processes are best described by the Langmuir isotherm.

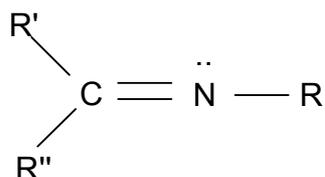
2.4 Immobilized ligands in the solid phase

2.4.1 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, nitriles), oxygen (e.g. O present in carboxylic, hydroxyl, phenolic, ether, carbonyl, phosphoryl groups) and sulphur (e.g. S present in thiols, thiocarbamates, thioethers). Chelating agents may be directly added to the sample for chelating trace elements, the chelates being further retained on an appropriate sorbent. An alternative is to introduce the functional chelating group into the sorbent. For that purpose, three different means are available: (1) the synthesis of new sorbents containing such groups (*new sorbents*); (2) the chemical bonding of such groups on existing sorbents (*functionalized sorbents*); and (3) the physical binding of the groups on the sorbent by impregnating the solid matrix with a solution containing the chelating ligand (*impregnated, coated or loaded sorbents*). The latter remains the most simple to be used in practice. Its main drawback is the possible flush of the chelating agent out of the solid sorbent during sample percolation or elution that reduces the lifetime of the impregnated sorbent.¹³

2.4.2 Schiff's bases

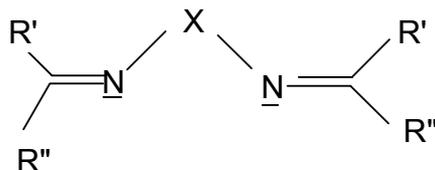
A *Schiff base* or Schiff's base is a functional group or type of chemical compounds containing a carbon-nitrogen double bond with the nitrogen atom connected to aryl group or alkyl group (R) but not hydrogen. The Schiff base is synonymous with an azomethine. These compounds were named after Hugo Schiff and have the following general structure:



Where R stands for a phenyl or alkyl group which makes the Schiff base a stable imine.

This kind of ligands is able to coordinate metal ions through the imine nitrogen and another group, usually linked to the aldehyde. The chemists still prepare Schiff bases and nowadays active and well-designed Schiff base ligands are considered “privileged ligands”.³⁴

The bridged Schiff's bases have the following structure which contains many functional groups able to change according to the purpose required.



Where R' = H or alkyl group

R'' = phenyl or substituted phenyl

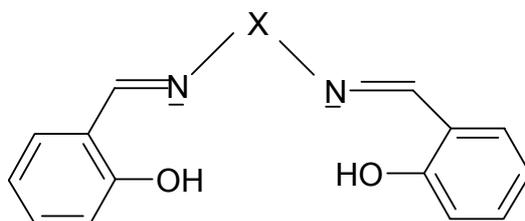
X = alkyl or aryl group

In fact, Schiff bases are able to stabilize many different metals in various oxidation states controlling the performance of metals in a large variety of useful catalytic transformations.^{35,36}

Although the Schiff bases are known to be easily prepared chelating ligands and are well characterized, little interest has been given to their uses for analytical purposes because of two serious drawbacks, they are insoluble in aqueous solutions and they decompose easily in acidic solutions, limiting their use to basic conditions.³⁷

2.4.3 Salen

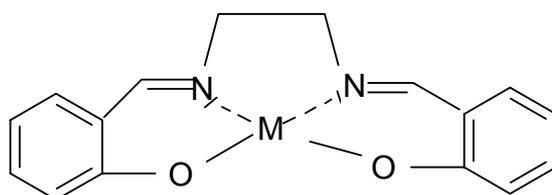
When two equivalents of salicylaldehyde are combined with a diamine, a particular chelating Schiff base is produced. The so-called Salen ligands with four coordinating sites and two axial sites open to ancillary ligands. Although the term Salen was used originally only to describe the tetradentate Schiff bases derived from ethylenediamine, the more general term Salen-type is used in literature to describe the class of [O,N,N,O] tetradentate Schiff base ligands.³⁴



Salen compounds are insoluble in aqueous solutions and they decompose easily in acidic solutions, limiting their use to basic condition.

Schiff's bases are able to coordinate metals through imine nitrogen, and there is a wide use of these metal complexes as catalysts.³⁴ In the last few years, various Schiff's base complexes of transition metals including Mn(III), Fe(II), Ni(II), Cr(III), Ru(III) etc. have been used as the catalysts for epoxidation of olefins and as mono oxygen sources for catalyst to carry out oxygen transfer to the olefins.^{35,37}

They are easy to synthesize (simply and cheaply) and multidentate. Substitution at the aromatic ring can modify the electronic and steric properties of the resulting complexes (fine-tuning) as it can form metal complexes of various sizes because of its open structure, as shown in the following structure.



Metal chelate of N,N'-Bis(salicylidene)ethylenediamine

2.4.4 Synthesis

There are several reaction pathways to synthesise Schiff bases.^{34,38,39} The most common is an acid catalysed condensation reaction of amine and aldehyde or ketone under refluxing conditions (Figure 2-6). The first step in this reaction is an attack of nucleophilic nitrogen atom of amine on the carbonyl carbon, resulting in a normally unstable carbinolamine intermediate. The reaction can reverse to the starting materials, or when the hydroxyl group is eliminated and a C=N bond is formed an imine can be formed. Many factors affect the condensation reaction, for example the pH of the solution as well as the steric and electronic effects of the carbonyl compound and amine. As amine is basic, it is mostly protonated in acidic conditions and thus cannot function as a nucleophile and the reaction cannot proceed. Furthermore, in very basic reaction conditions the

reaction is hindered as sufficiently protons are not available to catalyse the elimination of the carbinolamine hydroxyl group. In general, aldehydes react faster than ketones in Schiff base condensation reactions as the reaction centre of aldehyde is sterically less hindered than that of ketone. Furthermore, the extra carbon of ketone donates electron density and thus makes the ketone less electrophilic compared to aldehyde.³⁶

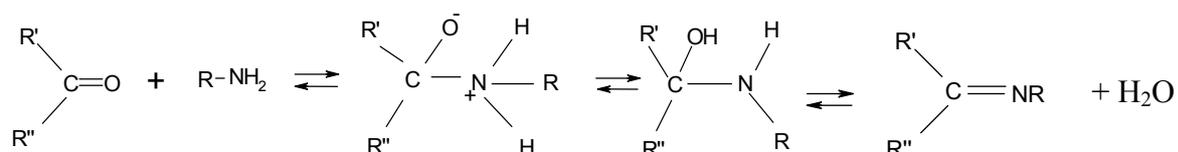
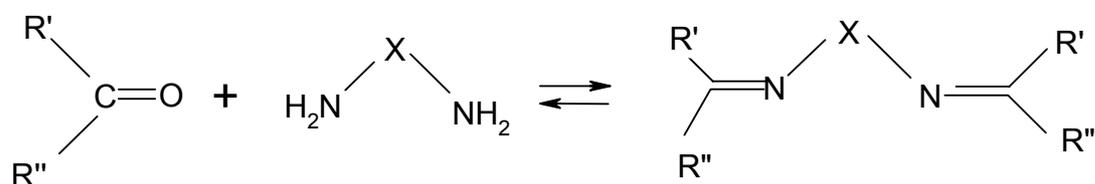


Figure 2-6 preparation of Schiff's bases.

For preparation of the bridged Schiff's bases a diamine compound is required with two molecules of aldehyde or ketone according to the structure of the product of interest as in the following reaction.



Where **R' = H or alkyl group**

R'' = phenyl or substituted phenyl

X = alkyl or aryl group

2.4.5 Metal salen complexes

Metal salen complexes have a tendency for square-planar geometry as the bridge between the imine moieties forces the *cis*-configuration around the metal centre, although in some cases a slightly distorted geometry towards tetrahedral form exists. When the metal complex has square-planar geometry, the metal ion is in the plane formed by the N₂O₂-donor atoms and the two axial positions are free for coordination of solvent or other molecules.^{40,41}

Metal salen complexes can have an umbrella, stepped, or planar molecular conformation (*Figure 2-7*) due to folding of the six-membered metallocycles and the metal center may deviate from the

plane defined by the N_2O_2 -donor atoms. The umbrella and stepped forms are the most common for salen complexes and the strictly planar conformation has been reported only for few complexes.³⁴

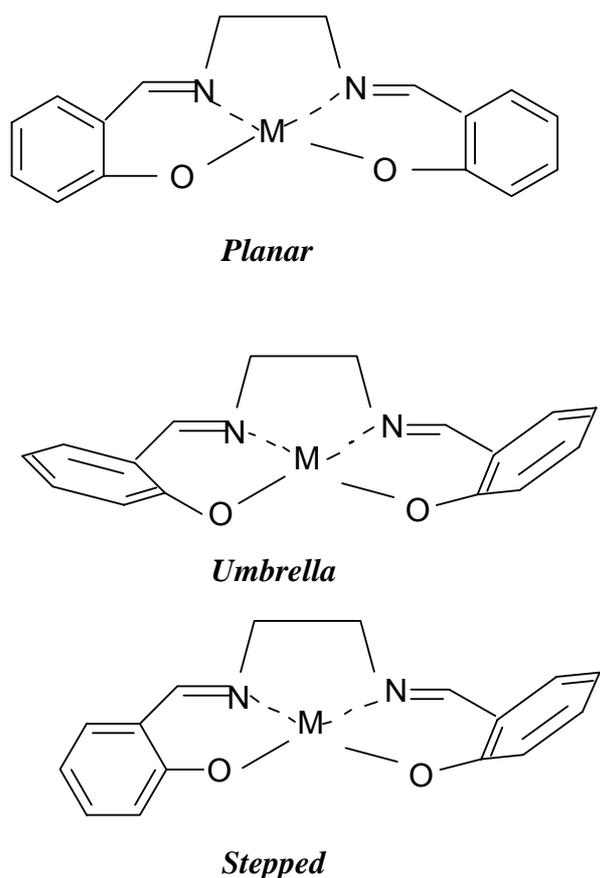


Figure 2-7 Schematic presentations of planar, umbrella, and stepped molecular confirmation of salen complexes.

Conformation of the ethylene-bridge is closely related to the overall conformation of the salen compound. Fully planar complexes might have an eclipsed conformation ($N(1)-CH_2-CH_2-N(2)$ torsion angle *ca.* 0°) but usually the conformation is *gauche*. In *gauche* conformation the carbon atoms of the ethylene-bridge are on the opposite sides of the MN_2O_2 -plane. The geometry around the metal centre in N,N' -bridged Schiff base complexes may be tuned by altering the length of the bridge, the molecular structure becomes more flexible as the number of methylene groups in the bridge increases.³⁰

In the salen complexes, which prefer square-planar geometry, dioxygen is coordinated in one axial position and the other axial position is occupied by a base or solvent molecule.⁴²

2.4.6 Analytical application of Schiff's bases

Jungreis and Thabet⁴³ have reported several applications for Schiff's bases in qualitative and quantitative classical analysis. All these applications did not cover a wide area in analytical chemistry. Schiff's bases were employed in chemical analysis in three principal ways:

- 1- Detection of amino or carbonyl compounds via formation of a Schiff base and the color or fluorescence of the latter, none of these tests, however, is based in chelation.
- 2- Use of a Schiff's base in a reaction involving chelation.
- 3- Use of a Schiff's base in a reaction not involving chelation.

2.4.6.1 Applications of Schiff's bases chelates in quantitative analysis

A number of Schiff's base chelating agents used for the detection of metal ions can also be applied for their quantitative determination. The procedures in most cases remain similar.

A. Gravimetric analysis.

B. Photometric methods.

Developments in photometric methods have been extensive, particularly for the determination of trace amount of metals. A number of these procedures are based on color formation resulting from the reaction between Schiff's base and the metal ion. For example, *o*-[N-(*o*-hydroxyphenyl)formimidoyl]phenol, known as Manganon, forms with manganese(II) at pH 9.1 to 11.6 a brown complex, the absorbance of which is measured at 428nm.

G. Tantaru et al. have prepared Salen-type Schiff's base by condensing ethyl-*o*-hydroxybenzene with ethylene diamine, and 1-ethyl-salicylidene-bis-ethylene diamine. These Schiff's bases present a good capacity of complexing Mn(II) ions, resulting brown complexes. They used the Schiff's base as reagent in spectrophotometric determination of the Mn(II). The Schiff base forms a brown complex with Mn(II) cation, with maximum absorbance at 460 nm. The complex with Mn(II) presents a maximum stability at pH 6.0. Spectrophotometric determination of Mn(II) using this Schiff base as reagent were successfully applied to pharmaceutical products containing Mn(II) cation.⁴⁴

C. Fluorometry

Fluorometric methods often provide excellent means for the detection and determination of inorganic ions. The reagents employed to produce fluorescence are often highly selective and very sensitive. In most cases a chelate is formed with the metal. There is often a dependence of fluorescence on the pH similar to that of acid-base fluorescence indicators. The analytical importance of such indicators lies in the fact that they make possible acid-base titrations in colored solutions when the use of conventional indicators would be precluded. Holzbecher reported that Schiff's bases obtained from salicylaldehyde and aniline fluoresce in the alkaline medium and therefore, be used for titration of acids with bases.⁴⁵ He also proposed O-[N-(O-hydroxyphenyl)formimidoyl]phenol, for quantitative determination of aluminium.⁴⁶

D. Schiff's bases as potentiometric sensors

A large number of different Schiff base ligands have been used as cation carriers in potentiometric sensors as they have shown excellent selectivity, sensitivity, and stability for specific metal ions.

Ganjali et al. (2004) have prepared potentiometric sensors for Dy(III) based on a Bis-pyrrolidene Schiff's bases, the electrode has been also used in the potentiometric determination of fluoride ions in mouthwash by the titration against Dy(III).⁴⁷

They also (2004) prepared a novel Bromide ion sensor based on Iron(III)-salen, this sensor has high bromide selectivity over a wide variety of organic and inorganic anions, specially iodide, chloride and hydroxide ions.⁴⁸

E. Schiff's bases as solvent extraction reagents

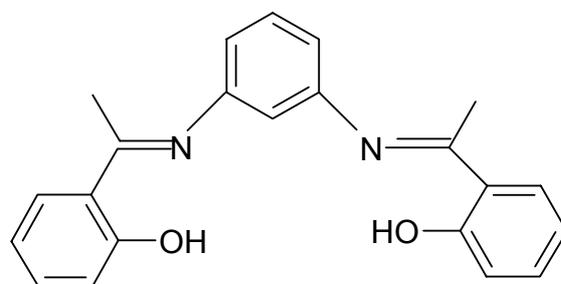
A solvent extraction using salophen was studied by Kim et al. and applied for determination of trace Ni(II), Co(II) and Cu(II) in water samples. The salophen is another type of salen derivative. Unlike the ethylenediamine of salen, salophen included a phenyldiamine as a backbone.⁴⁹ In another study the salophen has been used in solvent extraction for determination of trace Fe(II) and Fe(III) in water samples.⁵⁰

Gyo In et al. used salen (Net₂)₂ as a chelating agent for determination of Cu(II), Mn(II) and Zn(II) in water samples by FAAS.³⁷

F. Schiff's bases as solid phase extraction sorbents

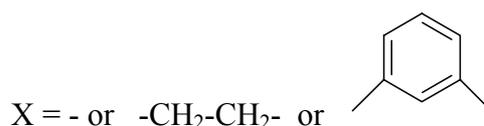
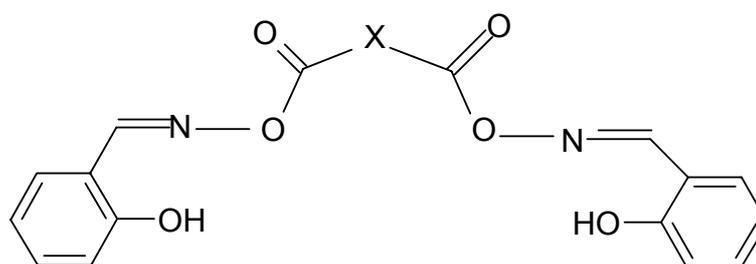
Ganjali et al.⁵¹ separated and pre-concentrated ultra-trace amounts of Cu(II) by solid phase extraction on octadecyl-bonded silica membrane disk modified with (Bis- (2-Hydroxyacetophenone) -2,2-dimethyl-1,3-propanediimine) SBTD followed by elution and ICP-AES detection in environmental and biological samples, the concentration factor was 500.

Shemirani et al.⁵² proposed a method for the preconcentration of Cd(II), Cu(II), Zn(II), and Ni(II) using a minicolumn filled with silica gel modified with (N,N'-bis(salicylene) phenylene-1,3-diamine Schiff' base. The proposed method was applied to the analysis of the metal ions in natural water samples and to a standard reference aluminium alloy material.



(N,N'-bis(salicylidene)-o-phenylenediamine (Salphen)

Shamsipur et al.⁵³ prepared highly selective Cr(III) PVC-Membrane electrodes based on some Schiff's bases, the electrodes have been applied to determine Cr(III) in water samples. The Schiff's bases used are as following:



The use of niobium(V) oxide, chemically adsorbed on silica gel surface (Nb₂O₅-SiO₂), as an adsorbent in a pre-concentration system of copper (II) and cadmium (II) by flow system with FAAS was proposed by Budziak et al.⁵⁴ The enrichment factor when using a sample volume of

10.2ml and 2 min of pre-concentration was 17.5 and 20.3 for copper (II) and cadmium (II), respectively. Good accuracy was obtained by the analysis of water reference materials and environmental samples.

Mashhadizadeh et al. have developed a selective method for extraction of Cu(II) by sorption on octadecyl silica membrane disk modified with bis-(3-methoxy salicylaldehyde)- 1,6-diaminohexane and determination with atomic absorption spectrometry. The retained copper eluted with a minimum volume of EDTA with a pre-concentration factor of 100. The maximum capacity of the disk was found to be 150 µg of copper ion on the disk.²²

In another work they have used octadecyl silica membrane disk modified by a recently synthesized Schiff base (Bis-(4-nitro phenyl azo) salicylidine-1,3-diamino propane) for flame atomic absorption spectrometric determination of µg amounts of Fe (III) ions in aqueous samples. The time taken for the separation and analysis of iron in 500 ml sample is 30 min at the most.⁵⁵

Dadfarina et al. have determined silver, zinc and copper employing FI-FAAS and a microcolumn of immobilized salen, 2,2'-[3-aza-1,5-pentanedyle bis (nitrilomethylidyne)]- bis-phenol, on surfactant-coated alumina. The practical concentration factors of 125, 210 and 166 for Ag, Zn and Cu, respectively and the detection limits of 0.3, 0.8 and 0.47 µg/L for Cu, Ag and Zn, respectively. The data obtained by their method agreed well with the results of independent analysis of GFAAS.⁵⁶

Jadid et al.⁵⁷ applied the modified activated carbon with 5 - ((4 – heptyloxyphenyl) azo) –N - (4-butyloxyphenyl)-salicylaldimine (HPBS) as a new sorbent for preconcentration of Cu(II) ions. The detection limit was 2.26µg/L. The procedure has been applied for the determination of copper in water samples.

Ahmadi et al.⁵⁸ have used octadecyl silica membrane disks modified by N,N'-disalicylidene-1,2-phenylendiamine (salphen) for extraction and determination of Zn by FAAS. The limit of detection was 14ng/L. Their method has been applied to the determination of zinc in natural water samples, the results has been checked by spiking experiments and comparing the results with data obtained by GFAAS.

2.5 Metal ions used in the study

The term heavy metal refers to any metallic element that has a relatively high density and is toxic or poisonous at low concentrations. Heavy metals polluted waters are produced by a variety of industrial activities associated with mining and smelting operations, metal processing, and use of metals and/or substances that contain metal pollutants. The environmental pollution by heavy metal containing effluents is of great concern, since they are not biodegradable and tend to accumulate in organisms, causing diseases and disorders.

The pollution of surface water with heavy metals, caused mainly by mining and heavy metal processing industry, is a serious problem in the developing countries. The inexistence of heavy metals polluted water treatment plants and the need of low-cost controlling and treatment process is one of the country's priority environmental protection issues.⁵⁹

2.5.1 Cadmium

The element is rare (2×10^{-5} % of the earth's crust) and is derived exclusively from zinc ores.⁶⁰ Cadmium is highly toxic element and it is now widely accepted that this metal is among the most dangerous of all the metal contaminants in food. This is not only because of its high level of toxicity but also because of its wide distribution and its many important applications. Therefore, it has been described as one of the most dangerous trace metals in the food and environment of human beings. The FAO/WHO Joint Expert Committee on Food Additives recommended provisional maximum tolerable daily intake of cadmium from all sources (water, food, and air) of 1.0–1.2mg/kg body mass.⁶¹ National and international regulations on food quality are lowering the maximum permissible levels of toxic metals in human food; thus the maximum permissible level of cadmium in drinking water is 5.0ng/L. Therefore, it is very important to determine this element in the environment such as drinking water and soil.

2.5.2 Zinc

Zinc is not abundant but widely distributed in nature. The chief ores are the sulphides and the carbonates. Zn^{2+} ion is a harder acid than Cd^{2+} or Hg^{2+} and resembles Mg^{2+} in forming strong complexes with oxygen donors. Zn also forms octahedral complexes with ammonia and ethylenediamine. Zinc complexes of sulphur are also known.⁶⁰

Also it is one of the important elements for the growth of the plants and animals and it is widely used for many industries. It is used for the protection of steel against corrosion, in dry batteries, photoengraving and lithiography.⁶² Therefore, sensitive, selective and rapid methods for the determination of zinc are in great demand. Spectrophotometry, atomic absorption spectroscopy,

neutron activation analysis, inductively coupled plasma-atomic emission spectroscopy, inductively coupled plasma-mass spectroscopy, direct current–inductively coupled plasma-atomic emission spectroscopy and X-ray fluorescence are widely applied for the determination of zinc in trace levels.

2.5.3 Copper

Copper does not occur abundantly in the nature (about 1×10^{-4} of the earth's crust). The chief ore is copper pyrites, CuFeS_2 . Copper(II) forms many complexes with nitrogen donors. The four coordinate complexes are usually much more stable than six-coordinate ones, although many of these exist.⁶⁰

Although copper (II) ions are extremely important for several biological processes, their levels in some situations are indications of a problem of disease in many living systems or environments. Copper accumulation in the liver is a characteristic of Wilson's disease producing neurological and psychiatric defects.⁶³

2.6 Detection system

After elution of the analyte from the sorbent, spectrophotometry, chromatography, atomic absorption and atomic emission are normally used to detect the amount of the analyte of interest.

2.6.1 Atomic absorption spectroscopy (AAS)

Spectrometric methods are a large group of instrumental methods that deals with atomic and molecular spectroscopy. The most widely used spectrometric methods are that which based on the interactions of the electromagnetic radiations and the matter. Atomic absorption spectrometry (AAS) uses the interaction of light with the analyte atoms to measure the absorption which related to the concentration of the analyte atoms in the gas phase.

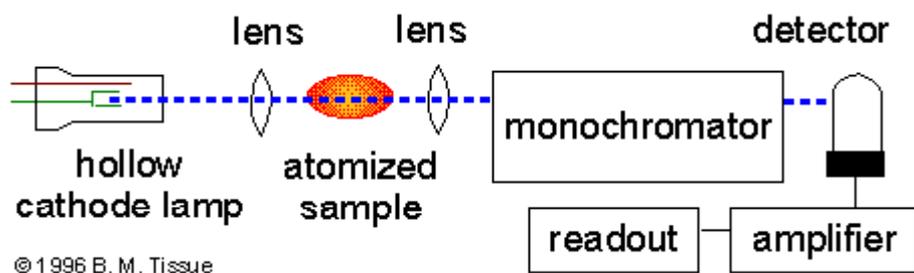
The light source is usually a hollow-cathode lamp of the element that is being measured, which uses a cathode made of the element of interest with a low internal pressure of an inert gas. A low electrical current (~ 10 mA) is imposed in such a way that the metal is excited and emits a few spectral lines characteristic of that element (for instance, Cu 324.7 nm and a couple of other lines). The light is emitted directionally through the lamp's window, a window made of a glass transparent in the UV and visible wavelengths.

Atomic Absorption spectroscopy requires that the analyte atoms be in the gas phase. Ions or atoms in a sample must undergo desolvation and vaporization in a high-temperature source such

as a flame or graphite furnace. Flame AA (FAAS) can only analyze solutions, while graphite furnace AA (GFAAS) can accept solutions, slurries, or solid samples.

The analyte concentration is determined from the amount of absorption. Applying the Beer-Lambert law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and nonuniformity of concentration and path length of analyte atoms (in graphite furnace AA). Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

The graphite furnace has several advantages over the flame. It is a much more efficient atomizer than a flame and it can directly accept very small absolute quantities of sample. It also provides a reducing environment for easily oxidized elements. Samples are placed directly in the graphite furnace and the furnace is electrically heated in several steps to dry the sample, ash organic matter, and vaporize the analyte atoms.



Schematic diagram of Flame atomic Absorption Spectrometer (FAAS).

Flame atomic absorption spectrometry (FAAS) is the most frequently chosen technique for this purpose as shown by thousands of papers published since its introduction in 1955. Flame atomic absorption spectrometry is often accepted because of its speed and ease of operation, but it has the major drawback of low sensitivity for direct determination of metals in many matrices. Therefore, for ultra trace determination of metals a separation/pre-concentration step is required. Cloud point extraction, solvent extraction, electrodeposition, ion exchange, coprecipitation are widely used pre-concentration and separation techniques for the trace metal ions in various samples, including water and biological media, which consist of high saline complex matrices. Solid-phase extraction based on adsorption has an important place in the pre-concentration studies because of its simplicity.⁵⁶

2.6.1.1 Interferences in AAS

Factors that may affect the ability of the instrument to read this parameter can also be classified as interference. Interferences of two types are encountered in atomic absorption methods.

Spectral interferences arise when the absorption or emission of an interfering species either overlaps or lies so close to analyte absorption or emission that resolution by the monochromator becomes impossible.

Chemical interferences result from various chemical processes occurring during atomization that alter the absorption characteristic of the analyte.

Interferences could be a real problem. It is important for the analyst to establish a set of analytical protocol which is appropriate for the sample to be analyzed and for the information required.⁶⁴

2.6.1.2 Calibration and standard curves

A working curve is a plot of the analytical signal (the instrument or detector response) as a function of analyte concentration. These working curves are obtained by measuring the signal from a series of standards of known concentration. The working curves are then used to determine the concentration of an unknown sample, or to calibrate the linearity of an analytical instrument.

The idealized calibration or standard curve is stated by Beer's law that the absorbance of an absorbing analyte is proportional to its concentration. It is based on the measurement of the transmittance T or the absorbance A of solutions contained in transparent cell (Flame in case of FAAS) having a path length of b cm. Ordinarily, the concentration c of an absorbing analyte is linearly related to absorbance as presented by the equation

$$A = -\log T = \log P_0/P = \epsilon b c$$

Where

P_0 is incident beam power

P is Emergent beam power

ϵ is Molar absorptivity

b is path length

c is molar concentration of the solution

2.6.1.3 Sample preparation for AAS measurements

The required sample preparation varies somewhat depending on which type of atomizer is to be used. Flame atomizer requires liquid samples of relatively low viscosity and with no suspended

particulate matter that could clog the nebulizer. Highly viscous liquids are not readily nebulized and must be diluted with low viscosity solvent. Relatively few solid samples are directly soluble in common solvents and require some chemical pre-treatment to convert the various constituents to soluble forms. Not only are these decomposition and solution steps time consuming, they are potential sources of error. When the analyte concentration is very low its important to ensure that the reagents used in the pre-treatment steps don't contaminate the sample with traces of the analyte. While the graphite furnace can handle both liquid and solid samples, matrix effects generally are more severe with this atomizer and more sample pre-treatments may be necessary. Chemists use the term "matrix" as a general reference to the analyte part of the sample or the environment in which the analyte exists.⁶⁵

2.6.1.4 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) for any analytical procedure, the point at which analysis is just feasible, may be determined by a statistical approach based on measuring replicate blank (negative) samples or by an empirical approach, consisting of measuring progressively more dilute concentrations of analyte. The LOD may be expressed as:

$$\text{LOD}=3 * \text{SD}/\text{S}$$

where

SD=the standard deviation of the response

S = the slope of the calibration curve

The limit of quantification (LOQ), or concentration at which quantitative results can be reported with a high degree of confidence, may likewise be determined by either approach. It is also defined as 3.33LOD.⁶⁴

3 OBJECTIVE

3.1 *General objective*

The main target of this study is the development of new sorbents for pre-concentration and separation of metal ions from aqueous solutions and to study the features of the sorbent in order to develop an analytical method for separation and pre-concentration of metal ions, using Schiff's bases.

3.2 *Specific objectives*

- To find a suitable solid support to be used in fixing the organic compounds, Schiff's bases on its surface by studying Silica gel, C18, Aminopropyl modified silica.
- To establish and synthesize Schiff's bases which can be used as ligand in the solid support surface to get a new sorbent with high sorption/adsorption efficiency; variables are the bridging group on one side and the functional groups on the imine carbon.
- To optimize the conditions for extraction.
- To study the effect of possible interferences.
- To improve trace element determinations of selected elements important from the environmental point of view.
- To develop simple and powerful alternatives for the high sophisticated techniques used for elemental analysis like GFAAS, ICP-AES, and ICP-MS.
- Application of the developed procedure for analysis of real samples.

4 EXPERIMENTAL

4.1 Instrumentation and Equipments

A Shimadzu model AA-6200 atomic absorption spectrometer was used for determination of metal concentrations.

For GFAAS experiments UNICAM 939 Graphite Furnace AA Spectrometer with Autosampler UNICAM FS90 has been used.

The pH measurements were made by a Knick pH-Meter 761.

A pre-concentration vacuum system 24G (7208-00) from J.T. Baker has been used for extraction, washing and elution steps.

The weight of the compounds was determined using electronic balance Mettler-Toledo AE 240 (sensitivity 0.0001 g to 40 g)

4.2 Materials

Commercial Silica gel cartridges from J.T. Baker (1000mg/6ml) (Product number 7086-07) were used as solid support.

Specification of Silica

Average Particle Diameter (APD): 55 μ m

Specific surface area: 537m²/g

Country of origin: USA

C18 polar plus silica from J.T. Baker (1000mg/6ml) (Product number 7066-07)

Aminopropyl modified silica (CHROMABOND) columns from Macherey-Nagel (500mg/3ml) (Product number 730 033)

Polyethylene volumetric flasks were used for preparation of metal ions solutions, and glass volumetric flasks for Schiff's bases solutions.

4.3 Chemical Reagents, Solvents and Water

Deionized water, purified by Elix3-MilliQ system (Millipore, USA) was used throughout.

The metal standard solutions were prepared from a standard solution (1000mg/l). Appropriate dilutions were made, whenever necessary, with deionized water.

Table 4-1 *List of the chemicals used.*

No.	Name	Company	Quality	purity
1	Acetone	KMF	For analysis	99.5%
2	Methanol	KMF	For analysis	99.8%
3	Nitric acid	KMF	For analysis	65%
4	Ethanol absolut	KMF	For analysis	>99.8%
5	Ammonium hydroxide	KMF	Puriss. p. a.	25%
6	Petroleum ether	Merck		Extra pure
7	Salicylaldehyde	Merck	For synthesis	99%
8	Ethylene diamine	Merck	For synthesis	>99%
9	1,3-diaminopropane	Merck	For synthesis	>99%
10	2,2-dimethyl-1,3-diaminopropane	Merck	For R&D	99%
11	2-hydroxyacetophenone	Fluka	Purum	>98%
12	Ammonium chloride	Merck		99.8%
13	Ammonium carbonate	Riedel de-Haen		Extra pure
14	Ammonium sulphate	Riedel de-Haen	For analysis	>98%
15	Ammonium acetate	Merck	For analysis	98%
16	Copper standard solution Cu(NO ₃) ₂	Merck		999-+2mg/l
17	Cadmium standard solution Cd(NO ₃) ₂	Merck		999.8-+2mg/l
18	Zinc standard solution Zn(NO ₃) ₂	Merck		1000-+2mg/l
19	Magnesium standard solution Mg(NO ₃) ₂	High purity standard		1000-+3mg/l
20	Calcium standard solution Ca(NO ₃) ₂	High purity standard		10-+ 0.03mg/ml
21	Stronium standard solution Sr(NO ₃) ₂	Merck		999-+2mg/l
22	Barium standard solution Ba(NO ₃) ₂	Merck		1001-+2mg/l
23	Palladium standard solution Zn(NO ₃) ₂	Merck		1001-+5mg/l
24	Silver standard solution AgNO ₃	High purity standard		1000-+3mg/l
27	Vanadium (IV) standard solution VO ₂ SO ₄	Merck		1001-+2mg/l
28	Vanadium (V) standard solution NH ₄ VO ₃	Merck		997-+5mg/l

4.4 Procedure

4.4.1 Preparation of the column

6ml columns filled with 1g silica gel each from J.T.Baker were used. 6ml of 10^{-2} M Schiff's base in acetone passed through the column by gravity action after cleaning with 3ml 2M HNO_3 , then 20ml Milli-Q water, then 2ml methanol, and finally 2ml acetone. The columns have been kept at room temperature for approximately 30 minutes to complete the solvent evaporation. In case of C18 polar plus silica and aminopropyl silica 6ml of n-Hexane was passed through the column by gravity after the methanol step to make the C18 chains uncoil.

4.4.2 General procedure for metal determination

After adjustment of metal sample solutions to pH 5-6 given volumes up to 1000ml were passed through the column at flow rate of 4-10ml/min. After a washing step with 3ml deionized water the adsorbed metal was eluted with 10ml of 2mol/L HNO_3 at an elution rate of 10ml/min. The concentration of metal ions in the eluate was determined by FAAS.

4.5 Preparation of the ligands

***N,N'*-Bis(salicylidene)ethylenediamine.=(ES)**

20,86ml (0,2mol) of salicylaldehyde was added dropwise with vigorous stirring to 6,7ml (0,1mol) ethylenediamine in ethanol. The product precipitated immediately. The product, re-crystallized from ethanol, was filtered and dried to give the desired Schiff's base as yellow crystals.

***N,N'*-Bis(salicylidene)1,3-propylenediamine=(PS)**

The same amount of salicylaldehyde was added to 8.4ml (0,1mol) of 1,3-diaminopropane in the same procedure, the product was yellow crystals.

***N,N'*-Bis(salicylidene)2,2-dimethyl1,3-propylenediamine=(DMPS)**

The same amount of salicylaldehyde was added to 10.218g (0,1mol) of 2,2-dimethyl-1,3-diaminopropane in the same procedure, the product was yellow crystals.⁶⁶

Bis-(2-Hydroxyacetophenone)-2,2-dimethyl-1,3-propanediimine=(SBTD)

2-hydroxyacetophenone (0.02 mol, 2.72 g) was mixed with 150mL of distilled ethanol in a 500 mL round bottom flask, which was stirred using a magnetic stirrer. 2,2-dimethyl-1,3-propanediamine (0.015 mol, or 1.02 g) dissolved in 25 ml of distilled ethanol was added drop by drop, using a dropping funnel to the above solution. The contents were refluxed for 2 h to get a yellow solid precipitate of Schiff's base ligand, which was cooled, filtered, washed with ether and dried. The ligand was then re-crystallized from ethanol.⁴⁸

4.6 Effect of pH on metal adsorption

For this study, the pH values of the element for 50 ml solutions were adjusted within the range of 1-10 using HNO₃ or NH₄OH then passed through the loaded columns and eluted with 10ml M HNO₃ and then measured by FAAS.

4.7 Effect of ligand amount

The ligand amount and the eluent volume are other important parameters obtaining quantitative recoveries. Quantitative retention is not obtained by smaller than optimum amount of the Schiff's base. Different volumes of the Schiff's base have been used 3, 4.5, 6 and 7.5ml which contain 3×10^{-5} , 4.5×10^{-5} , 6×10^{-5} , and 7.5×10^{-5} moles, respectively.

4.8 Effect of sample volume

Studies were also performed with metal solutions with different sizes to find the possibility to enrich solutions at low concentrations with a high concentration factor. A series of 50,100,250,500, and 1000ml solutions spiked with 20 μ g Zn have been tested.

4.9 Capacity of the sorbents

In order to study the adsorptive capacity of Schiff's base-immobilized silica gel for metals, column method was used. 50ml of 20ppm metal solution at pH 5.00 was passed through the column and then eluted with 10ml 2M HNO₃ and diluted to 50ml and determined by FAAS. The absolute amount of the metal is then calculated in the final solution which is the maximum amount adsorbed on the surface of the sorbent.

4.10 Interference effect

Various salts and metal ions are added individually to 50ml of solutions containing 20 μ g Zn(II), and 50 μ g Cd(II) or Cu(II) in different ratios (1:1), (1:10), (1:100), (1:1000) and (1:10000) and the general procedure was applied.

4.11 Experimental part of GFAAS measurements

4.11.1 The Temperature Programme

The temperature programme suggested by the SOLAAR-software for Cadmium-analysis revealed two major problems. The first one was a slight peak tailing during the atomisation phase preventing a proper determination of the peak area and consequently the Cadmium concentration. In order to catch the whole sample several runs were conducted to find the optimal temperature and length of the atomisation phase. A length of 5 s at unchanged 1200 °C gave the best results. Another problem was the accumulation of Cadmium. The Peak area increased from run to run probably because the Cadmium was not completely atomized. In order to fix that problem a cleaning step was added to the furnace programme. Table 4-2 compares the suggested and the optimised programme.

Table 4-2 Temperature programme of Cd determination by GFAAS

Step	Suggestion by Software	Optimised
Drying	120 °C for 30 s Gas flow: 2	120 °C for 30s Gas flow: 2
Pyrolysis	800 °C for 20 s Gas flow: 2	800 °C for 20 s Gas flow: 2
Atomisation	1200 °C for 3 s Gas flow: none	1200 °C for 5 s Gas flow: none
Cleaning	None	2200 °C for 5 s Gas flow: 3

4.11.2 Effect of Schiff's base volume

Volumes of 1, 2, and 3 ml of 0.01M PS solution have been used to coat the silica columns. 50 ml of a sample containing 2 ppb Cadmium were passed through the columns. The Cadmium was eluted with 5 ml 2 M nitric acid into 50 ml plastic flasks which were filled up to the mark with Millipore water afterwards.

4.11.3 Effect of sample concentration

Five different samples with Cadmium-concentrations of 10, 20, 30, 40 and 50 ppb were prepared and the pH was adjusted to 5. 50 ml of each sample were passed through a 1000 mg silica column, eluted with 5 ml 2 M nitric acid and then filled up to a total volume of 50 ml with Millipore water. The samples were all diluted to a concentration of 2 ppb before measurement to be in the linear range of GFAAS.

4.11.4 Effect of sample volume

50, 100, 1000 and 2000 ml of MQ water containing the same amount of Cd ion (0.1 μg of cadmium) were sucked through the sorbent columns. The columns were eluted with 1.5 ml 2 M nitric acid into 50 ml flasks and filled up with Millipore water. Hence every eluent should have a final concentration of 2 ppb.

4.11.5 Effect of eluent volume

50 ml samples containing 20ppb of Cd were sucked through the sorbent columns. Then the columns were treated with 0.5, 1, 2.5 and 5 ml of 2 M nitric acid.

5 RESULTS AND DISCUSSION

5.1 Pre-concentration/separation of Zn, Cd, and Cu on different Schiff's bases loaded on silica gel

The system used in the study consists of three main parts, the solid support, Schiff's base as chelating agent, and the detection system.

In the first part we are going to discuss the effect of the structure of the Schiff's bases on the pre-concentration/separation of the metal ions using silica gel as a solid support and FAAS as a detection system in order to study sorption/desorption behaviour of the Schiff's bases.

In the second part we will discuss the use of the same system but with GFAAS as a detection system with low limit of detection for ultra trace element analysis.

The third part is a study of the effect of the solid support on the pre-concentration/separation process. A comparison has been made between three different solid supports, silica gel, C18 polar plus silica, and aminopropyl silica using all the Schiff's bases and FAAS as a detection system.

5.1.1 Structure of the Schiff's bases

Four Schiff's bases different in their structure have been used in our study are shown below in *Figure 5-1*. The main difference between them is NN bridge which is ethylene in case of ES, propylene in case PS, and 2,2-dimethyl propylene in case of DMPS and SBTD. SBTD has two more methyl groups connected to the carbon atom of imino group. These differences can vary the efficiency of the Schiff's bases to interact with the solid support and the metal ion of interest by means of changing the electronic density of the lone pair of nitrogen atom and the steric hindrance occurred by the presence of methyl groups. The steric and electronic effects around the metal core can be finely tuned through the appropriate selection of electron withdrawing or electron donating substituents in the Schiff's bases. Salens are N_2O_2 donating Schiff bases, these N and O atoms induce two opposite electronic effects: the phenolate oxygen is regarded a hard donor, which stabilizes the higher oxidation states while the imine nitrogen is a softer donor and will hence stabilize the lower oxidation states of the metal ion.⁶⁷

Molecular weights and pKa values of Schiff's bases are shown in Table 5-1 .

The elements Zn and Cd follow Cu and Ag respectively. Each has a filled (n-1)d shell plus two ns electrons. While Cu and Ag give rise to ions or complexes in which one or even two d electrons are lost, that is to compounds in oxidation states II and III, no such compounds have ever been isolated for the group 12 metals. Thus while Cu and Ag are classified as transition elements Zn and Cd are not.⁶⁸

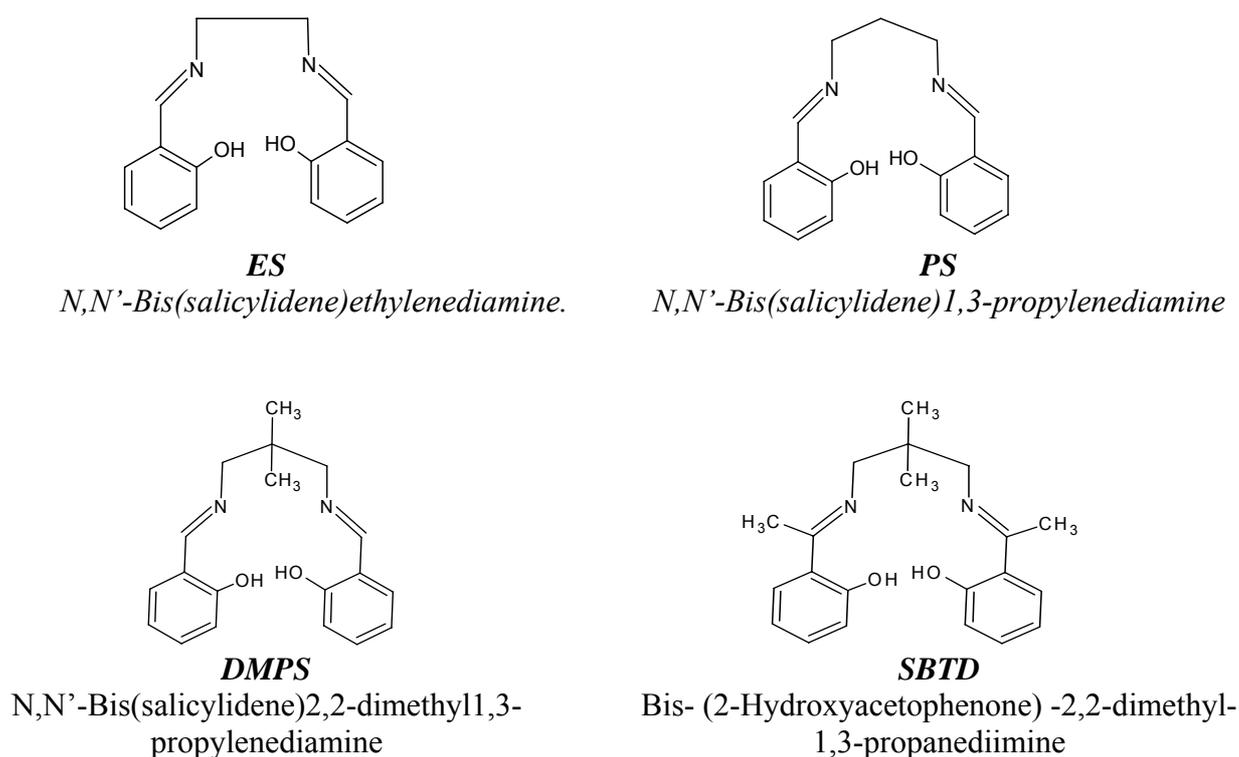


Figure 5-1 Structure of Schiff's bases used in the study.

Table 5-1 Molecular weights and pKa values of Schiff's bases.

Ligand	Formula	Molecular Weight	pKa
ES	C ₁₆ H ₁₆ N ₂ O ₂	268.31	12.58-+0.5
PS	C ₁₇ H ₁₈ N ₂ O ₂	282.34	12.54-+0.5
DMPS	C ₁₉ H ₂₂ N ₂ O ₂	310.39	12.62-+0.5
SBTD	C ₂₁ H ₂₆ N ₂ O ₂	338.44	13.18-+0.5

5.1.2 Interaction between metal ions and sorbents

In order to understand the behaviour of Schiff's bases toward ions present in the solution many factor must be taken in consideration, such as type of interaction between Schiff's bases and silica surface and between Schiff's bases and metal ions.

There are three parts of interaction in our system, firstly the bonding between silica gel surface and Schiff's bases which is hydrogen bonding between the silanol group and the phenyl group in Schiff's base, and this part is affected by the structure of Schiff's base used, ES and PS can bind easily with silanol group but DMPS and SBTD have extra methyl groups which hinder the formation of H-bonds.

The second part of interaction is chemical interaction between Schiff's bases and metal ion which is supposed to be a complex formation. This type of interaction is affected by pH, charge on metal ion, ionic size and ionic form of metal ion, concentration of the metal ion, and interferences.

The third part is the interaction between the metal ions and free silica silanol groups directly which is kind of cation exchange. Divalent transition metals are bound as monodentate (S-O-M^+) and bidentate ((SOO)M) surface complexes or as hydroxyl species (SO-M-OH).⁶⁹

The nature of the functional group will give an idea of the selectivity of the ligand towards trace elements. In practice, inorganic cations may be divided into 3 groups:

- *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 (Ca^{2+} , Mg^{2+} , Na^{2+}) 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*: 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 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 atoms: (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.^{13,70}

The ligand coordinates to metal ion in a tridentate manner through the deprotonated phenolic oxygen and the imine nitrogen. The coordination sphere of metal is completed by a water molecule and a nitrate ion that binds in an asymmetric chelating mode.⁷¹

The structures of metallosalen complexes range from essentially planar to highly twisted. Depending on the oxidation state of the central metal ion of the complex, the ligand structure and nature of the possible counterion, salen complexes have coordination geometries from four coordinate square planar to eight coordinate dodecahedron with varying degree of distortion.

If the salen ligand has substituents at the imine carbons conformation is adopted to avoid steric repulsion.⁷²

5.1.3 Effect of pH on metal adsorption

The Zn, Cd and Cu uptake by the sorbent as a function of pH value of the solution is presented in Figure 5-2, 5-3, and 5-4.

The recovery of the analytes was calculated from the ratio of the concentration found by FAAS and the concentration calculated theoretically as following

$$\text{Recovery\%} = \frac{\text{analyte concentration in eluent (FAAS)} \times \text{volume of eluent}}{\text{analyte concentration in sample solution} \times \text{volume of sample solution}} \times 100$$

The pH of the metal ion water solution is an important controlling parameter in the sorption process. The pH of the solution affects the adsorbent capacity, the metal adsorption typically increases with increase of pH; and the mechanism of metal ions binding, by changing it from ion exchange and/or adsorption at acidic region to adsorption and/or complexation and possible precipitation at the basic region.⁵⁹ The formation of a stable chelating complex requires that the chelating agent of a Lewis base has a functional group. The group could donate its electron pair to metal ions, and it is an advantage for the agent if it has a negative charge. The Schiff's bases used as chelating agents have two hydroxyl groups and two donating electron pairs, so it can form stable chelates with metal ions. Here two of hydroxyl groups can be ionized in a basic solution.³⁷

The pH values of the element solutions were adjusted within the range of 1-10 using HNO₃ or NH₄OH. The optimum retention pH range of the sample solution was 4-8.

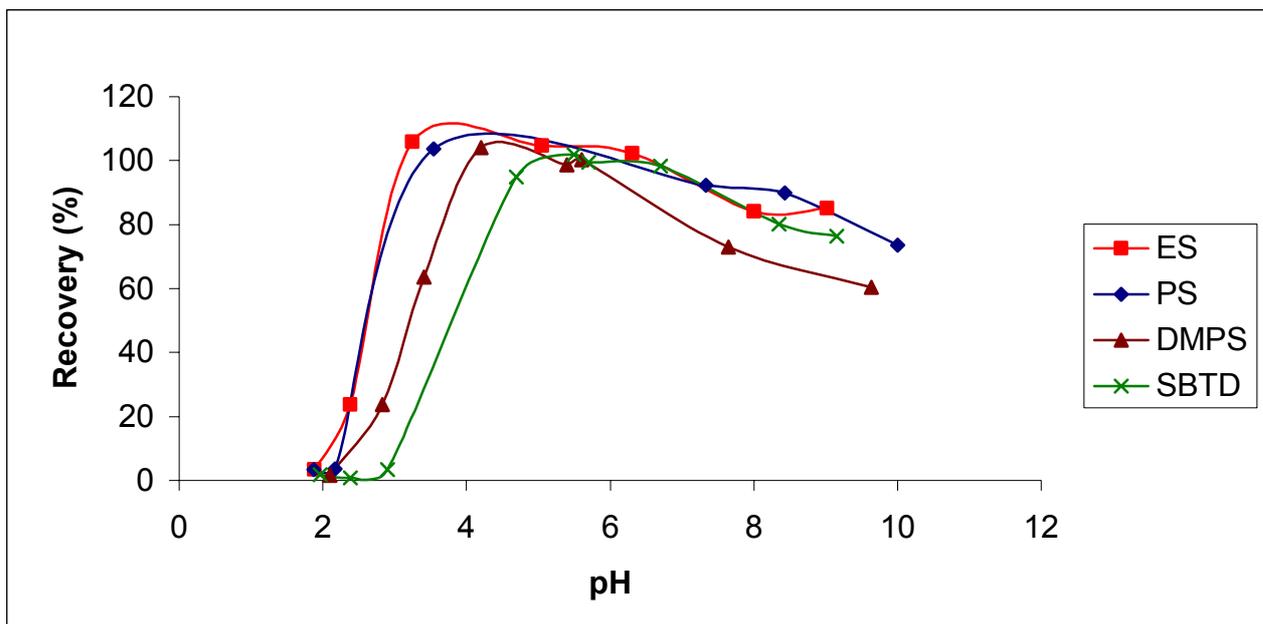


Figure 5-2 Influence of sample pH on the recovery of Zn(II) from silica gel coated with Schiff's bases ligands.

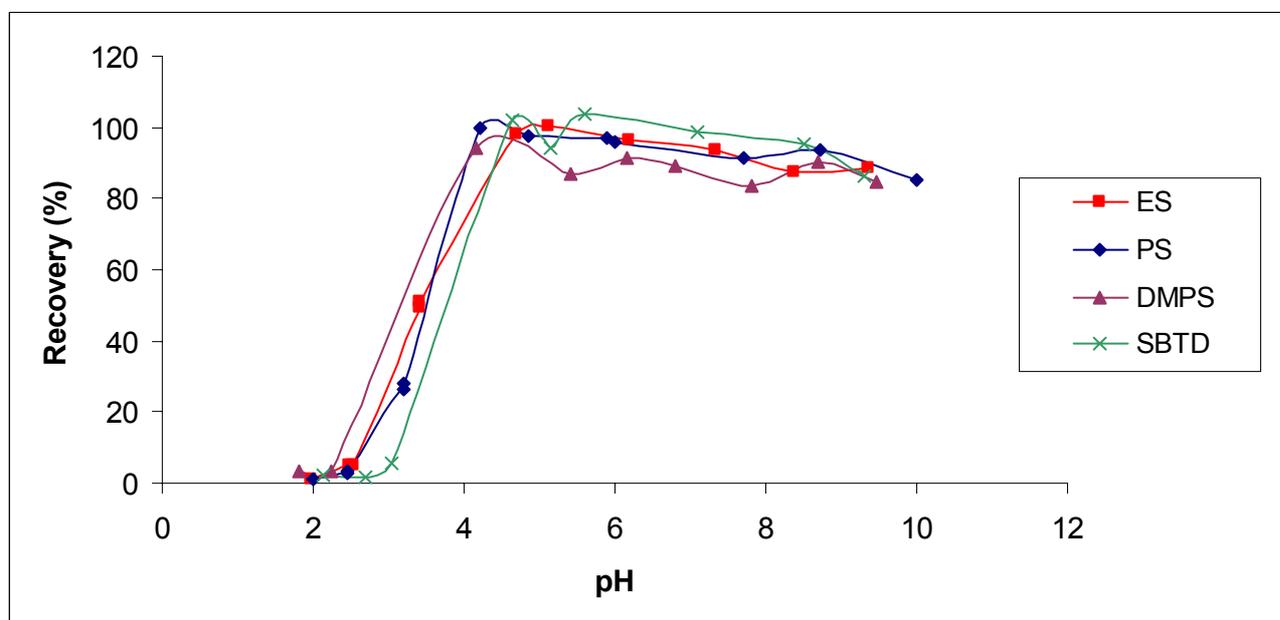


Figure 5-3 Influence of sample pH on the recovery of Cd(II) from silica gel coated with Schiff's bases ligands.

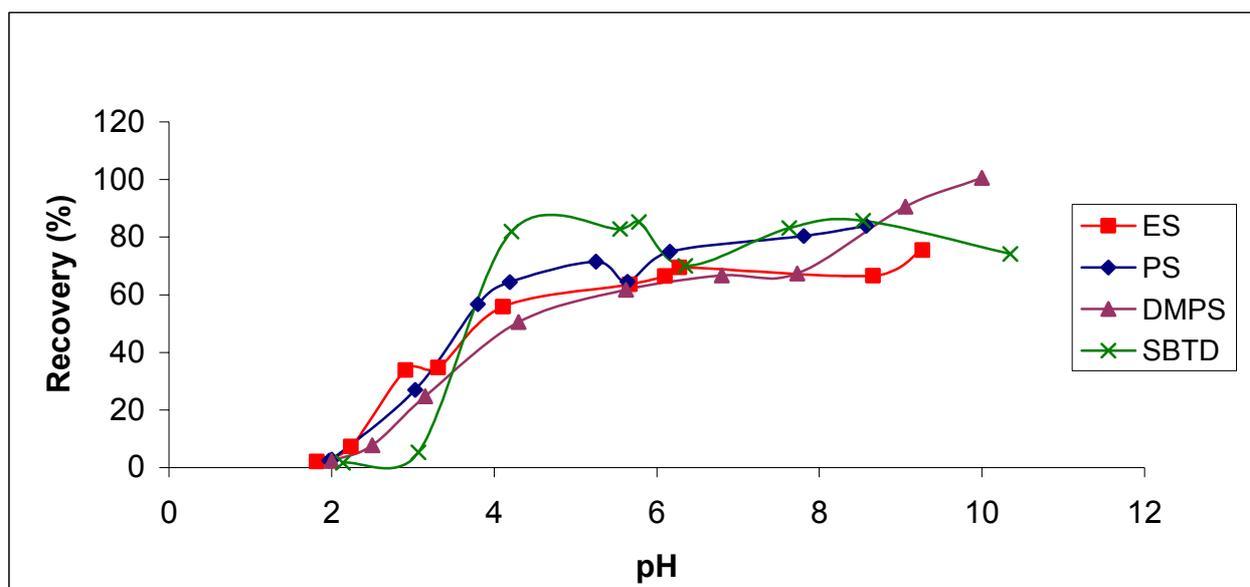


Figure 5-4 Influence of sample pH on the recovery of Cu(II) from silica gel coated with Schiff's bases ligands.

Two types of functional group, Lewis base ($=\text{CO}, -\text{OH}$) at acidic pHs and Lewis acids ($-\text{NH}_2$) at basic pHs, seems to be involved in the binding of the metal studied. With very strong acidic ($\text{pH} < 2$) or basic ($\text{pH} > 10$) solutions, the metal is not retained by the adsorbent. The retention of the metal is very low, either because acidic functional groups are strongly protonated in acidic solution or the metal is bound to hydroxyl groups.^{48,52}

The increase in metal ion removal as increased pH can be explained on the basis of a decrease in competition between H^+ and positively charged metal ion (Zn^{2+} , Cd^{2+} , or Cu^{2+}) at the surface sites which results in a lower electrostatic repulsion of the adsorbing metal ion and consecutively increased metal ion removal.⁵⁹

The low recovery at lower pH can be associated with the protonation of functional groups of the sorbent and a low degree of swelling.⁵⁹ At such a pH value, M(II) ions occur in a hydrated form $[\text{M}(\text{OH}_2)_n]^{2+}$ form. The swelling capacity grow as the solution pH increases ($\text{pH} 4-6$), which favours the interaction between the metal ions and coordinatively active groups of the sorbent. The formation of aqua and hydroxo-complexes species of given metal ion depends on the pH value of the solution and the metal ion concentration. Depending on the pH level of the metal ion aqueous solution the total concentration of the metal ion in the solution is the sum of the concentration of the metal ion (M^{2+}) and present metal-hydroxy species.

The speciation of a metal has a significant impact on the adsorbent extraction efficiency and can influence the mechanism of metal ion binding. The metal ion adsorbent selectivity is also influenced by the character of the metal complex that predominates at a particular solution pH.⁵⁹ Along with the cation form, M(II) ions in aqueous solutions with pH>5 can occur in the form of $[M(OH)(OH_2)_{n-1}]^+$ hydroxo complexes. At higher pH values, hydrolysis is accelerated and, as a consequence, the degree of M(II) sorption is gradually reduced.^{73,74} In addition to water and hydroxide, other dissolved species can act as ligands and form soluble complexes with metal ions. In all such cases, at least a portion of the ligand molecule must have a local excess of electronic (negative) charge which it donates or shares with the metal ion, thereby forming a chemical bond. For instance, ammonia can form strong complexes with some metal ions because the nitrogen atom in an ammonia molecule has an orbital containing an unshared pair of electrons. These electrons, which cause the nitrogen atom to carry a slightly negative charge in free NH_3 molecules, can be shared with metal ions to form the M- NH_3 bonds.

pH range (5-6) has been taken for all ligands to ensure the complete adsorption of metal ions. In the case of zinc, PS and ES have an optimum recovery over a wide range of pH, but pH >5 have been taken for all ligands to ensure the complete adsorption of metal ions. The differences in the behaviour of metal ions are attributed to the differences in their ionic size and their stability with Schiff bases.

In case of Zn ion (Figure 5-2) the variation of the ligand behaviour arises from the difference in their structure. ES and PS have the same trend, they reach the maximum recovery in relative low pH. This possibly indicates that the complex formation constants of Zn with ES and PS are higher than the complex formation constants with DMPS and SBTD. DMPS has two methyl groups attached to the NN bridge leading to some hindrance in complex formation. The hindrance is greater in the case of SBTD because of the extra two methyl groups attached to the imine group carbon although they are electron pushing groups expected to increase the electron density on the imine group.

In case of Cd ion (Figure 5-3) there was no significant difference in the behaviour of the Schiff bases. At high pH values the recovery of Cd ion was about 80% for all sorbents, comparing with Zn where the recovery was different for each sorbent, this could be attributed to the relative large volume of Cd ion compared with Zn and Cu and its hydroxo complexes. In case of Zn ions the governing factor is the structure of the Schiff's base.

Copper has the lowest recovery among the three metal ions under study. This can be due to the addition of NH_3 to adjust the pH of the solution could lead to the formation of complexes of successive displacement of water molecules, for example the species $[\text{Cu}(\text{NH}_3)(\text{H}_2\text{O})_5]^{2+}$ until $[\text{Cu}(\text{NH}_3)_4(\text{H}_2\text{O})_2]^{2+}$ are formed in the normal way. Addition of the fifth NH_3 can occur in aqueous solution, but the sixth occurs only in liquid ammonia.⁶⁸ At higher pH the adsorption decreases due to the precipitation of metal hydroxide, in this case the sorbent works as a normal sieve.

In general, the interesting pH range is separated into two parts; (1) the pH range of mostly strong increasing adsorption and (2) the pH of slightly increasing desorption. The best pH setting has been found to be in the maximum of the curve.

5.1.4 Effect of ligand amount and eluent volume and concentration

Quantitative recoveries for the examined volumes were obtained in the range of 6-7.5ml for both ligands used (Figure 5-5, 5-6, and 5-7). 6ml was chosen to minimize the consumption of the ligands and the solvent. Different concentrations 0.5, 1, and 2M of HNO_3 have been examined on the elution of Zn ion from PS sorbent the recovery percents were 85.57, 87.30, and 99.48 respectively, 10ml of 2M was the maximum values obtaining quantitative recovery, therefore this concentration has been fixed for all metal ion under study in order to make a comparison between their adsorption behaviour on the sorbents and the analytical performance of the sorbents used. The reason for the choice of nitric acid as eluent was that the nitrate ion is reported to be a more acceptable matrix for both flame and electrothermal AAS experiments than chloride and sulphate ions.²³

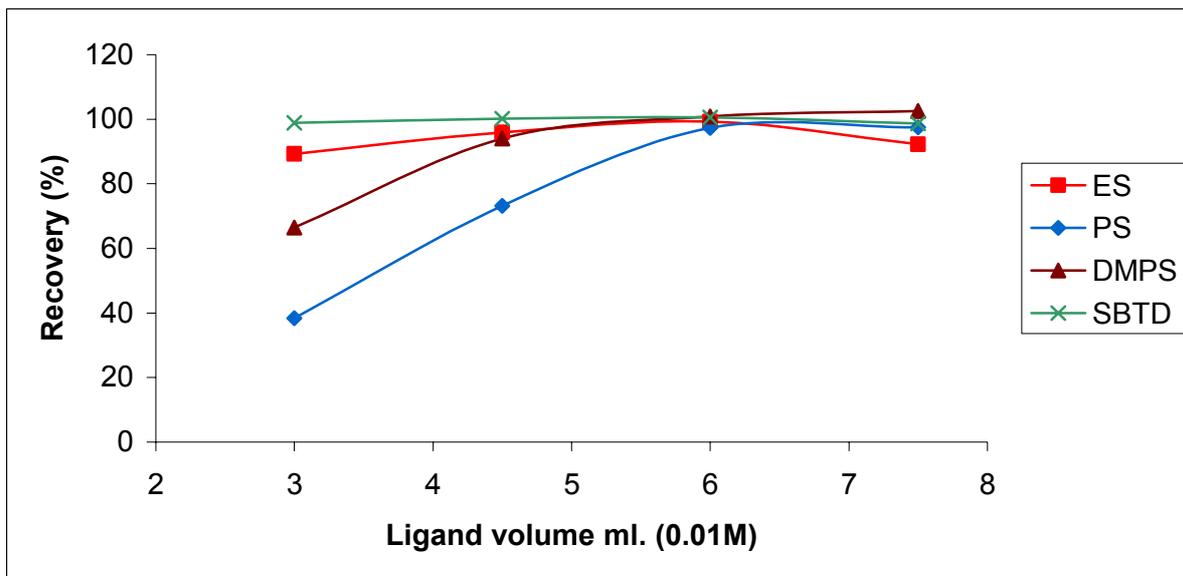


Figure 5-5 Influence of Schiff's base volume on the recovery of Zn(II) ion.

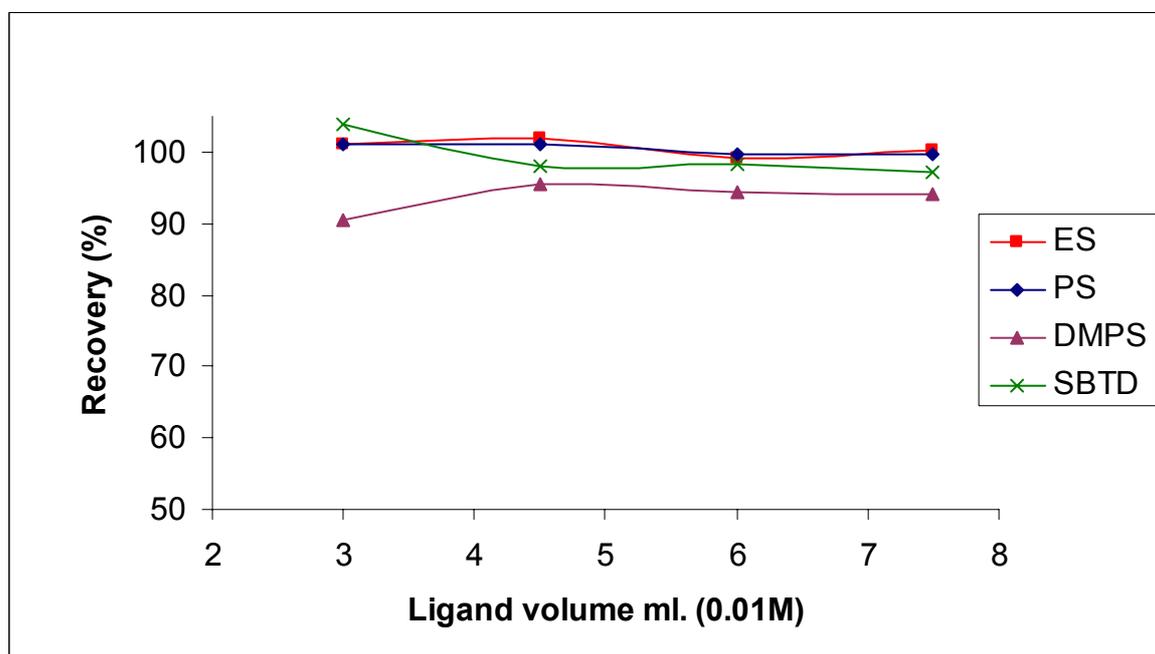


Figure 5-6 Influence of Schiff's base volume on the recovery of Cd(II) ion.

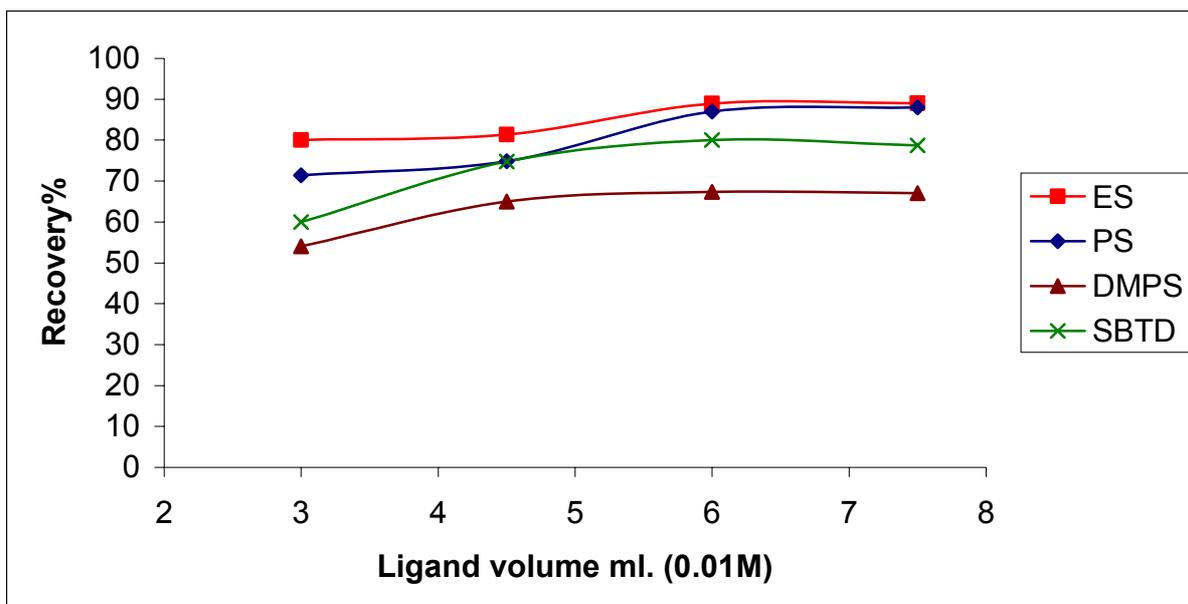


Figure 5-7 Influence of Schiff's base volume on the recovery of Cu(II) ion.

5.1.5 Effect of sample volume

The effect of changes in the volume of sample solution passed through the column on the recovery of metal ions was investigated in order to determine an applicable sample volume or a minimum analyte concentration. To get a high pre-concentration factor, a large volume of sample solution is required. From Figure 5-8 and 5-9 the recovery values were quantitative up to 1000ml (The case of Zn and Cd), the highest pre-concentration factor was found to be 100. Larger volumes have been avoided because of dynamic elution of Schiff bases by the sample is expected after 1000ml.

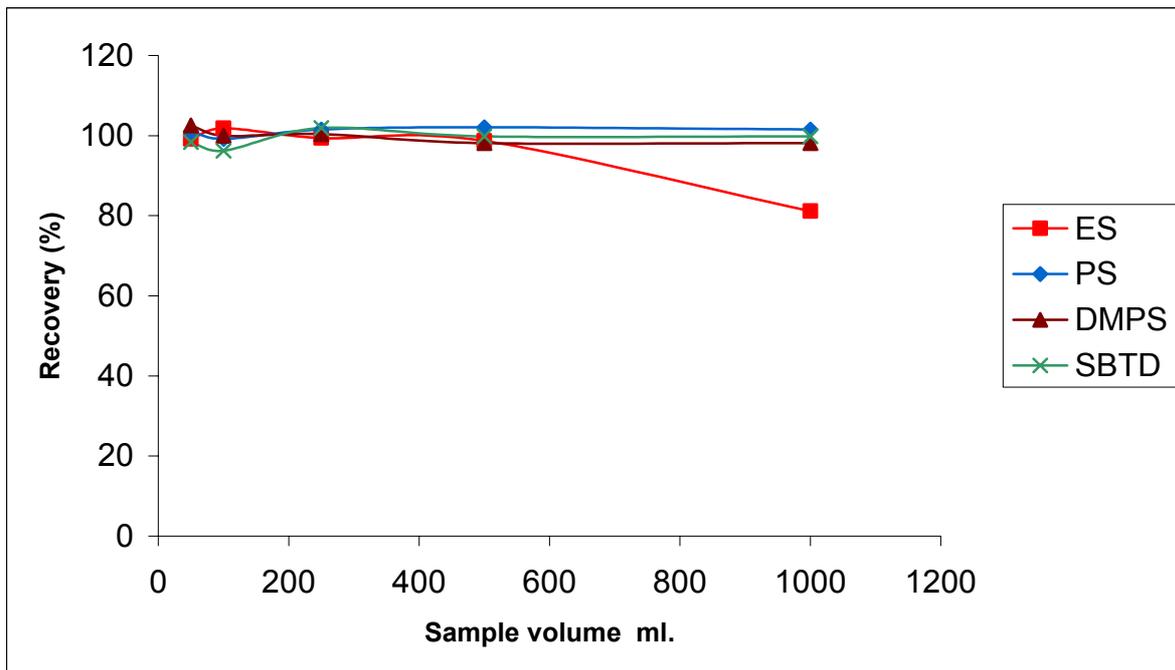


Figure 5-8 Effect of sample volume on the recovery of Zn(II) ion.

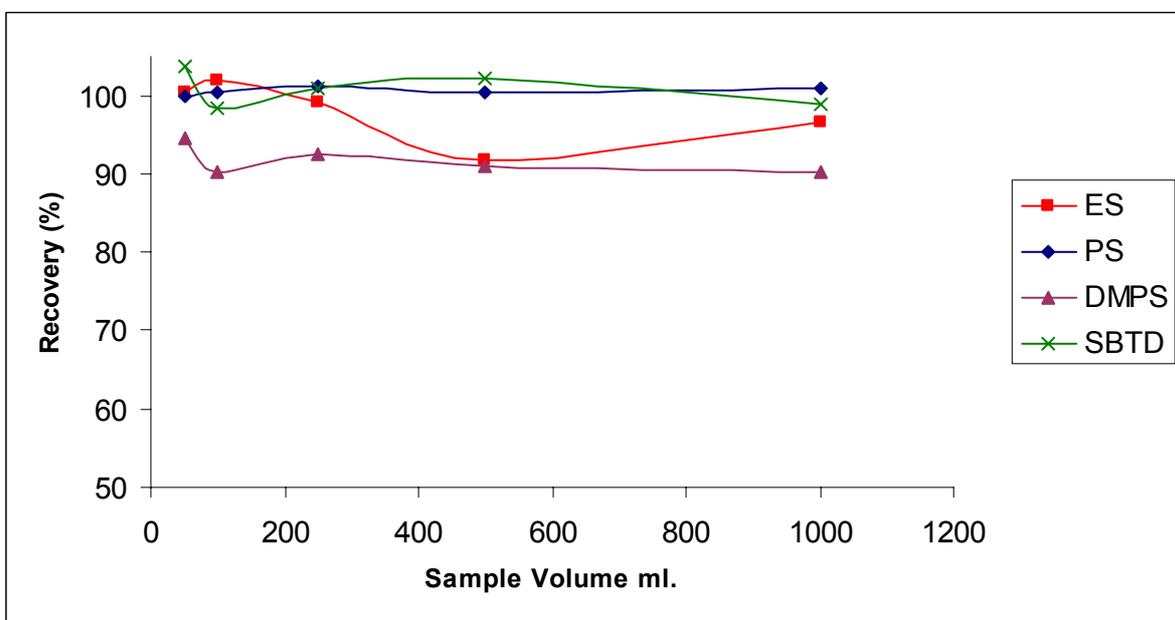


Figure 5-9 Effect of sample volume on the recovery of Cd(II) ion.

Figure 5-9 shows that DMPS is less stable than PS and SBTD. PS has less steric hindrance than DMPS and SBTD. SBTD has two electron pushing groups connected to the imine group which

enhance the stability of the complex formed with Cd ion, therefore both PS and SBTD chelates are more stable than DMPS.

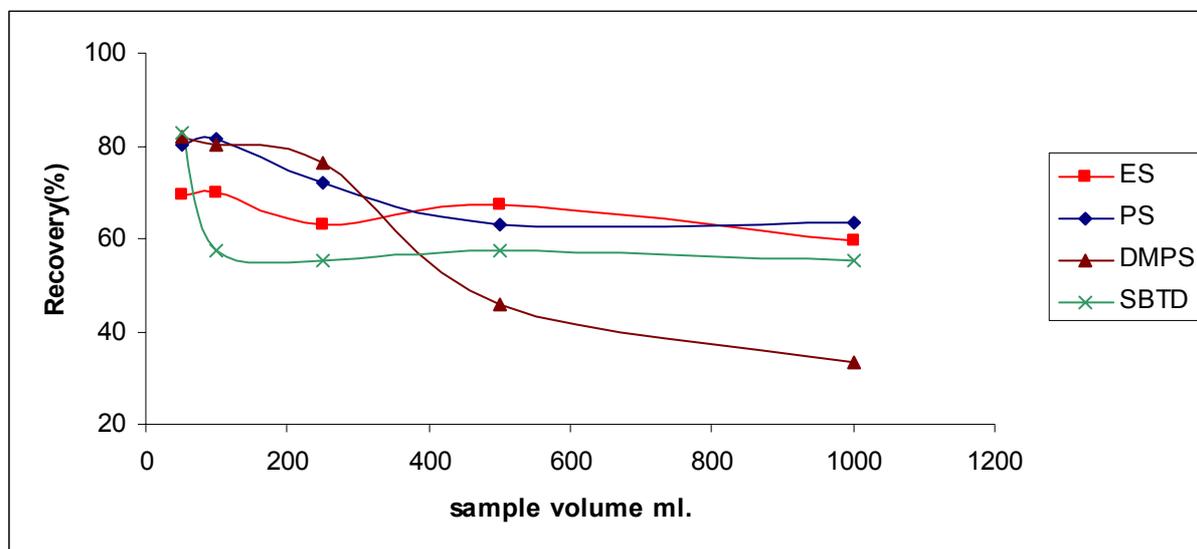


Figure 5-10 *Effect of sample volume on the recovery of Cu(II) ion.*

In case of copper ion (Figure 5-10) it shows some stability in case ES, PS, and SBTD after 100 ml although the low recovery. The highest values were in case of PS and DMPS until 250ml. The lower recovery of copper ion is attributed to the high stability copper complexes according to Irving-Williams series, which refers to the relative stabilities of complexes formed by a metal ion. It is independent of ligand choice (Mn(II) <Fe(II) <Co(II) <Ni(II) < Cu(II)> Zn(II)), in this case Cu ions work as eluent for Schiff's bases from silica surface.⁷⁰

It can be concluded that 0.02µg/ml Zn(II) and 0.05µg/ml Cd(II) could be determined by this method in 1000 ml sample volume. However, these concentrations cannot be determined directly by FAAS with sufficient accuracy.

5.1.6 Capacity of the sorbents

Adsorption capacity is an important factor to evaluate the sorbent, as it determines how much solid phase is required for a given solution. The pre-concentration and determination of the analyte is possible if the total load of the analyte is less than the sorption capacity. The capacities of the used sorbents towards metal ions used are shown in Table 5-2.

Experimental values of the capacity allow us to discuss the dependence of the chromatographic efficiency on both the properties of the stationary phase and the solutes to be separated and the experimental conditions.

The sorbent capacities toward metal ions was in the following order PS>ES>DMPS>SBTD in case of all metal ions used except for Cu the order was PS>ES>SBTD>DMPS. This sequence was expected because of the structure differences between the four ligands. PS can form six membered ring complexes with metal ions and ES forms five membered ring. Although the six membered ring could be formed by DMPS and SBTD the formation of this ring will be hindered by the presence of methyl groups on the propylene bridge in addition to presence of two extra methyl groups on both sides of SBTD close to imine group. The capacities shown in Table 5-2 have been calculated in $\mu\text{g/g}$. This is useful for analytical purposes but to explain obvious difference in the capacity it is needed to discuss the distribution of metal chelates on the sorbent surface, therefore it is needed to calculate the capacity in $\mu\text{mol/g}$.

Table 5-2 Sorbents capacities towards Zn, Cd and Cu ions ($\mu\text{g/g}$ sorbent).

METAL/LIGAND	ES	PS	DMPS	SBTD
Zn	445	473	418	302
Cd	513	549	503	490
Cu	483	583	110	137

The variation in sorption capacities of the various metal ions probably arises due to the difference in their sizes, degree of hydration and binding constants with the chelating matrix.⁷⁵

Table 5-3 Sorbents capacities towards Zn, Cd and Cu ions ($\mu\text{mol/g}$ sorbent).

METAL/LIGAND	ES	PS	DMPS	SBTD
Zn	6.80	7.23	6.39	4.61
Cd	4.56	4.88	4.47	4.36
Cu	7.60	9.17	1.73	2.15

From this point of view the capacity of Zn is greater than that of Cd with some variance due to the moderate size of Zn ion relatively to Cd.

Cd has low capacity in all cases except DMPS and SBTD. The capacity values of Cd in all sorbents are close to each other this is could be attributed to the large size of Cd ion.

The capacity of DMPS and SBTD towards Cu ion was very low, this is maybe attributed to the different basicities of the donor atoms of Schiff bases caused by inductive and steric effects. The variation in sorbent capacities towards Cu ion may be due to the stability of Cu complexes which is the highest among Irving-Williams series, also the complex formation with ammonia used to adjust the pH of the sample.

Capacity is an important factor to choose the proper sorbent for real sorption process, but there are more conditions influencing the adsorption i.e. interference effects.

5.1.7 Interference effect

Foreign ions in the sample solution can interfere with the solid phase extraction of metal complexes in a trace analysis because the ions may compete with analyte ions in the process of complex formation. In this study the influence of presence of some anions and cations with different sizes, different charges and different abilities to form metal complexes with Schiff's bases has been examined in order to know more about the sorbent behaviour.

The form of metal ion in solution changes according to pH level. At low pH values the metal ions prefer the hydrated form but the metal hydroxo species becomes more predominate as the pH increase.

Before start discussing the interference effect on the metals adsorption we should have a look on the chemistry of the metal ions in aqueous solutions.

Copper(II) forms many complexes with nitrogen donors. The four coordinate complexes are usually much more stable than the six-coordinate ones, although many of these exist also there are numerous complexes with chelating oxygen donors. In general, neutral four coordinate complexes containing chelating ligands have planar coordination.

The Zn(II) ion is a harder acid than Cd(II) in forming strong complexes with oxygen donors. These complexes usually have tetrahedral coordinated zinc atoms. It also forms octahedral complexes with ammonia and ethylenediamine. Different ionic radii of Cu, Zn, and Cd induce certain differences in the bond distances and angles of the metal salen complexes.^{36,76,77,78}

The stability of the formed complexes shows a complicated dependence on the nature of the metal ion and on the nature of the ligand. The main part of the metal-ligand bond strength derives from σ -bonding, whose extent depends on the donor properties of the ligand and the acceptor properties of the metal ion. There is no simple explanation for the observed strengths of metal ion-water interactions. As stability constants represent the differences between metal ion-water and metal ion-ligand interactions, it follows that there will be no simple universal rationalisation. Moreover, there is the added complication of significant π -bonding between certain combinations of ligands and of metal ions. Quantitative expressions with appropriate equations and parameters, and a real understanding of the various chemical factors and their interplay are still in an early stage of development. Semi-quantitative correlations of stability with various ligand and metal ion properties are often quite successful when they are restricted in scope to certain groups of ligands or metal ions.⁷⁰

5.1.7.1 Interference effect on zinc ion

In case of ES and PS the ions examined did not interfere even at very high concentration levels (Figure 5-11,13,14,15). The presence of anions such as chloride, sulphate and carbonate and also some cations such as Mg, Ca, Sr and Ba did not affect the recovery of Zn even in 10000-fold related to the Zn concentration in case of ES and PS sorbents, which means that Zn ion could be determined even in these elements samples and means also the high selectivity of ES and PS toward Zn metal ion. DMPS and SBTD are more affected by the presence of other ions in the solution. This is due to the presence of more methyl groups which hinder the complexation of DMPS and SBTD with metal ions in addition to the competition from the foreign ions in the solution.

The presence of vanadium has a high influence on the sorption of Zn ion in case of DMPS and SBTD. This could be attributed to the competition of the vanadium ions on the sorbent surface and the same thing happens in case of Ag and Pd. The different sorption pattern in the presence of vanadium could be explained by its complex hydrolysis behaviour and slow decomposition of the formed species. In the extremely diluted solutions ($<10^{-6}$ M) V(V) exists only in monomeric forms VO^{2+} (pH $<$ 3.5), H_2VO_4^- (pH 3.5–7.5), HVO_4^{2-} (pH 7.5–13). The V(IV) species in the acidic pH range are: VO^{2+} , $\text{VO}(\text{OH})^+$, $(\text{VO}(\text{OH}))_2^{2+}$, $\text{VO}(\text{OH})_2$. At $\sim 10^{-2}$ M alkaline V(IV) solution the formation of polyanionic species $\text{V}_4\text{O}_9^{2-}$ was found. In the sample solutions stored for analysis (pH 1–2) in the presence of reducing components (e.g. citrate) vanadium(V) can be spontaneously reduced to vanadium(IV). At higher pH V(IV) is exposed to slow oxidation to V(V).⁷⁹

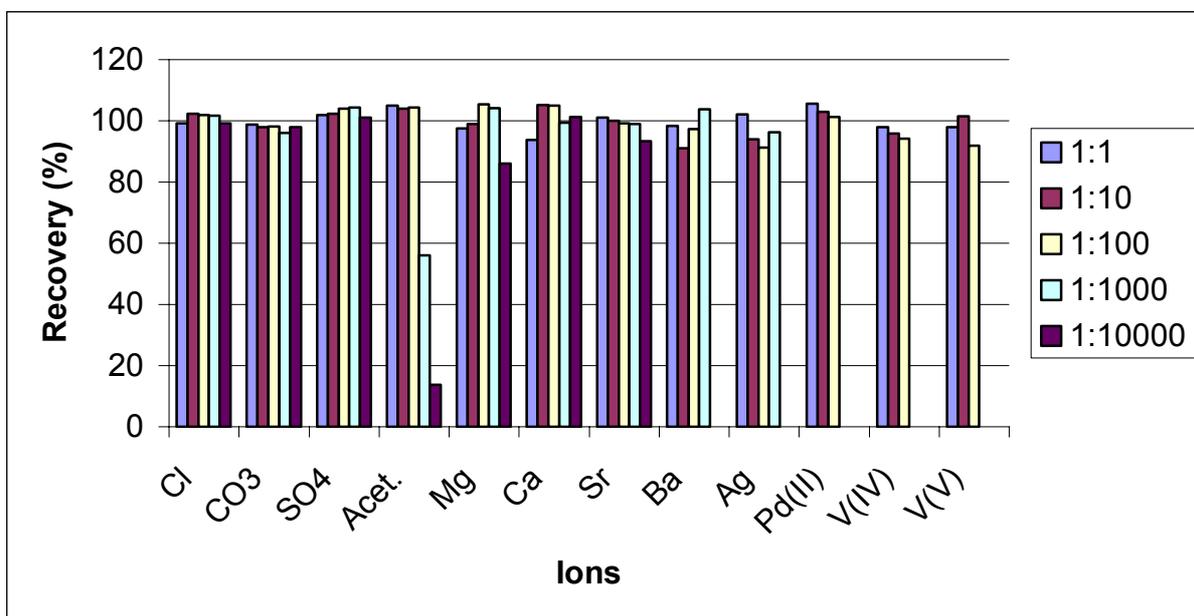


Figure 5-11 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with ES Schiff's base.

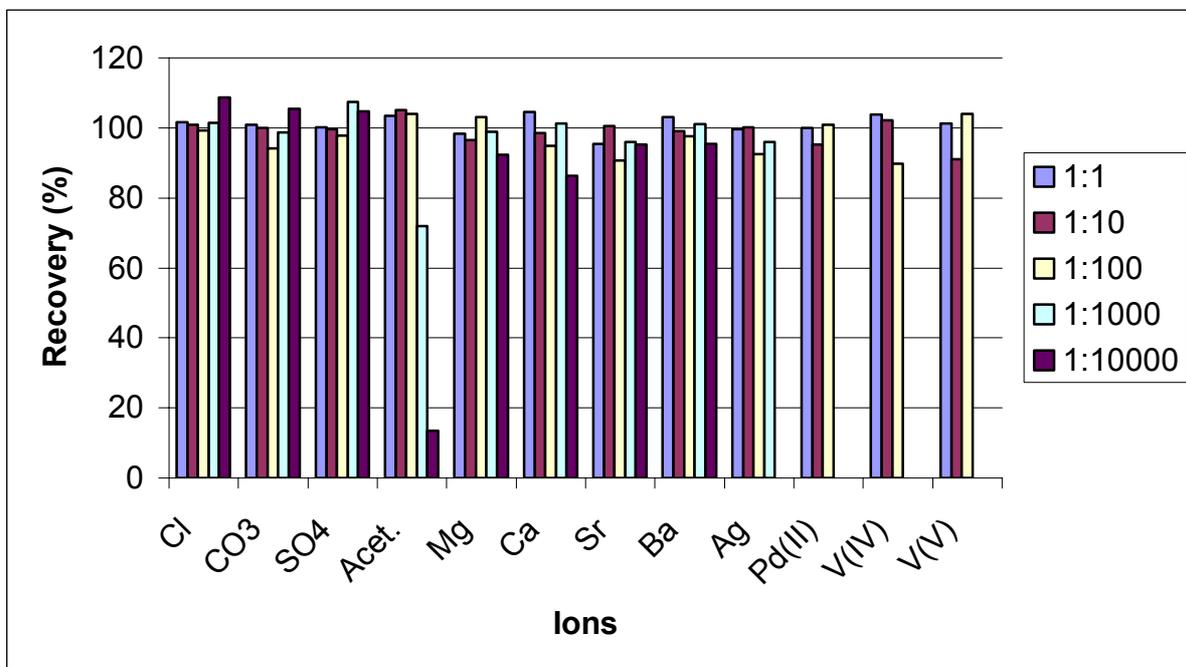


Figure 5-12 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with PS Schiff's base

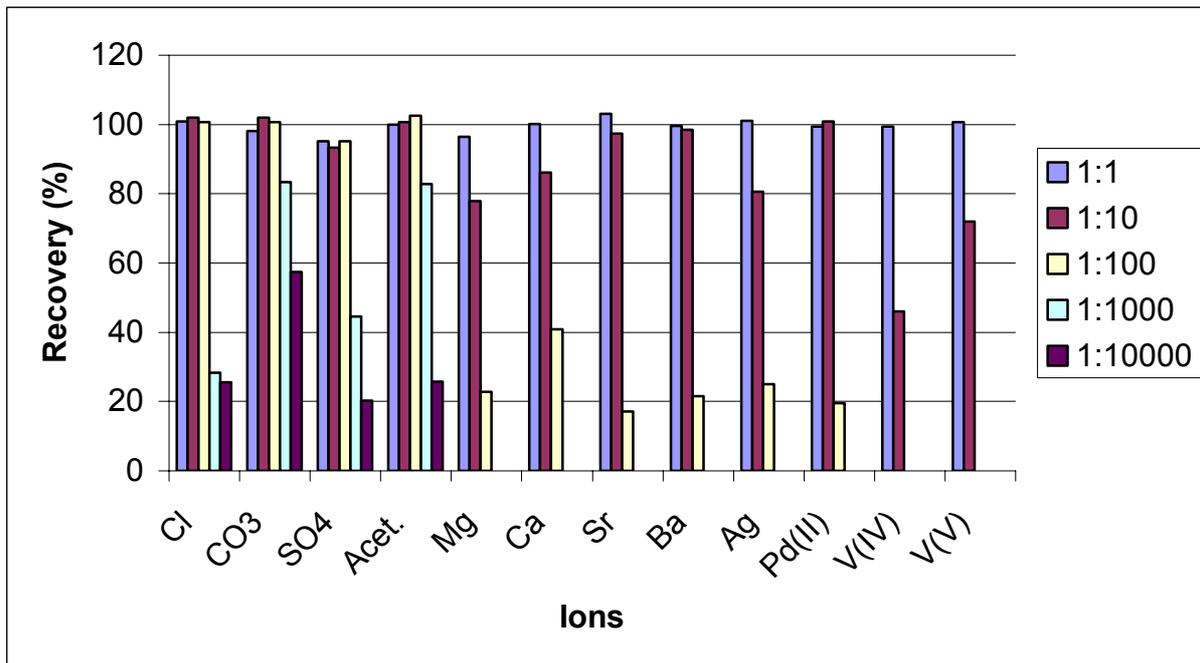


Figure 5-13 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with DMPS Schiff's base.

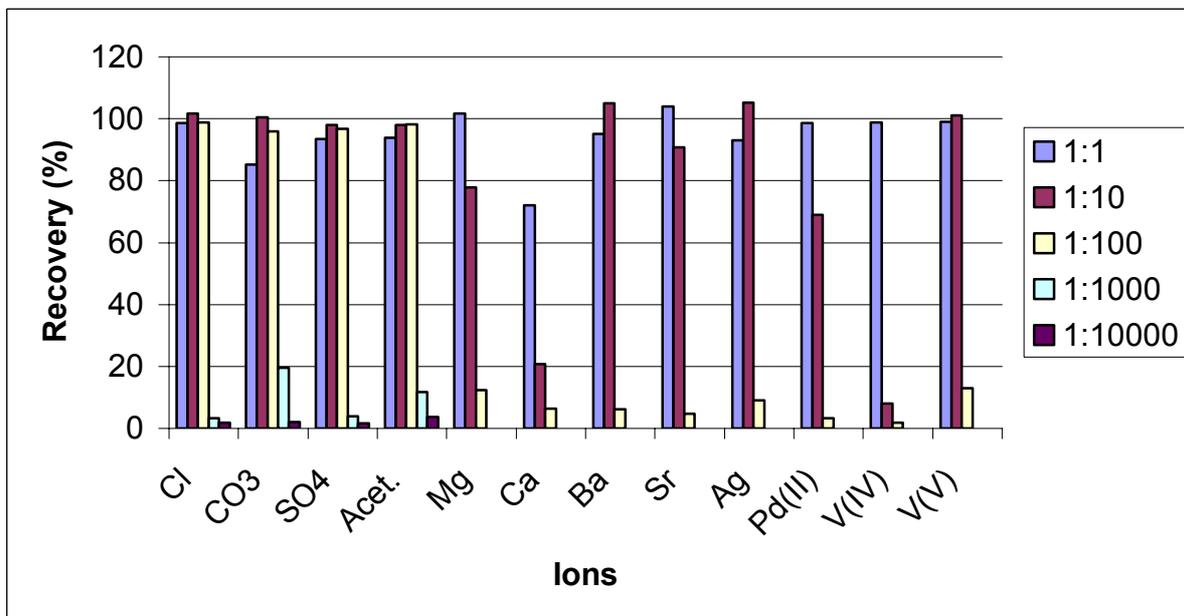


Figure 5-14 Effect of foreign ions on adsorption of Zn(II) ion on silica gel coated with SBTD Schiff's base.

It has been noted that the effect of presence of V(IV) is greater than that of V(V) in all cases specially at higher ratios due to the charge density and size of the vanadyl ion. The vanadyl ion dominates vanadium (IV) chemistry, it forms complexes with Schiff's bases of distorted trigonal bipyramidal structure.⁶⁸ Moreover the Schiff's bases have a semi-cavity of proper size, and be able to form wraparound complexes with vanadyl ions. This is very clear in case of Zn-SBTD.⁸⁰ Although the precipitation of the interfering ions is expected at the working pH the sorption of Zn was quantitative in the most cases. Vanadate ion (V(V)) can make metavanadates of MV_2O_6 formula where M = Mg, Ca, Mn, Co, Zn, and Cd.⁶⁸

The lower recovery of Zn in case of DMPS and SBTD in presence of the foreigner ions is attributed to the weak interaction between the Schiff's bases and silica gel surface because of presence of extra methyl groups on the basic skeleton.

5.1.7.2 Interference effect on Cd ion

In the case of ES and PS (Figure 5-15 Figure 5-16) there was no significant change in presence of all ions in all cases at 1:1 ratio except V(IV), this is maybe due to the variety of species of V(IV) ion formed in the solution of working pH. The redox reactions make the complex chemistry of vanadium more difficult.⁶⁸ In case of 1:10 ratio there was no change in the presence of anions used, but the recovery decreased dramatically in the presence of the cations onwards.

The figure for DMPS (Figure 5-17) shows less stability towards the presence of the cations even at 1:1 ratio. This difference in selectivity towards Cd ions between the ligands under study could be attributed to the stereochemistry of the ligand molecules attached to the surface of silica gel. Although SBTD (Figure 5-18) shows more stability than DMPS at 1:1 ratio, the maximum ratio for cadmium appearance was 1:100 in presence of many ions. The great effect was coming from Ca ions in case of DMPS and SBTD this may be due to the similarity in ionic radii of Ca and Cd and the weak interaction of DMPS and SBTD with silica surface.

In general the adsorption of Cd ion was affected by the increase in the interfering ions concentration although the high recovery in case of pure Cd solution or in 1:1 ratio in some cases. For real sample measurements this problem could be overcome by increasing the amount of the sorbent or using a pre-cleaning column or using masking agents.

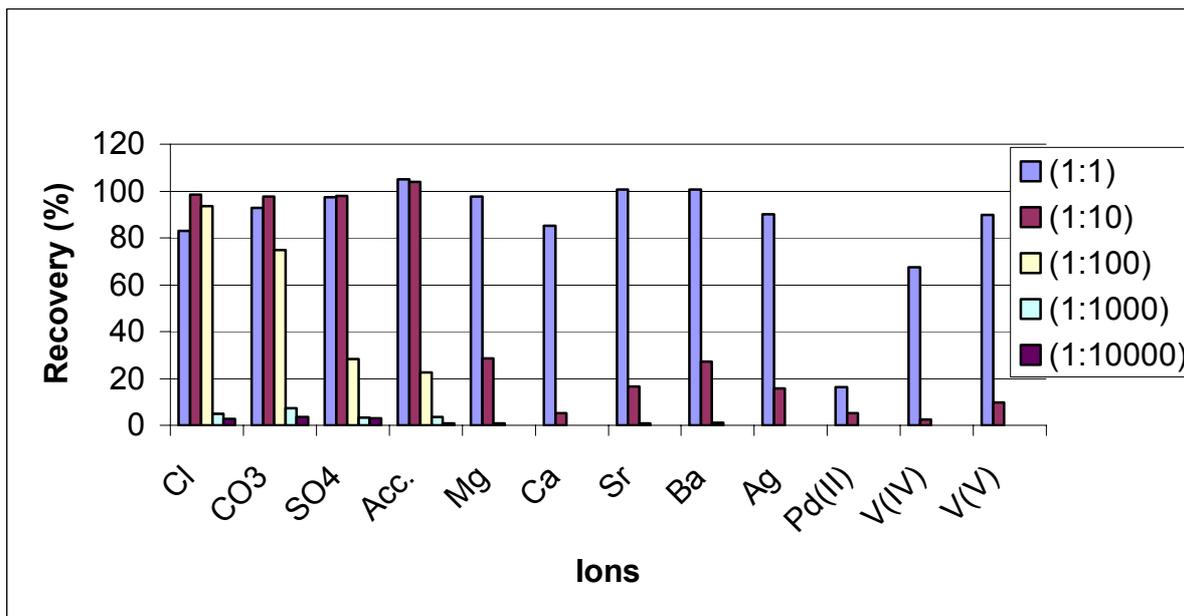


Figure 5-15 Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with ES Schiff's base.

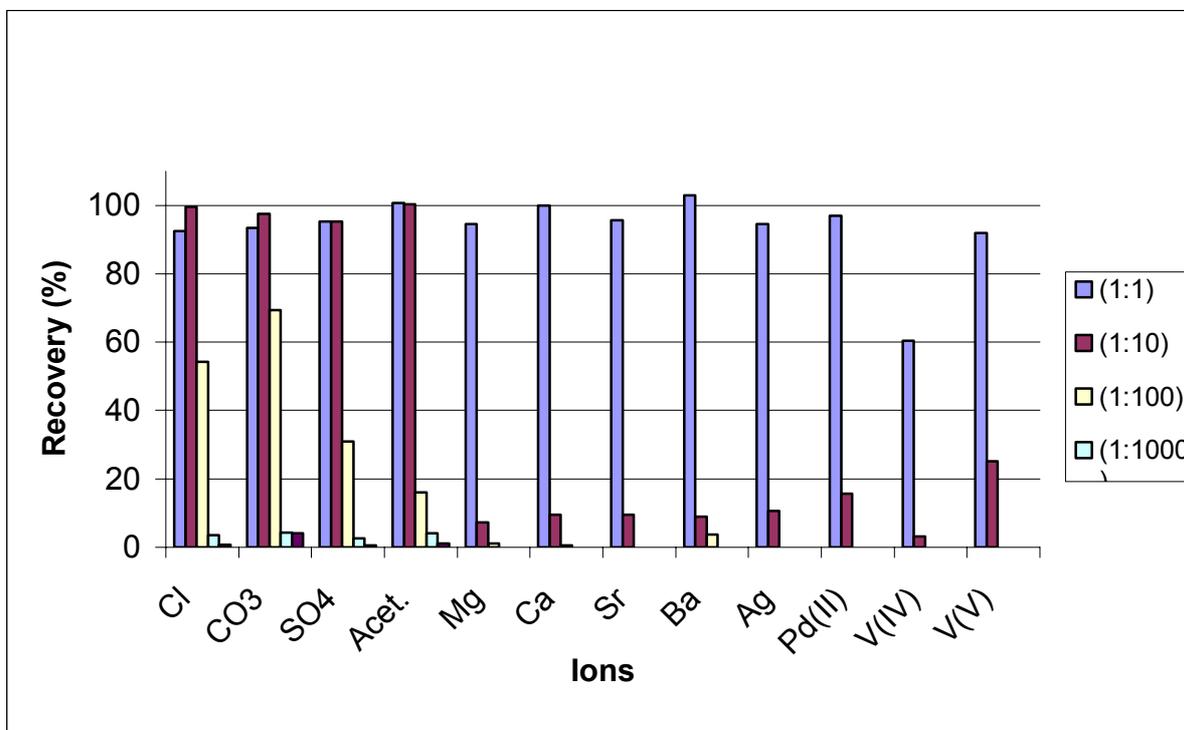


Figure 5-16 Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with PS Schiff's base.

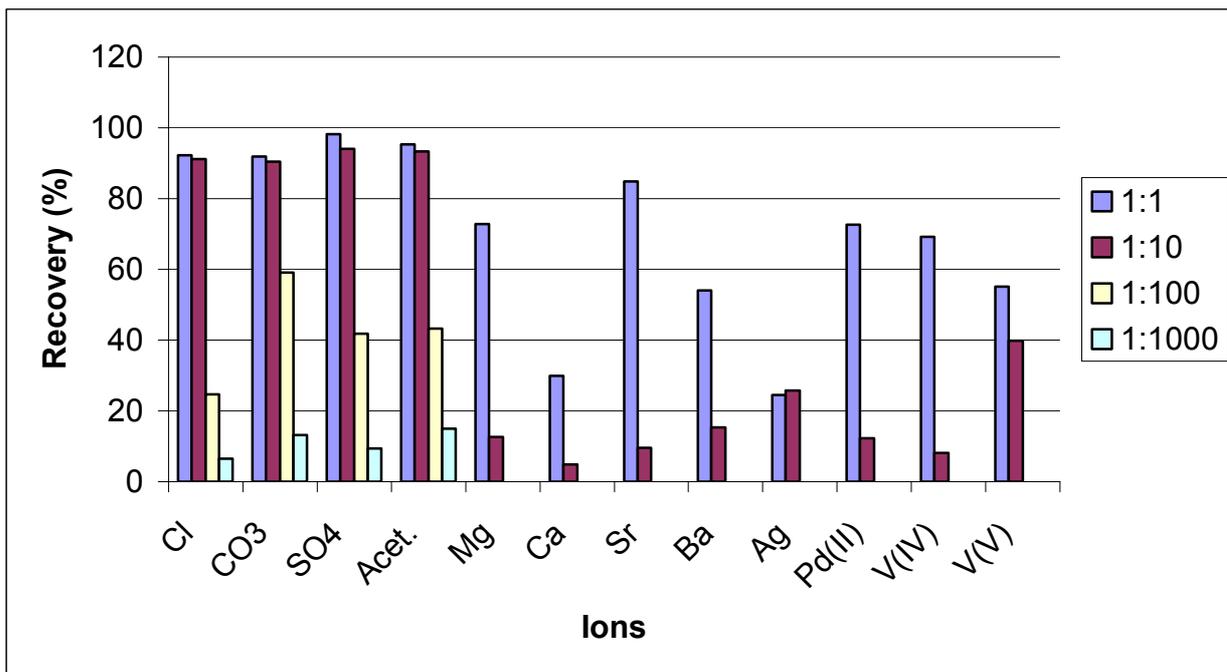


Figure 5-17 Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with DMPS Schiff's base.

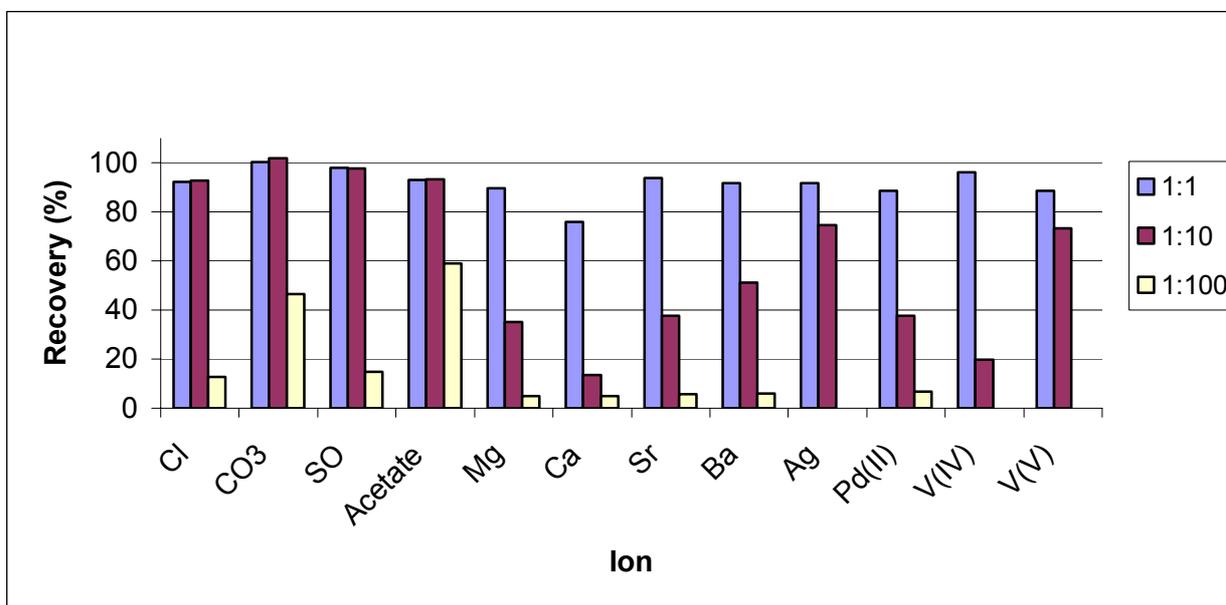


Figure 5-18 Effect of foreign ions on adsorption of Cd(II) ion on silica gel coated with SBTD Schiff's base.

5.1.7.3 Interference effect on Cu ion

Copper ion was less sensitive to the presence of the interfering ions than Cd ion although its low recovery even when it is alone in the solution, this is due to the high stability of copper complexes with N and O donors. As shown in Figures (Figure 5-19 and 5-20) Cu ion appears in low recovery in high concentration of interfering ions (1:100). DMPS and SBTD have higher recovery than ES and PS since the recovery enhanced by silanol groups on silica surface. In general the sorbents were not stable or selective for Cu ion.

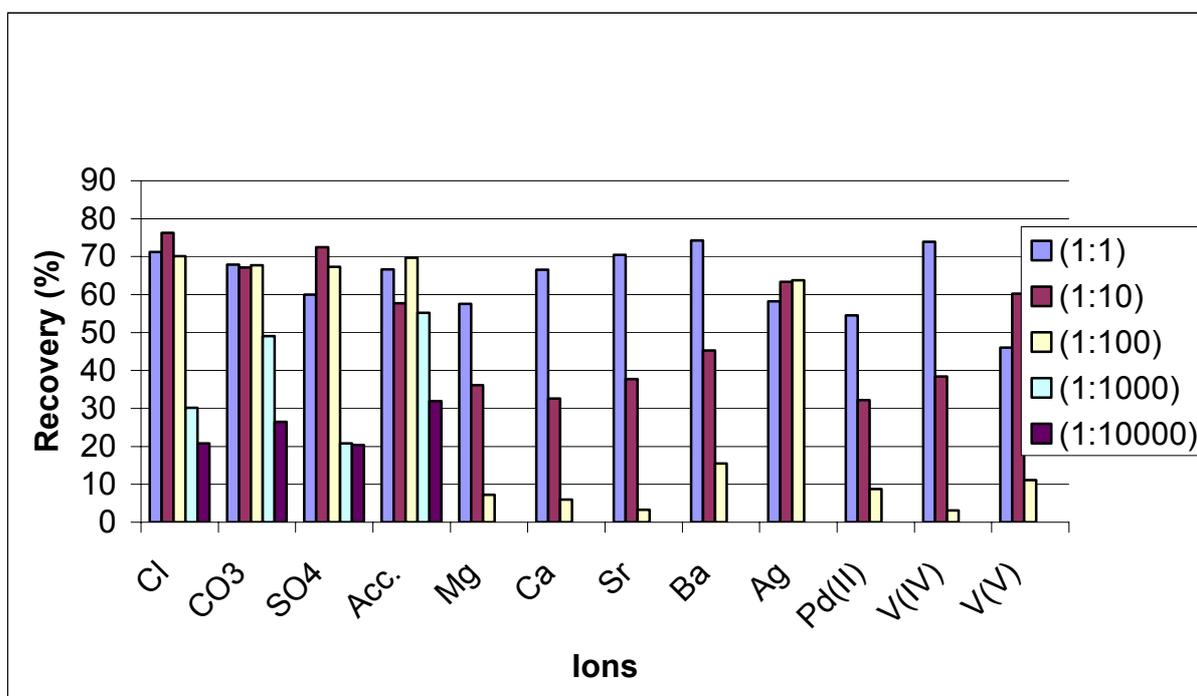


Figure 5-19 Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with ES Schiff's base

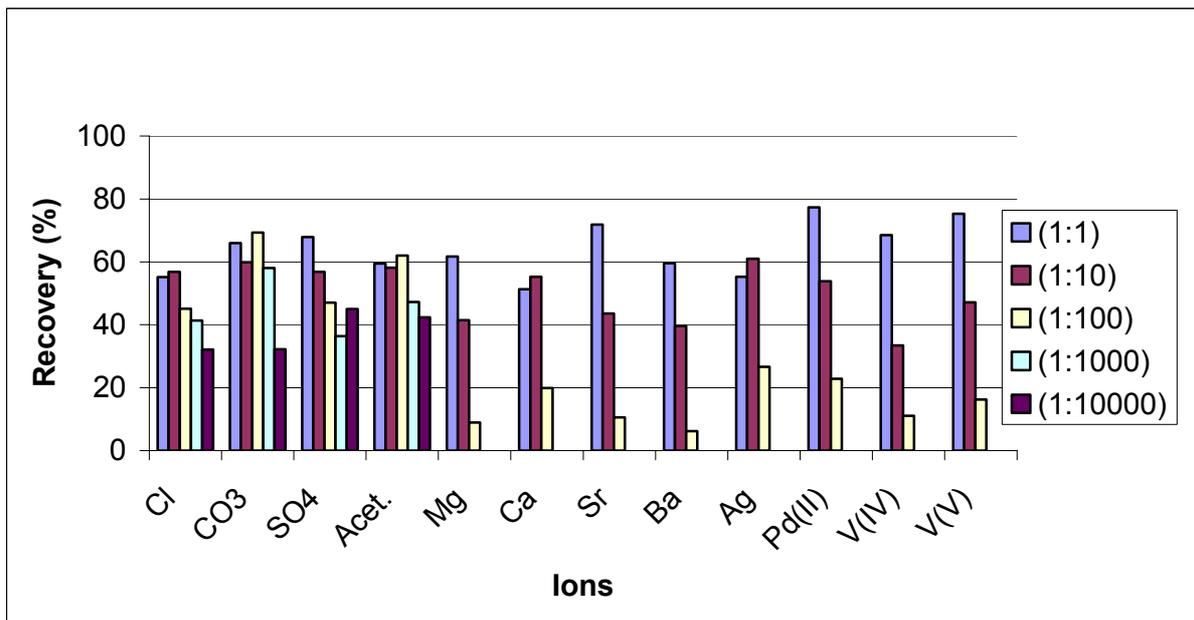


Figure 5-20 Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with PS Schiff's base

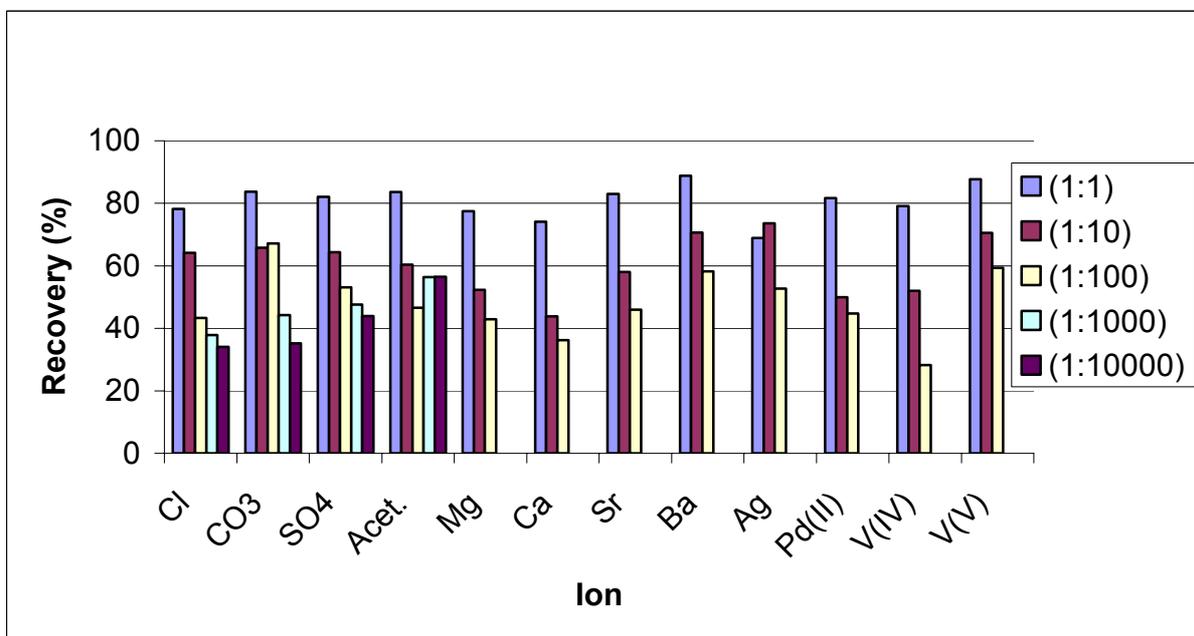


Figure 5-21 Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with DMPS Schiff's base.

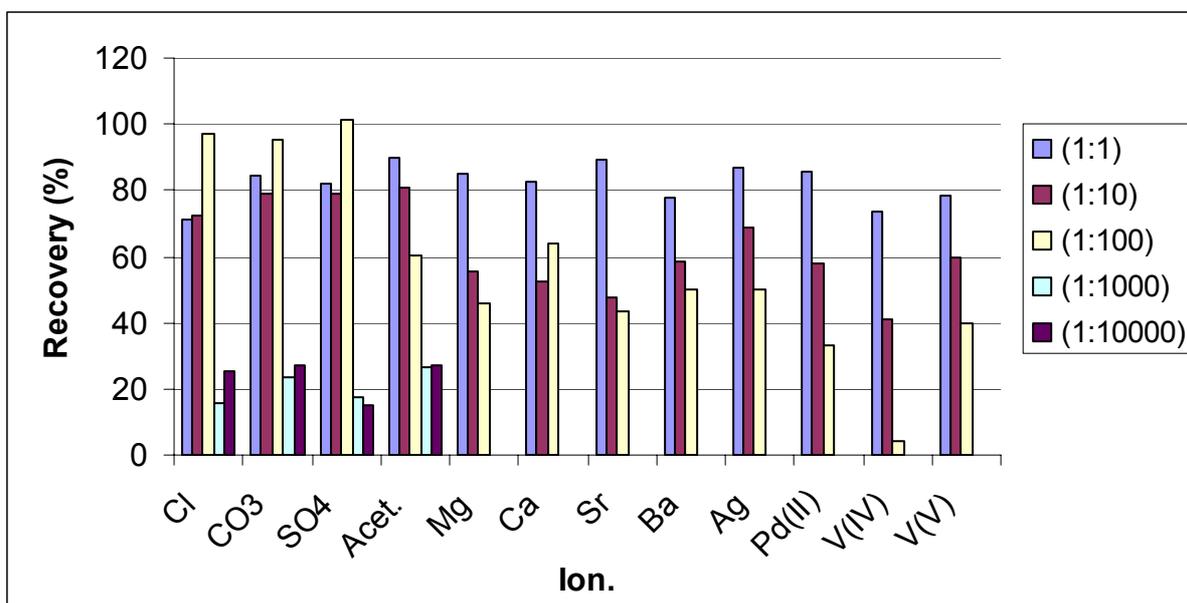


Figure 5-22 Effect of foreign ions on adsorption of Cu(II) ion on silica gel coated with SBTD Schiff's base.

Using acetate ion as a buffering agent is very limited because of the great influence of acetate ion on percent recovery of Cu. Therefore it is more preferred to adjust the pH in all cases by using ammonium hydroxide or nitric acid.

Despite the similarity in electronic structures and ionization potential, the chemistries of Ag and Cu differ more than might be expected, this could explain the very small effect of Ag(I) ion on the adsorption of Cu(II) ion, this could be attributed to the large covalent radius of Ag. Therefore, Ag is less reactive than copper, except toward sulphur and hydrogen sulphide.⁶⁸

The differences between the recoveries of Zn, Cd and Cu in presence of foreign ions in the solution indicate that the competition between Zn or Cd or Cu and the foreign ions is a competition to form complexes with Schiff's bases adsorbed on the silica surface and not a competition between the foreign ion and the Schiff's bases on silanol groups of silica surface.

Competition among metals for whatever ligands are available is assumed always be relevant and, therefore, not particularly noteworthy. By contrast, adsorption processes are often viewed with a specific target adsorbate in mind, and experiments are often conducted to compare adsorption of

that species in systems where it is the sole adsorbate with that in adsorption of that species in systems containing multiple adsorbates.⁸¹

Comparing the three metal ions Cd was very sensitive to the presence of foreign ions. The metal ions with larger ionic radius ($Cd > Zn > Cu$) have a lower electrostatic attraction that limits the interaction of the metal ions with the adsorption sites.³⁵ From the results obtained in this study was very clear the sorbents were robust toward Zn ion adsorption (on a recovery basis), which means that the sorbents prepared are promised to use in determination of Zn ion in aqueous solution. The same is for Cd and Cu with some additional pre-treatments and considerations.

5.1.8 Selectivity

The primary requirement for an economic separation process is a sorbent with sufficiently highly selectivity, capacity, and number of sorption experiments. The selectivity of a sorbent is a measure of relative affinity for different ions. It is determined by both the properties of the ion and the sorbent, with the solvent playing an important intermediate role.

The selectivity sequence is an important clue to the microenvironment of the adsorbed ion and the behaviour of the sorbent toward the adsorbed ion. It also controls the extent uptake of a particular cation and the effect of one cation on the uptake of another.

Divalent cation adsorption across a range of pH values at equal initial cation and adsorbent concentrations by the equation $\log D = a + b (\text{pH})$, Where a and b are empirical constants and D the distribution ratio, which defined as the ratio of the fraction of added metal that is adsorbed to the fraction of the added metal in the solution

$$D = \frac{\% \text{ Adsorbed}}{(100 - \% \text{ Adsorbed})}$$

A plot of $\log D$ vs. pH is called a Kurbatov plot. The pH at which $D = 1$, where 50% of the added metal is adsorbed and 50% is in solution, is designated pH_{50} . The pH_{50} values are a relative measure of the selectivity of an adsorbent for a particular series of bivalent metal cations; the smaller the pH_{50} value the more selective the adsorbent for the metal cation. (Figure 5-23, 5-24, and 5-25).

Divalent transition and heavy metals cations are invariably considerably more strongly adsorbed than the alkaline earth cations, but their selectivity sequences vary (Table 5-4). While there is a broad relationship between relative affinity and the tendency to hydrolyze, there are also many exceptions, as revealed by the different sequences found on different sorbents.

Because of the similarities between adsorption and precipitation, it is not surprising that cation selectivity is also reasonably well correlated with the solubility of the corresponding metal hydroxide. At high concentration also it is difficult to determine whether the cation is adsorbed or precipitated. Moreover there is no unifying principle governing the selectivity of the sorbents for divalent cations.^{69,82}

There are basic factors, which can effectively change the selectivity of the ligand including: the ligand topology which can be sub-classified into the ligand dimensionality, its connectivities, shape, size, conformation, chirality and also its cavity or ring size. The degree of flexibility of the ligand holding of the donor atoms by the host backbone, which makes their positions suitable to match the shape of the coordination sphere of the target species, is another factor. Finally, the presence of lariats (functional groups, arms or chains added to the ligand to change selectivity) has a great effect on binding. Another very important factor that affects the complexation properties of the ligand binding sites which depends on the nature and number of the binding sites, is their shape, size, arrangement and reactivity. The planarity of the ligand is also important in its complexation properties. Some factors, related to the cation and influencing the selectivity behavior, include the geometric shape of the ion, its charge density and its size (the ionic radius). The last factor seems to have a large influence on the selectivity.

Another effective parameter includes the effect of the counter ion, which is of value in the case of the charged complexes. The neutralization of the charged complex is necessary to the transportation of the charged complex, which is normally done by the counter ion.⁶⁷

Table 5-4 Selectivity sequence for the adsorption or co-precipitation of a variety of divalent transition and heavy metal cations on several sorbents.⁶⁹

Sequence	Sorbent
Pb > Zn > Cd	Fe gel
Zn > Cd > Hg	Fe gel
Pb > Cu > Zn > Ni > Cd > Co	Fe gel
Cu > Zn > Co > Mn	α - FeOOH
Cu > Pb > Zn > Co > Cd	α - FeOOH
Cu > Co > Zn > Ni	MnO ₂
Pb > Zn > Cd	MnO ₂
Zn > Cu > Co > Mn > Ni	Si gel
Zn > Cu > Ni = Co > Mn	Si gel

There is apparently little consistency in the selectivity sequences. The selectivity sequence may also vary with pH, for example, on MnO₂, Zn > Cd at pH 6.0 and 8.0 but Cd > Zn at pH 4.0. Differences in H⁺/Mⁿ⁺ stoichiometry for different cations could lead to reveal in selectivity with changing pH since the ion with higher H⁺/Mⁿ⁺ stoichiometry would tend to be increasingly favored at higher pH.⁶⁹ But in general we can have a general sequence for certain group of elements with a sorbent under restricted conditions.

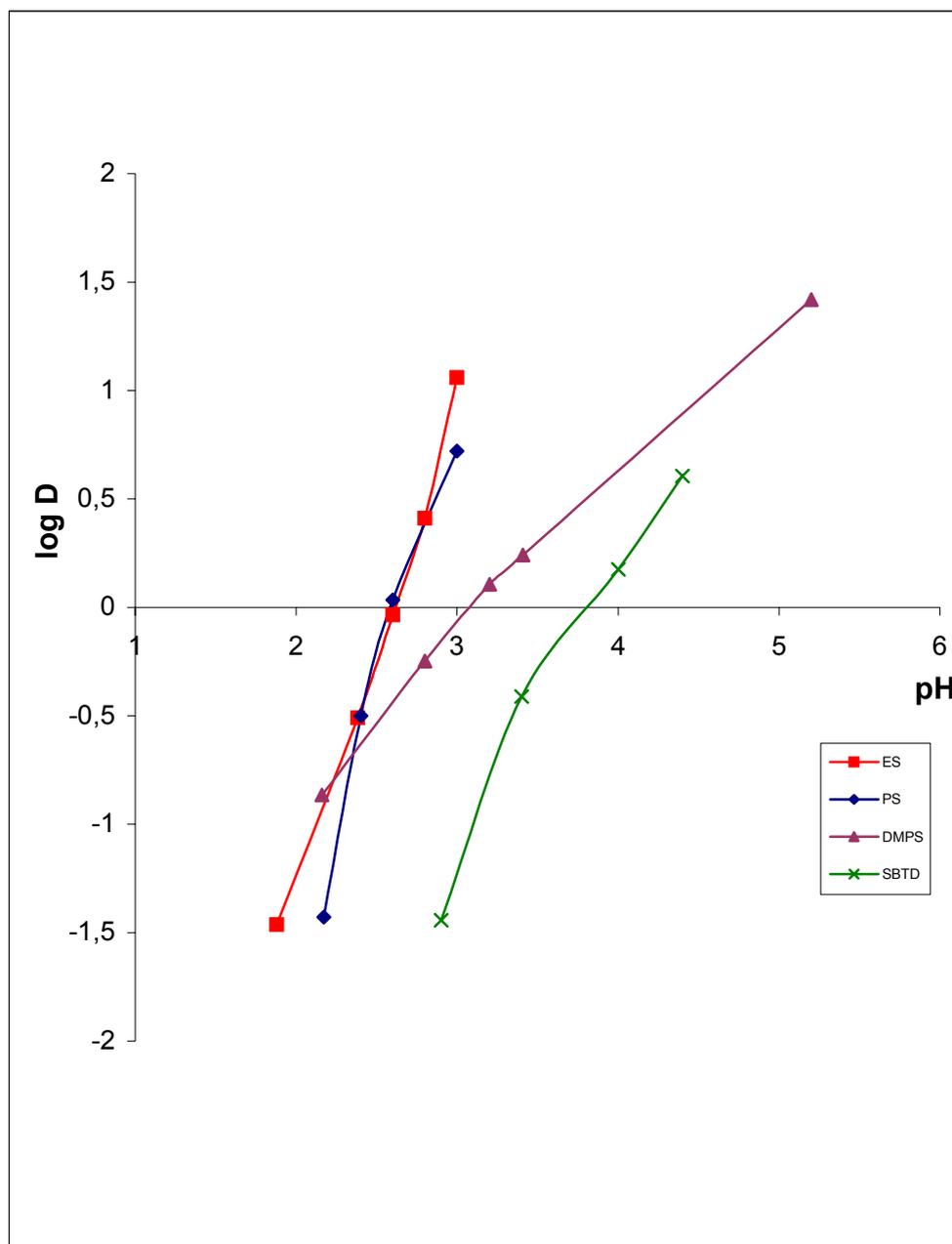


Figure 5-23 Kurbatov plot for Zn adsorption on silica gel coated with Schiff's bases.

ES and PS have the same and high selectivity toward Zn ion then DMPS and at last SBTD, this can attributed to the differences in structure of the Schiff's bases and, therefore, the type of the total interaction (solid support - Schiff's bases - metal ion) since the interaction of ES and PS with silica is stronger than the interaction DMPS and SBTD, respectively. So the sequence of selectivity of Schiff's bases toward Zn ion is in this way ES = PS > DMPS > SBTD.

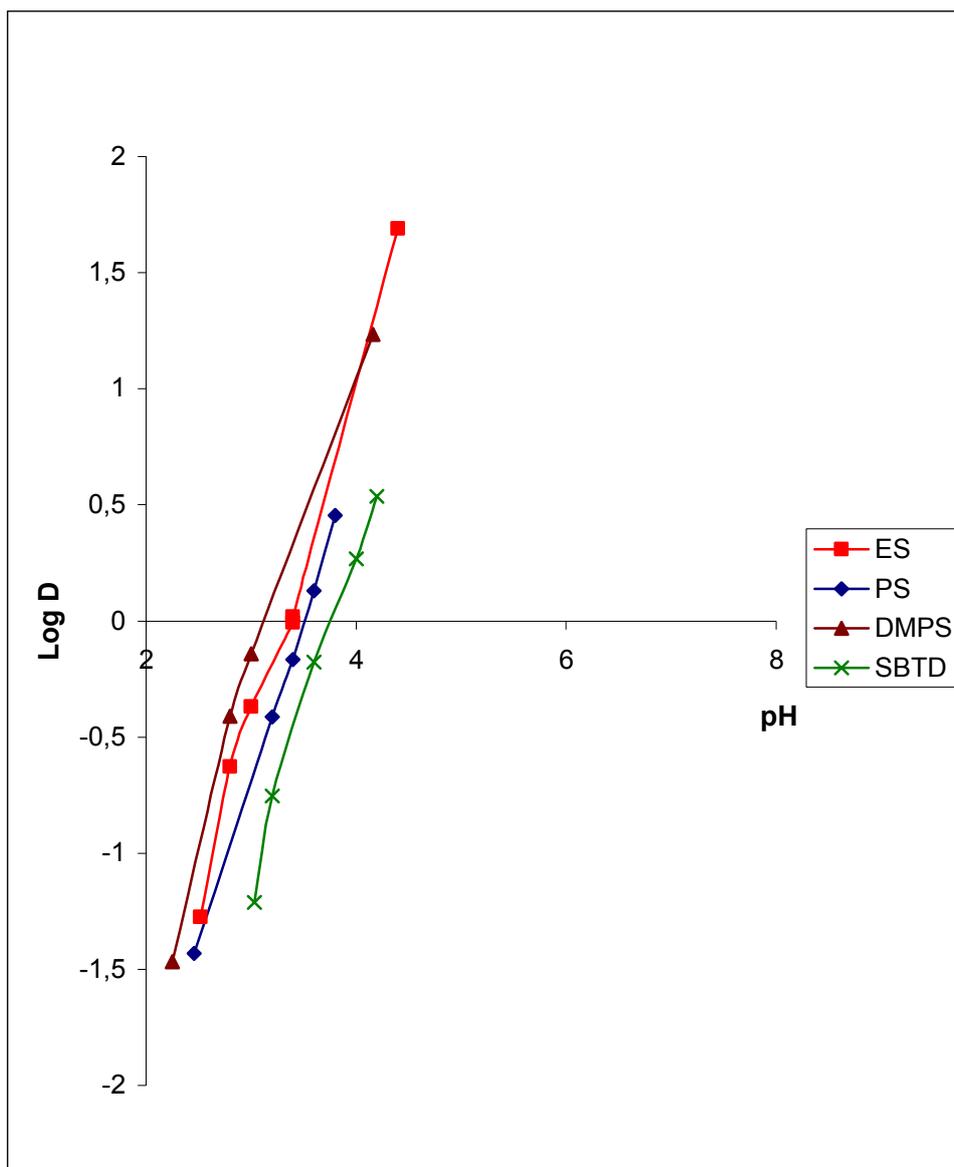


Figure 5-24 Kurbatov plot for Cd adsorption on silica gel coated with Schiff's bases.

From Kurbatov plot, the order of selectivity toward Cd ion was found to be the following DMPS > ES > PS > SBTD.

DMPS is more selective although its less stability but actually there are many factors be taken in consideration before choosing the proper sorbent such as the type of matrix, amount of interference, concentration range of metal ion of interest, and the stability of the sorbent. The stability of the sorbent or the stability of the complex formed is more important factor than the sensitivity of the sorbent toward metal ion.

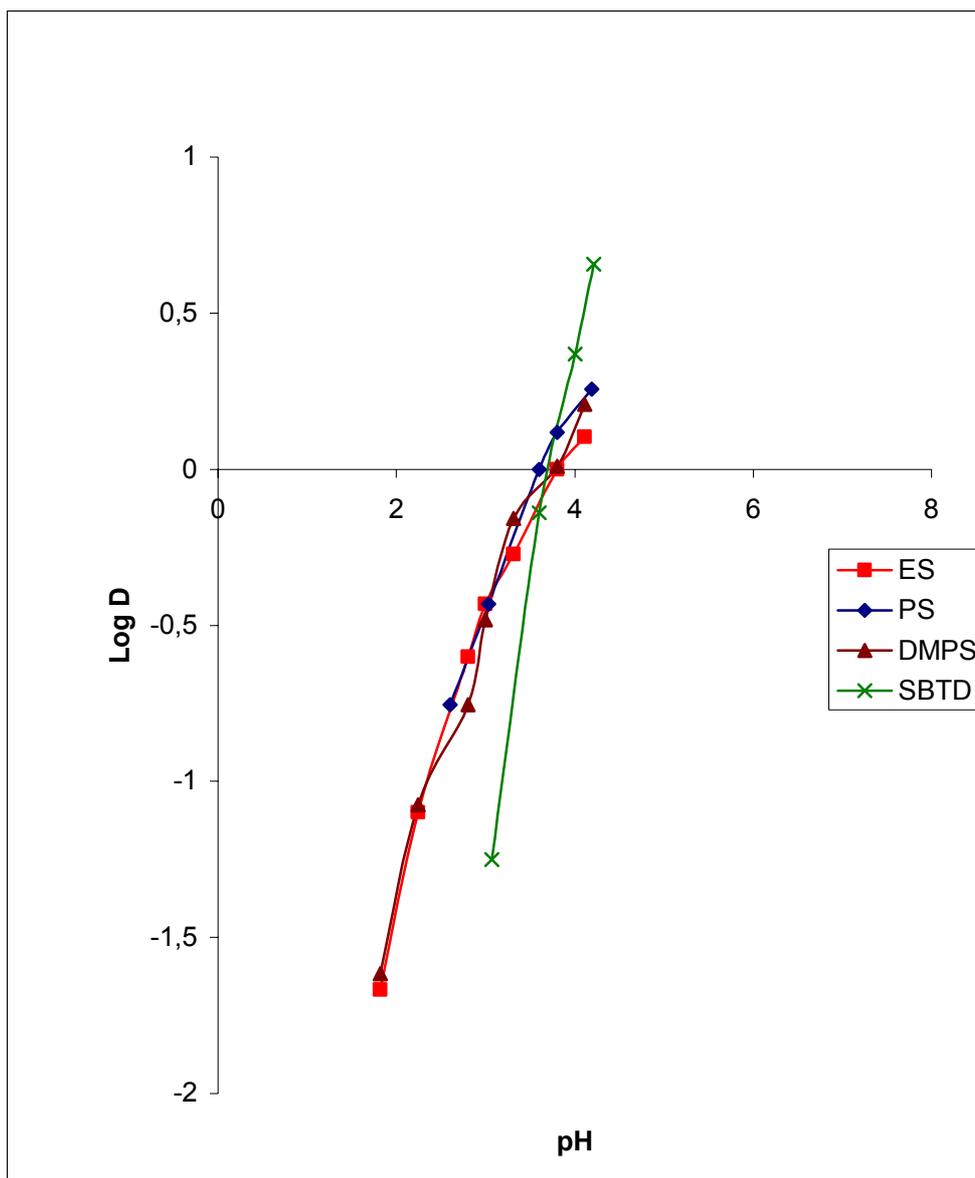


Figure 5-25 Kurbatov plot for Cu adsorption on silica gel coated with Schiff's bases.

In general the most important factors that influence the relative selectivity of cations in solution are valence and ionic radius. While selectivity behaviour for group IA and IIA elements of the periodic table can generally be explained using these factors, no such generalization can be made for the heavy metals.

Table 5-5 Selectivity of silica gel coated with Schiff's bases toward metal ions.

Metal/ Ligand	PS	ES	DMPS	SBTD
Cu	3.60	3.81	4.80	3.70
Zn	2.62	2.64	3.05	3.80
Cd	3.50	3.40	3.10	3.70

The ionic potentials (Z^2/r) of the three metals estimated based upon the charge (Z) and radius of the ion (r) follow the order: $\text{Cu} > \text{Zn} > \text{Cd}$. If the metal adsorption on the sorbents were entirely electrostatic, ions of higher ionic potentials should have been adsorbed more strongly. The selectivity sequence observed in the present study, therefore, suggests that the sorbent metal bonds are not entirely electrostatic. In our case the sequence in all the sorbents was as follows: $\text{Zn} > \text{Cd} > \text{Cu}$ except the case of SBTD $\text{Cd} = \text{Cu} > \text{Zn}$. There is no certain rule governing the selectivity of the sorbents toward metal ions in solution, the behaviour of the sorbent is the summation of all the factors together in the same time.

5.1.9 Adsorption isotherms

The relationship between the amount of a substance adsorbed at constant temperature and its concentration in the equilibrium solution is called the adsorption isotherm.

The adsorption isotherms of all Schiff' bases with Zn on Silica gel surface or we can say the total adsorption isotherms are shown in the following figures (5-26-36). Adsorption of metal ions showed much stronger pH dependent, as the pH rose above 3, metals adsorption on the sorbents steeply increased and reached plateau levels at pH between 4 and 8 when adsorption was almost 100%.

The adsorption isotherm is important from both a theoretical and a practical point of view. Its shape is related to the energy of adsorption and the number of the adsorption sites, but the relationship is not necessarily simple. We assume that the adsorbed metal ions are bound directly to deprotonated sites of Schiff's bases. Actually, equilibrium analysis in aqueous solutions does not permit differentiation between "inner sphere" and "outer sphere" complexes.⁸³

At higher concentrations, adsorption is reaching the maximum, a result arising partly by the filling up of a significant number of the total possible adsorption sites. This results in a smaller chance of a solution ion that impinges on the surface of finding a vacant site, and therefore, being adsorbed. The Langmuir isotherm takes this into account and has been widely used to describe the concentration dependence of cation uptake.⁶⁹

On the bases of classification of adsorption isotherms studied in the chapter II, all cases are following Langmuir isotherm. This represents the adsorption of metal salen complex on the surface of silica gel, which is the overall adsorption isotherm since the kind of interaction between Schiff's bases and silica gel is hydrogen bonding while the interaction between the metal ions and Schiff's bases is complex formation which is chemical interaction (complex formation).

The effect of complex formation on the overall adsorption of a metal ion will depend on the stability of the complex formed and the relative affinity of the surface for the free and complexed forms. If the stability of the cation-solid surface complex is large compared with the stability of the cation-solution ligand complex(es), then complex formation will have little effect on the overall extent of adsorption. Steric and charge factors will mean that not all of the possible surface-complex cation interactions will be important and, in practice, it will be probably unnecessary to consider the whole matrix of possible interactions.⁶⁹

It is difficult to generalize about the extent of cation uptake by the solid phase since not only does this depend on the concentration of the cation in solution and the pH, but it can also depend on the method of preparation of the solid, kind of interaction and presence of other ions in solution. At high concentrations, reactions such as surface induced hydroxide precipitation may be important, and these can obscure the extent of the underlying adsorption reaction.⁶⁹

The main problem facing adsorption is precipitation. Precipitation and adsorption are intimately related, with adsorption a necessary first step of heterogeneous surface precipitation. The difference between the two as follows: (1) adsorption is a two dimensional process (a surface layer) whereas precipitation is three dimensional (crystal buildup), and (2) solution adsorbate concentration is controlled by surface site concentration in adsorption and solution concentration in precipitation. Generally adsorption reactions are considered in terms of the formation of a stable surface compound in equilibrium with the solution.⁸⁴

With cations that form very stable hydroxyl complexes, increasing pH actually lead to a decrease in adsorption because of the formation of stable hydroxyl metal complexes.

Because the species controlling adsorbate extraction from solution are different in adsorption and precipitation, there is no assurance that monolayer coverage will be complete at an adsorbate

concentration less than that required for the onset of precipitation. It is important, therefore, in adsorption studies, to ensure that the observation of a decrease in the solution adsorbate concentration is actually caused by the adsorption rather than precipitation.

The pH at which divalent cation adsorption becomes significant varies with the particular cation and solid involved, the solid solution ratio, the specific area of the solid, the total cation concentration, and the concentration of other interacting species. Therefore it is obviously difficult to generalize.

In adsorption models that focus on the similarity between adsorbed species and other species that truly in solution, binding of the adsorbate to the adsorbent is viewed as similar to the binding of a proton to a base, a metal to a ligand, or an electron to an oxidant. The metal/ligand analogy is the one that is most often made, with the adsorbent sometimes described as a collection of surface ligands and the adsorption reaction referred to as a surface complexation reaction. The surface ligands are presumed to correspond to specific sites on the adsorbent surface, so the model is sometimes referred to as a site-binding model. In some of the complex versions of such models, different adsorbed molecules are envisioned to reside at different locations in the interfacial zone.⁸¹

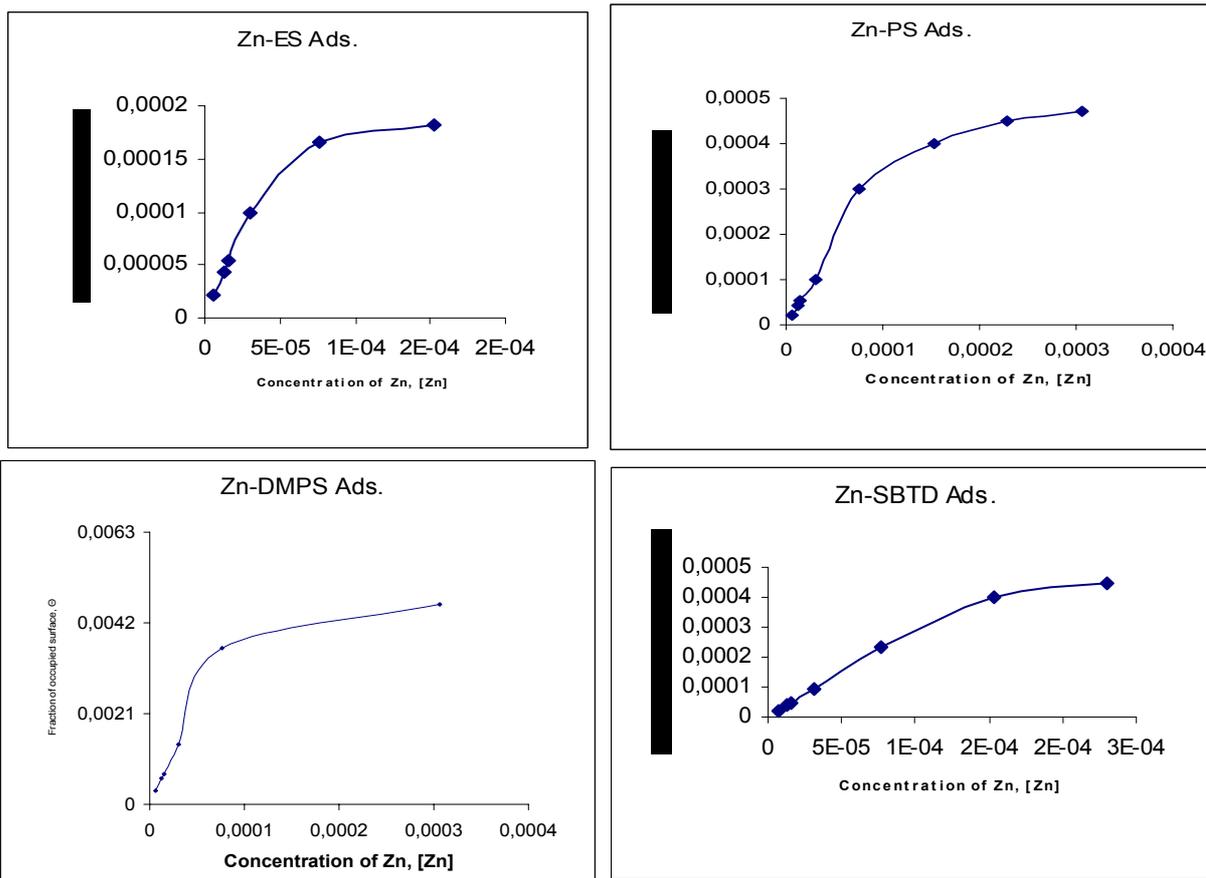


Figure 5-26 Adsorption isotherm of Zn ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.

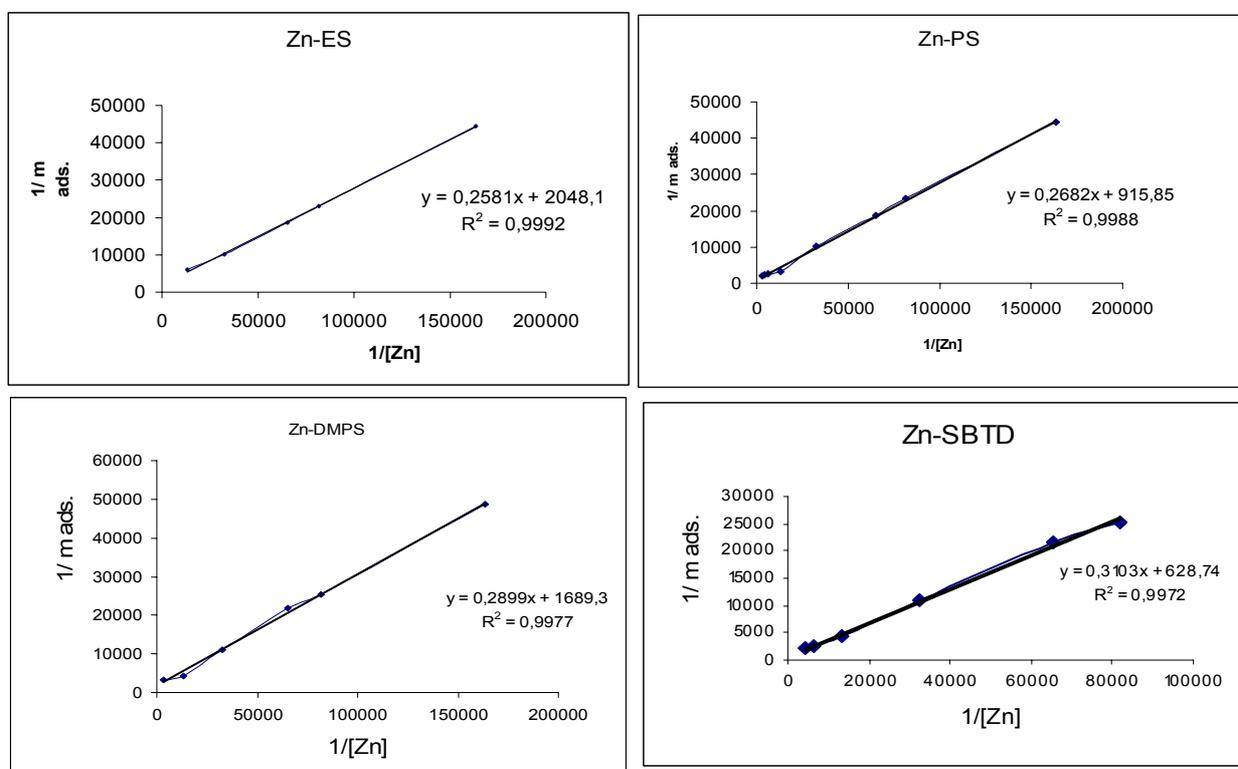


Figure 5-27 Langmuir isotherm of Zn ion (pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.

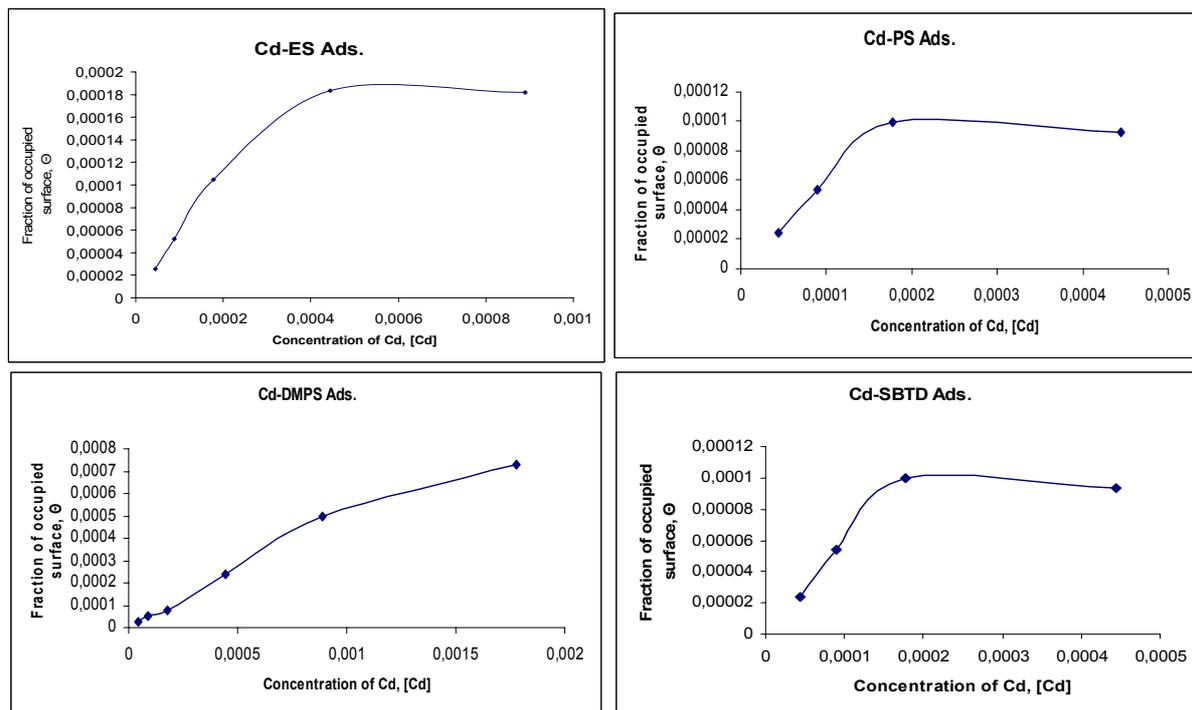


Figure 5-28 Adsorption isotherm of Cd ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.

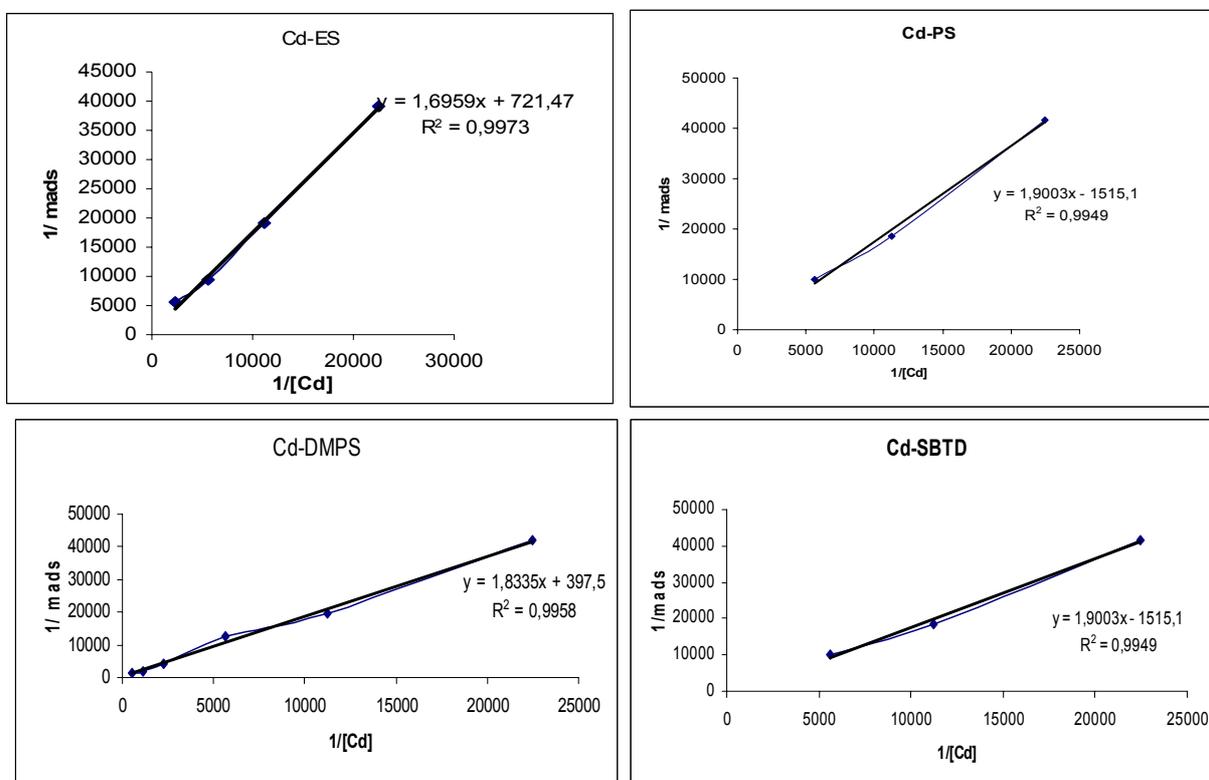


Figure 5-29 Langmuir isotherm of Cd ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.

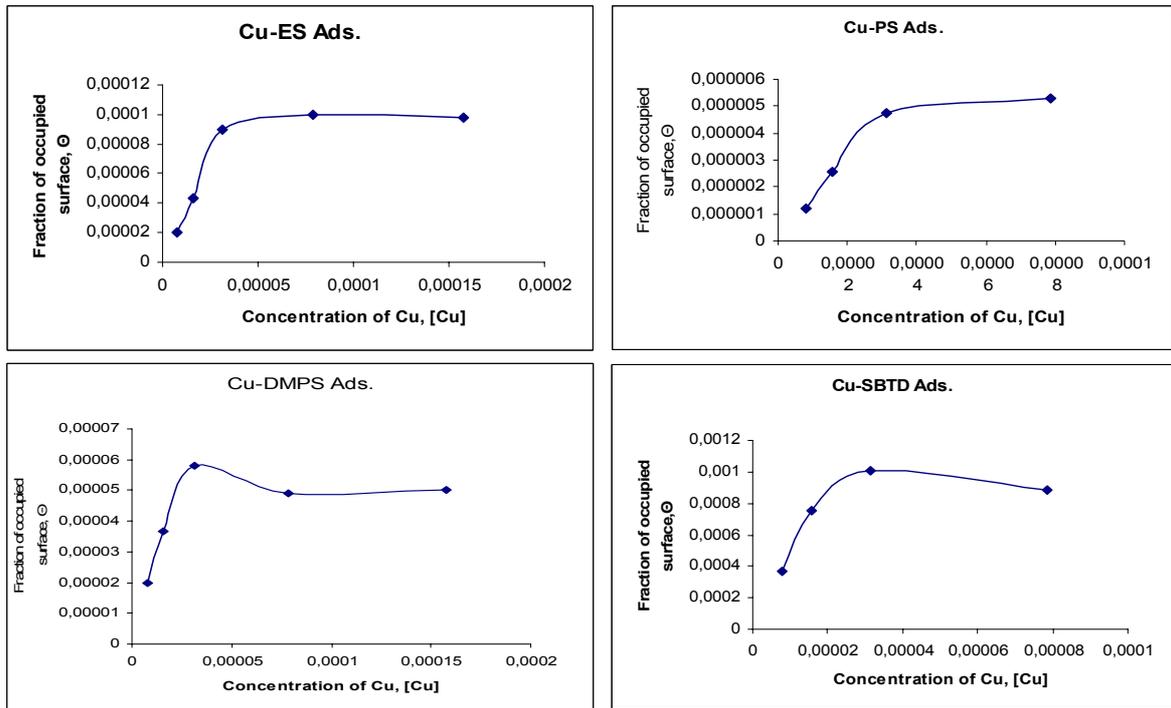


Figure 5-30 Adsorption isotherm of Cu ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.

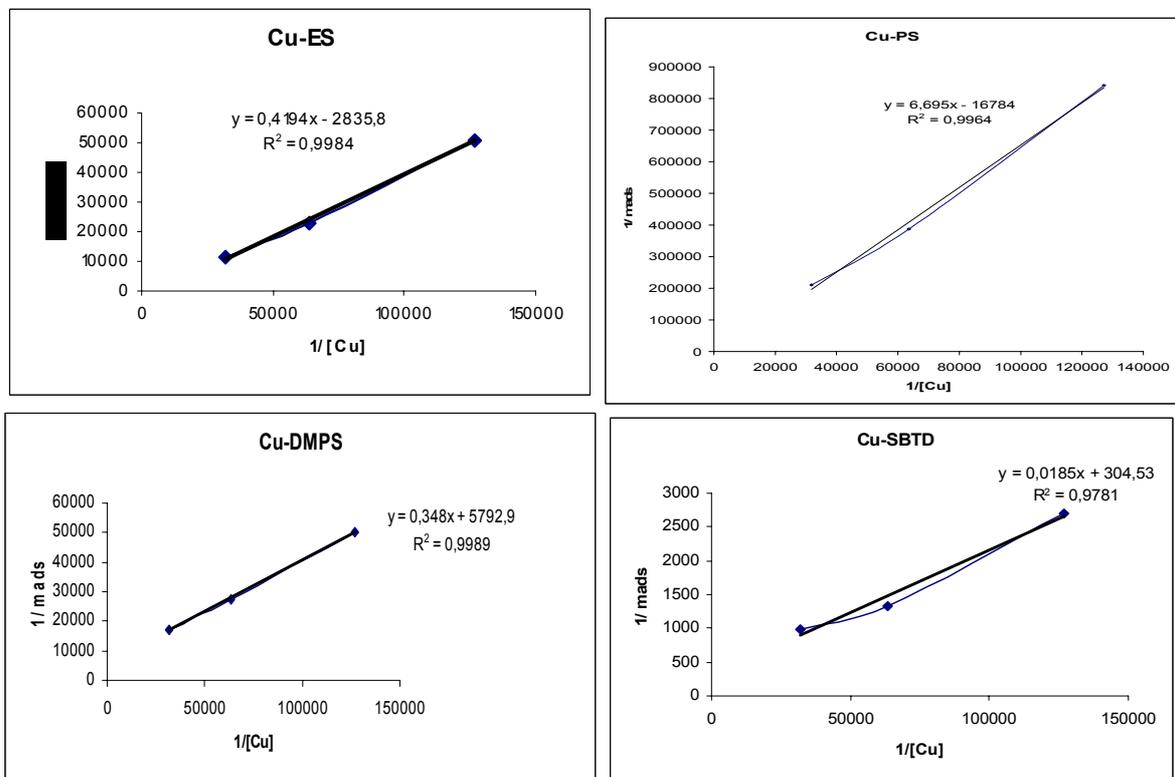


Figure 5-31 Langmuir isotherm of Cu ion ,(pH=5, sample volume 50ml., Temp25°C) on silica gel coated with Schiff's bases.

It would be expected that the resultant metal organic complex would display adsorption properties more closely related to those of the organic molecule than of the free of metal ion. This would allow the pre-concentration of the low concentrations of metals by using SPE similar to those used for pre-concentration of trace organics. The metal interacts with Schiff's base which is physically adsorbed on the silica surface. Therefore, the overall adsorption behaviour is physical adsorption according to the Langmuir isotherm, which means that the adsorption takes place only at specific localized sites on the surface and the saturation coverage corresponds to complete occupancy of these sites, according to the major assumption of the Langmuir isotherm. The Langmuir model represents the surface as a collection of identical and non-interacting ligands that can bind to adsorbates to form non-interacting complexes.⁸¹

5.1.10 Analytical performance

The evaluation of linearity is necessary to be performed in order to verify that the response signal is linearly proportional to the analyte in a defined range of concentration. The final calibration curves for all metal ions used with all sorbents are presented in figures (5-32,5-33, and 5-34). The calibration curves were constructed with 50ml standard solutions containing 0.2 – 2 µg/ml of metal ion according to the general procedure. The linearly ranges, regression equation and correlation coefficient were obtained by the method of least squares. The limits of detection (LOD) and the limits of quantification (LOQ) were evaluated as the concentration corresponding to three times and ten times the standard deviation of the blank signal, respectively. The results are presented in Table 5-6

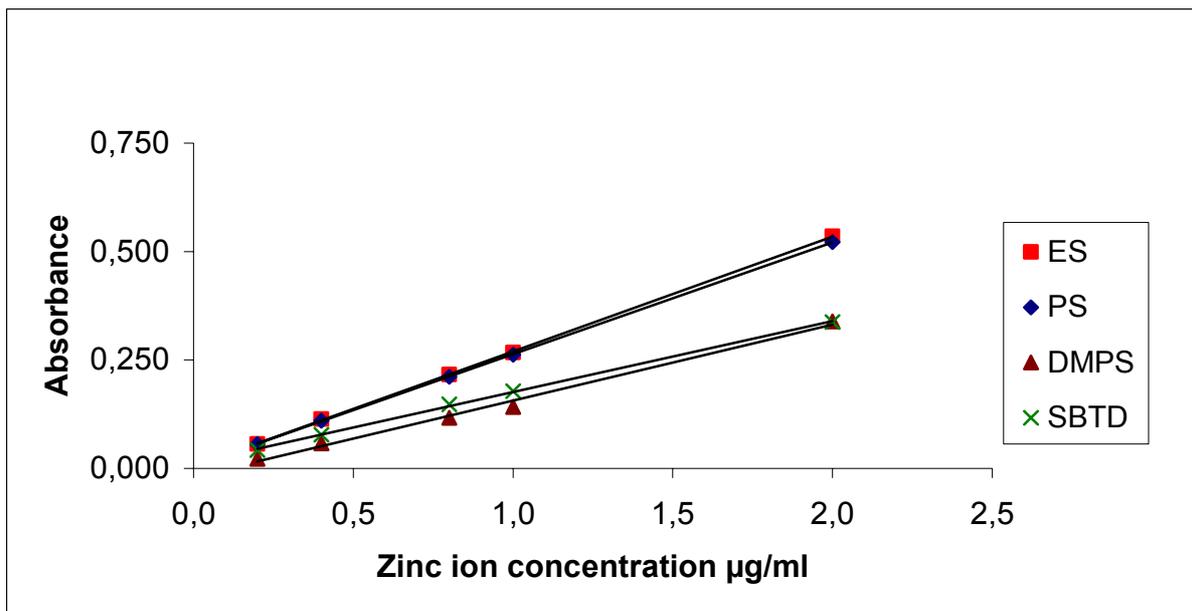


Figure 5-32 Calibration curves of Zn(II) ion.

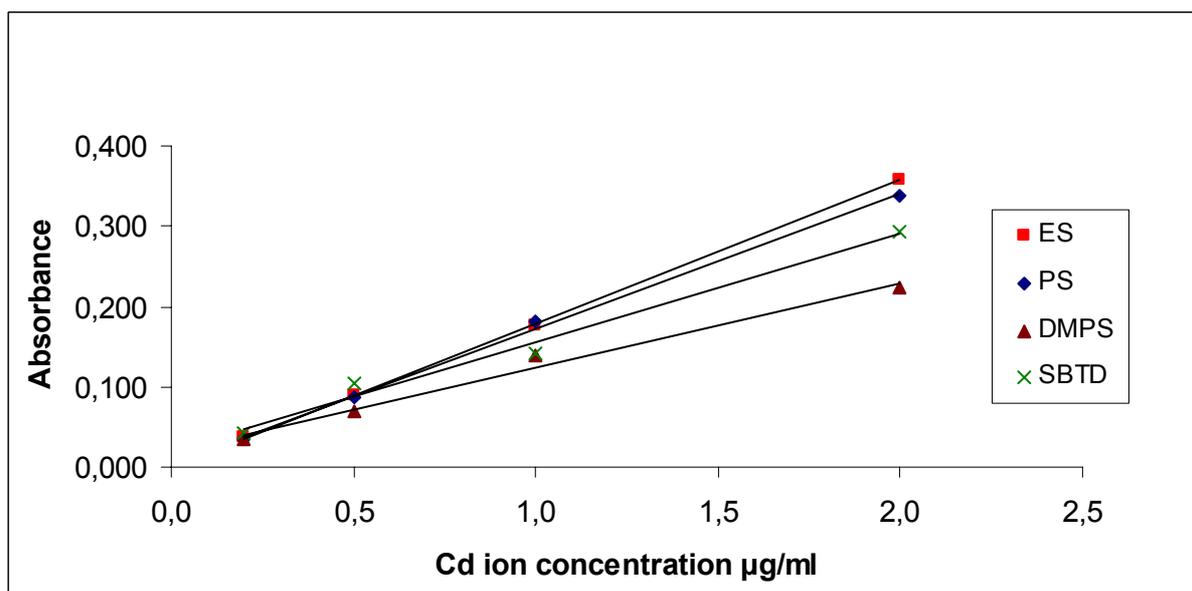


Figure 5-33 Calibration curves of Cd(II) ion.

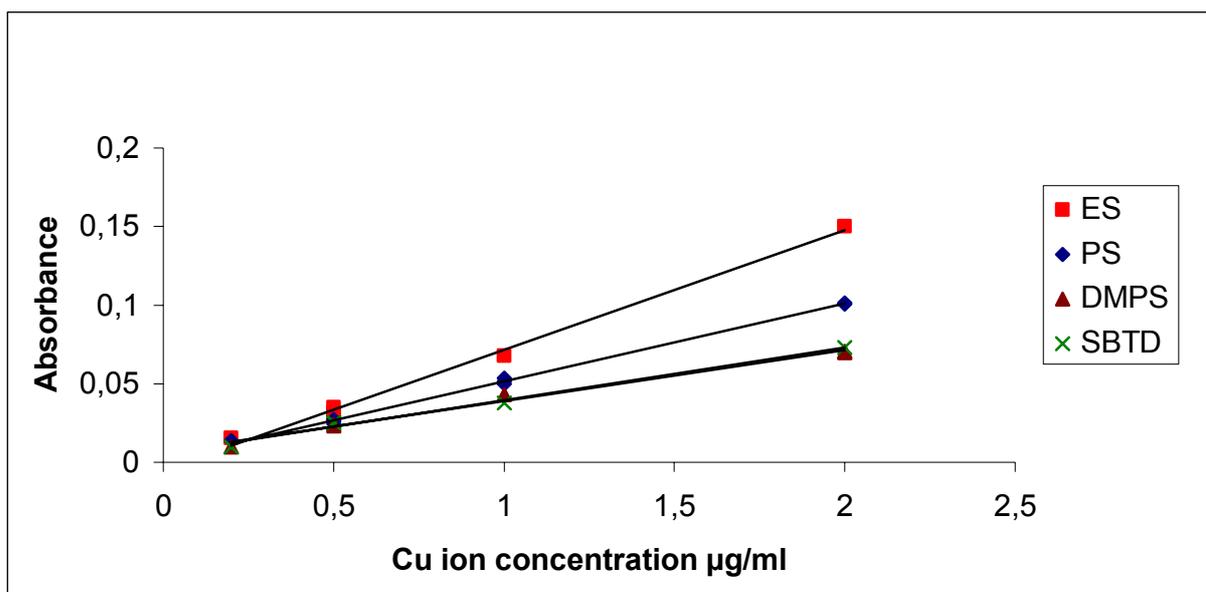


Figure 5-34 Calibration curves of Cu(II) ion.

Table 5-6 Analytical limits of the metal ions.

Metal-Schiff's base	Regression equation	Corr.	LOD ng/ml	LOD (Ave.)	FAAS LOD ng/ml	Linearity range (µg/ml)
Zn-ES	Abs. = 0.2662C + 0,0029	$R^2 = 0.9992$	7.60	7.00	84	0.0253 – 2
Zn-PS	Abs. = 0.2589C + 0,004	$R^2 = 0.9997$	7.30			0.0242 – 2
Zn-DMPS	Abs. = 0.1699C - 0,0116	$R^2 = 0.9913$	13.40			0.0448 – 2
Zn-SBTD	Abs. = 0.1661C + 0,009	$R^2 = 0.9985$	11.70			0.0391 – 2
Cd-ES	Abs.= 0.1776C + 0.0009	$R^2 = 0.9997$	57.60	36.27	100	0.1918 – 2
Cd-PS	Abs.= 0.171C + 0,0006	$R^2 = 0.9972$	58.30			0.1942 – 2
Cd-DMPS	Abs.= 0.1105C + 0.0116	$R^2 = 0.9821$	9.60			0.0320 – 2
Cd-SBTD	Abs.= 0.1396C + 0.0136	$R^2 = 0.9851$	19.50			0.0652 – 2
Cu-ES	Abs.= 0.07C + 0.0016	$R^2 = 0.997$	10.00	10.25	450	0.0332 – 2
Cu-PS	Abs.= 0.0468C + 0.003	$R^2 = 0.9981$	6.90			0.0229 – 2
Cu-DMPS	Abs.= 0.0348C + 0.0028	$R^2 = 0.9895$	13.80			0.0460 – 2
Cu-SBTD	Abs.= 0.0362C + 0.0025	$R^2 = 0.9878$	10.30			0.0343 – 2

The limits of detection of Zn ions were as following PS<ES<SBTD<DMPS, the values are relatively close to each other with average LOD of 7.0ng/ml. This is 12 times lower than the LOD of normal FAAS without SPE as shown in Table 5-6.

In cases of Cd ion the sequence was DMPS<SBTD<<ES<PS with average LOD of 36.27ng/ml and they are also about 3 times lower than the LOD of FAAS.

The LOD values of Cu ion were close to each other and to LOD values of Zn ion with average LOD 10.25ng/ml. compared with LOD of FAAS, they are about 43 times low.

But, additionally the concentration factor calculated by volume of sample and low volume of final measurements solution (see 5.1.5) has been found to be 100 for Zn, 100 for Cd and 5 for Cu. Thus the obvious improvement of the LODs has been achieved as combination of both improvement factors as shown in Table 5-7. The combination SPE-FAAS is suggested as analytical procedure for trace element analysis with LOD comparable to GFAAS.

Table 5-7 Comparison between LODs of SPE-FAAS and FAAS.

Metal	Total improvement factor	SPE-FAAS LOD ng/ml	FAAS LOD ng/ml	GFAAS LOD ng/ml
Zn	12000	0.007	84	0.02
Cd	276	0.362	100	0.007
Cu	4411	0.102	450	0.014

We will discuss later a very interesting combination between SPE and GAAS to go very deeply in limits of detections.

5.2 SPE-GFAAS with Schiff's bases for ultra trace analysis

In this part of the study we are going to apply the optimized solid phase extraction procedure for ultra trace amounts of metals in water samples using graphite furnace atomic absorption spectrometry GFAAS in order to reach the very low limit of detections. As powerful example we have chosen cadmium determination in water samples. The combination of SPE and GFAAS for Cd analysis is very powerful since we can work in the concentration range of ICP-MS.

5.2.1 Effect of Schiff's base volume

In order to know the amount of Schiff base required to give the best adsorption rate 1000 mg silica columns were coated with different volumes of PS Schiff base solution (0.01M). 50 ml of a sample containing 2 $\mu\text{g/L}$ Cadmium were passed through the columns. The Cadmium was eluted with 5 ml 2 M nitric acid into 50 ml plastic flasks which were filled up to the mark with Millipore water afterwards. The results are show in 5-35.

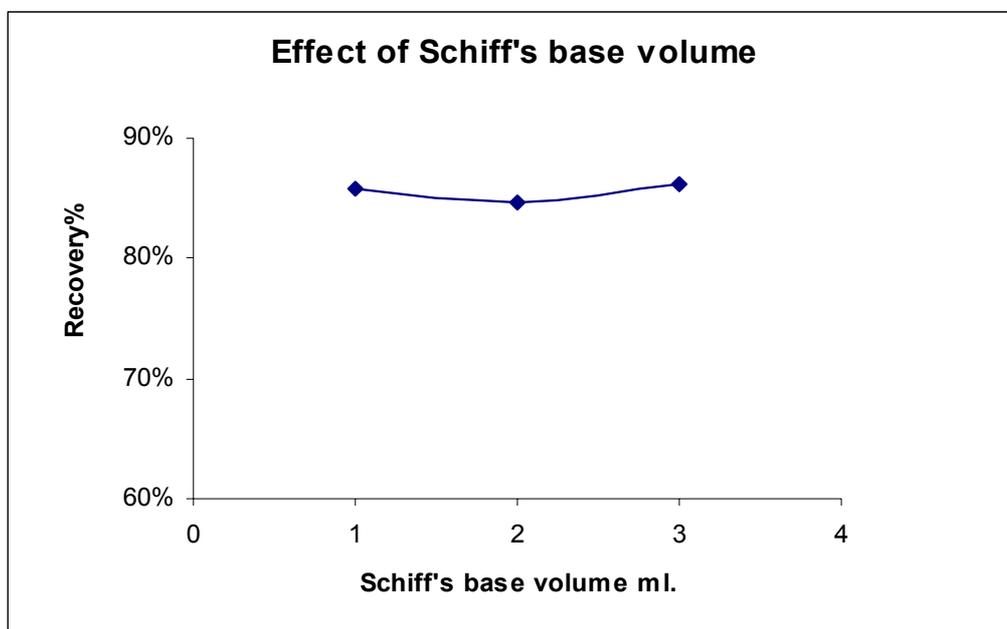


Figure 5-35 Effect of Schiff's base volume on Cd adsorption.

5.2.2 Effect of sample concentration

The recovery percentage stays the same with higher concentrations of cadmium or if the sorbent might be overloaded. There were no significant differences in the recovery percentage of the different concentrations. The results are illustrated in 5-36.

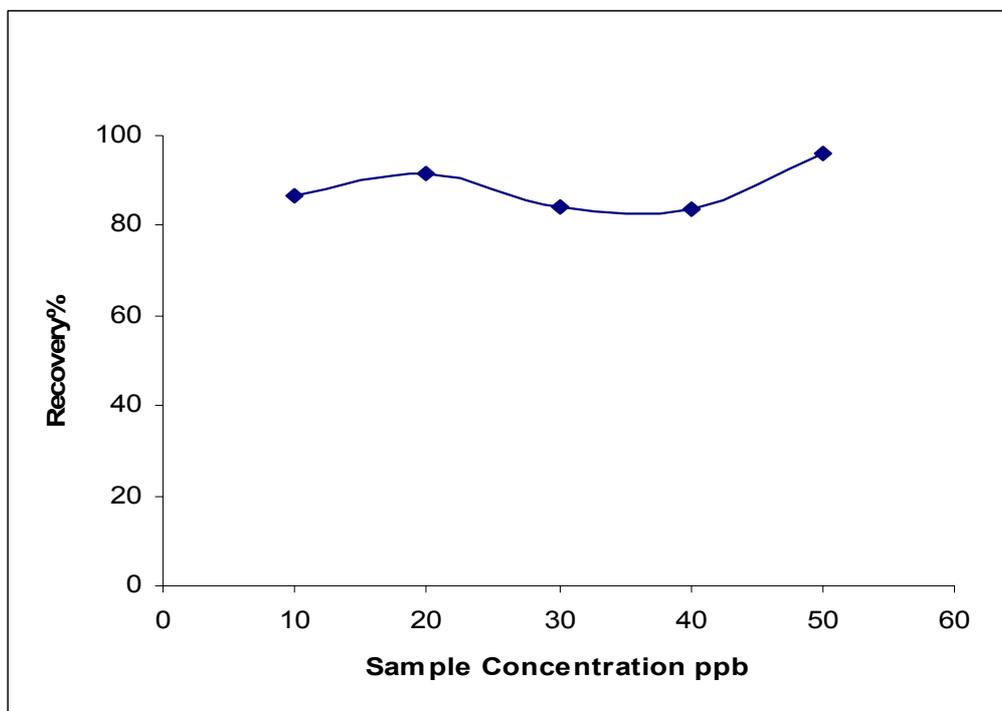


Figure 5-36 *Effect of sample concentration.*

5.2.3 Effect of eluent volume

Different volumes of 2M nitric acid has been used to elute the Cd ions from the sorbent to know the minimum volume being used for this purpose, the smaller the elution volume the higher the concentration factor, the results are show in 5-37. The volume of 1.50 ml was chosen.

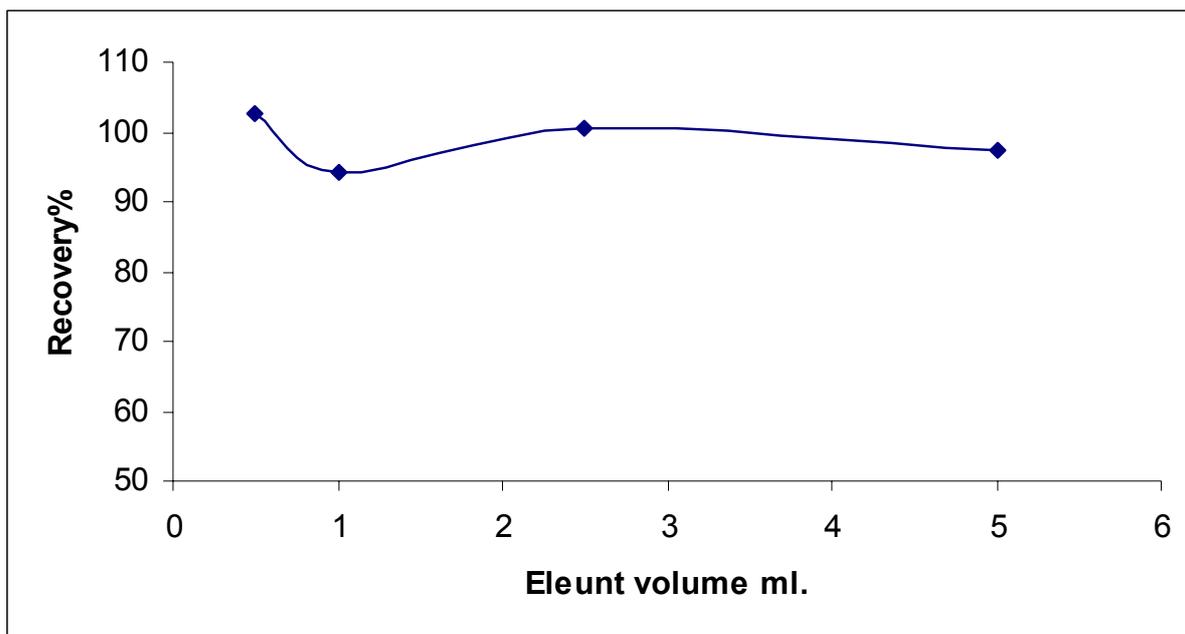


Figure 5-37 *Effect of eluent volume.*

5.2.4 Effect of sample volume

The importance of this experiment is to know the maximum concentration factor and the minimum concentration reached.

The same absolute amount of cadmium was spiked into different amounts of water. Theoretically the eluent should always have the same concentration if the complete sample volume has been sucked through the column. The columns were eluted with 1.5 ml 2 M nitric acid into 50 ml flasks and filled up with Millipore water in order to bring the Cd concentration to the linear range of GFAAS. Hence every eluent should have a final concentration of 2 ppb. The results are shown in 5-38. Concentration factor of 1334 has been achieved. This means that we can measure a concentration of 1.5 ng/L (ppt) which belongs to the concentration range of ICP-MS which meets the target of this study.

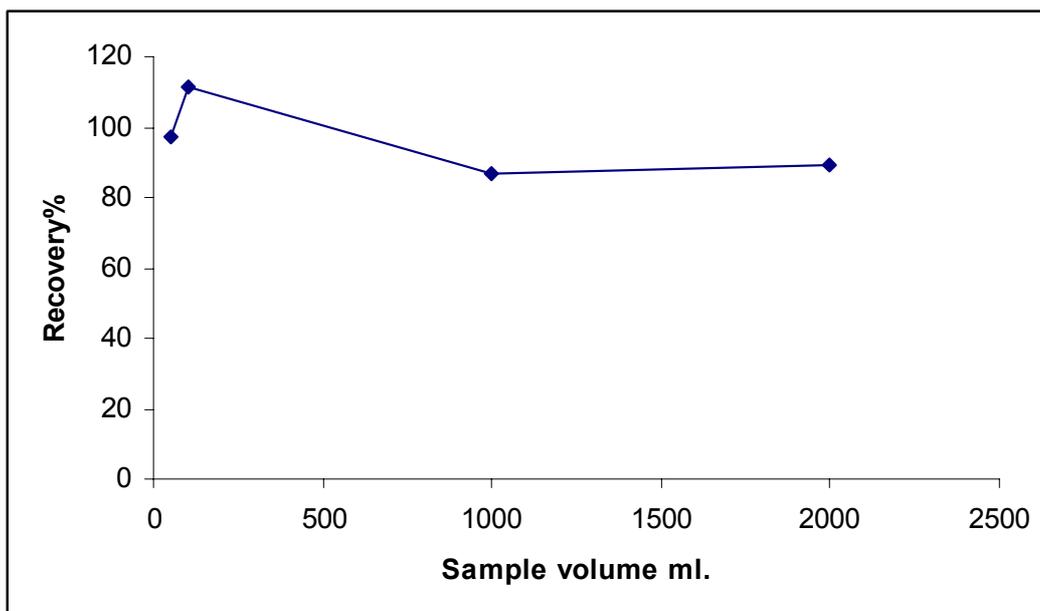


Figure 5-38 *Effect of sample volume.*

GFAAS allows the analysis of very small amounts of an analyte. This two is a great advantage compared to the FAAS that was used for this type of experiments before.

Considering the detection limit of 7ng/L Cd for GFAAS and the concentration factor of 1334, 0.005ng/L could be detected by SPE-GFAAS system, and this value is even lower than the detection limit of ICP-MS for Cd which is 0.09ng/L.

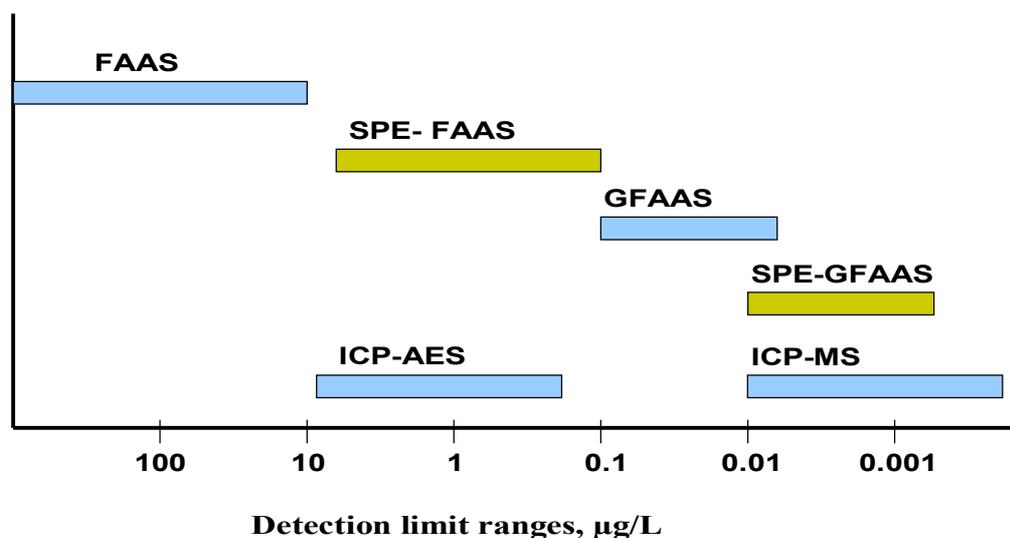


Figure 5-39 Comparison between The detection limit ranges of SPE combinations with some major atomic spectroscopy techniques.

Figure 5-39 illustrates the detection limit ranges of some major atomic spectroscopy techniques including the detection limits of the combination of SPE with FAAS and GFAAS of the method used in the study. It clearly shows that the detection limit range of SPE-FAAS lies in the range of GFAAS. Also the detection limit range of SPE-GFAAS in the range of ICP-MS. The most important advantage of the SPE combinations is that the measurements are matrix free measurements, moreover they are covering the sample preparation step in a very clean and simple way which meets the environmental requirements and the needs of the analytical chemists specially in the developing countries.

The combination of SPE and GFAAS is a very interesting subject and further experiments should be conducted in order to further by improve the whole process.

5.3 Comparison between different silica supports loaded with Schiff's base on pre-concentration/separation.

In this part we will discuss a comparison between three different solid supports, silica gel, C18 polar plus silica gel, and Aminopropyl silica gel under the same condition of the last part using the same procedure in order to find the differences and similarities between them. Before discussing the results of this part let us have a look to the possible kinds of interaction between metal ions and the sorbent. The interaction in case of silica gel occurs between metal ions and the functional groups of the Schiff's bases also with the silanol groups on the silica surface.

Trace elements could be adsorbed on C18 solid phases through van der Waals forces or hydrophobic interaction. Hydrophobic interaction occurs when the solid sorbent is highly non-polar plus the interaction with Schiff's bases, but we should not forget the presence of some extents of silanol groups which could interact with both metal ions and Schiff's bases by means of hydrogen bonding in addition to the van der waals interaction between the Schiff's bases and the C18 aliphatic chain.

In case of Aminopropyl sorbent it contains amino group which takes place in the interaction with metal ions and Schiff's bases.

We have Zn as chemical analogue for the divalents in order to develop a quantitative understanding for the interaction of the divalent organic complexes with silica based sorbent.

5.3.1 Effect of pH on adsorption of Zn(II) ion

The pH value of the medium is one of the most important factors controlling the limit of extractability of metal ions by the proposed modified support due to the competitive reactions between chelate forming group and hydrogen ion in solution. The results are illustrated in figures (5-40, 41, and 42)

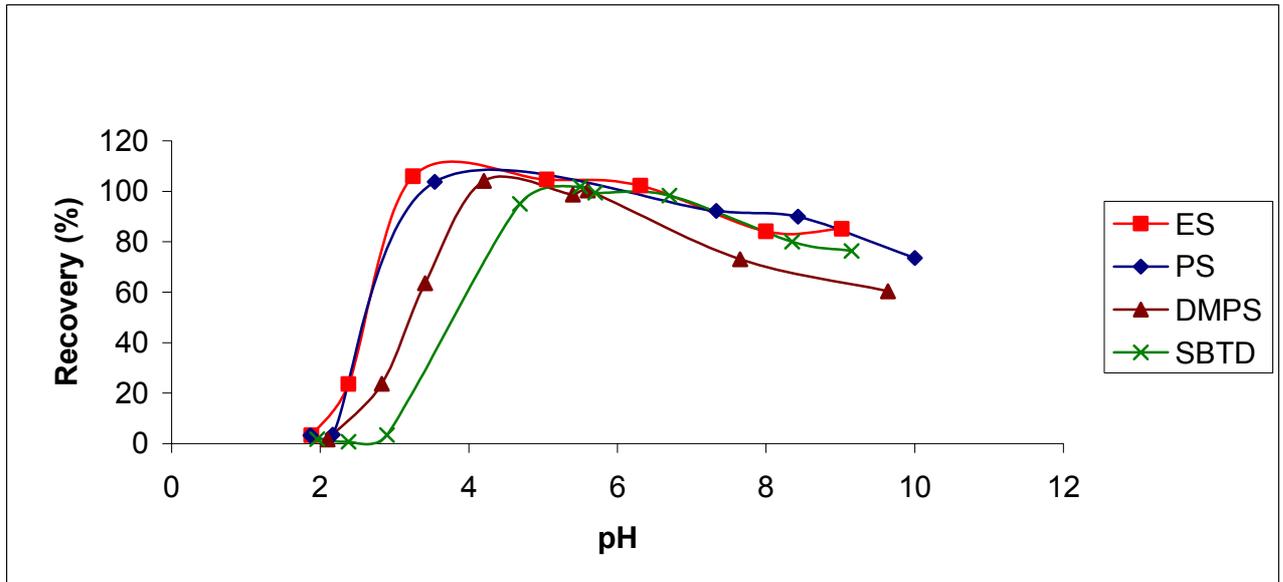


Figure 5-40 Influence of sample pH on the recovery of Zn(II) from silica gel coated with Schiff's bases ligands

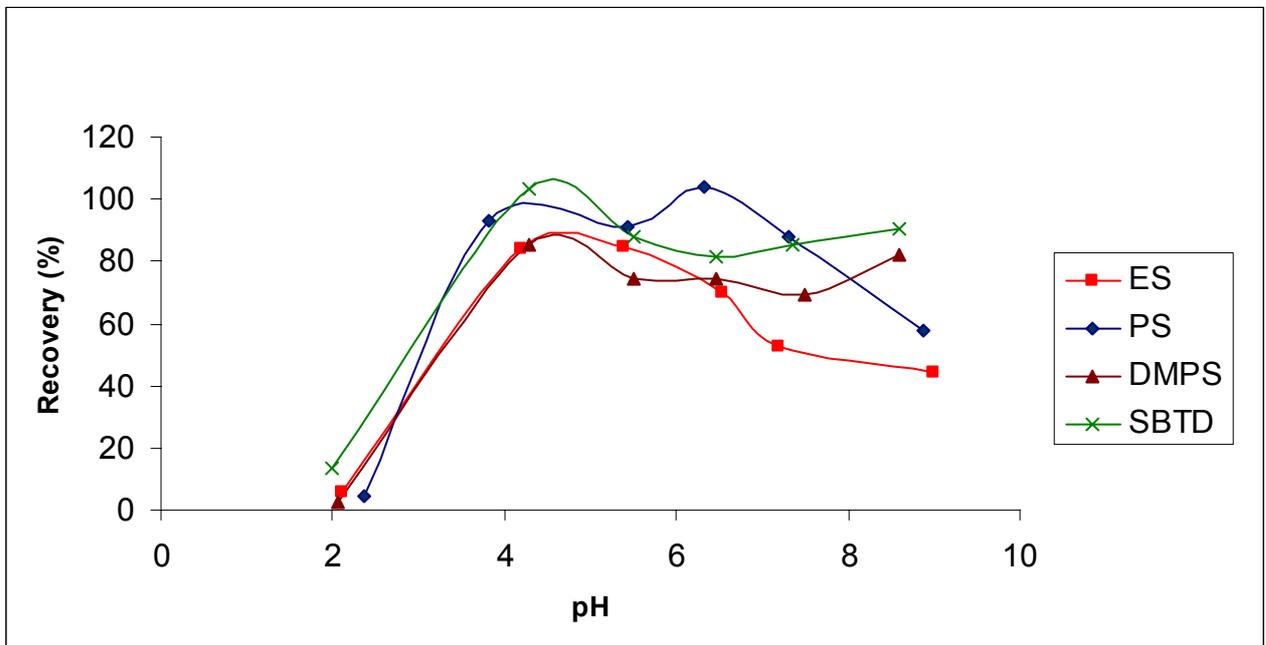


Figure 5-41 Influence of sample pH on the recovery of Zn(II) from C18 polar plus silica gel coated with Schiff's bases ligands.

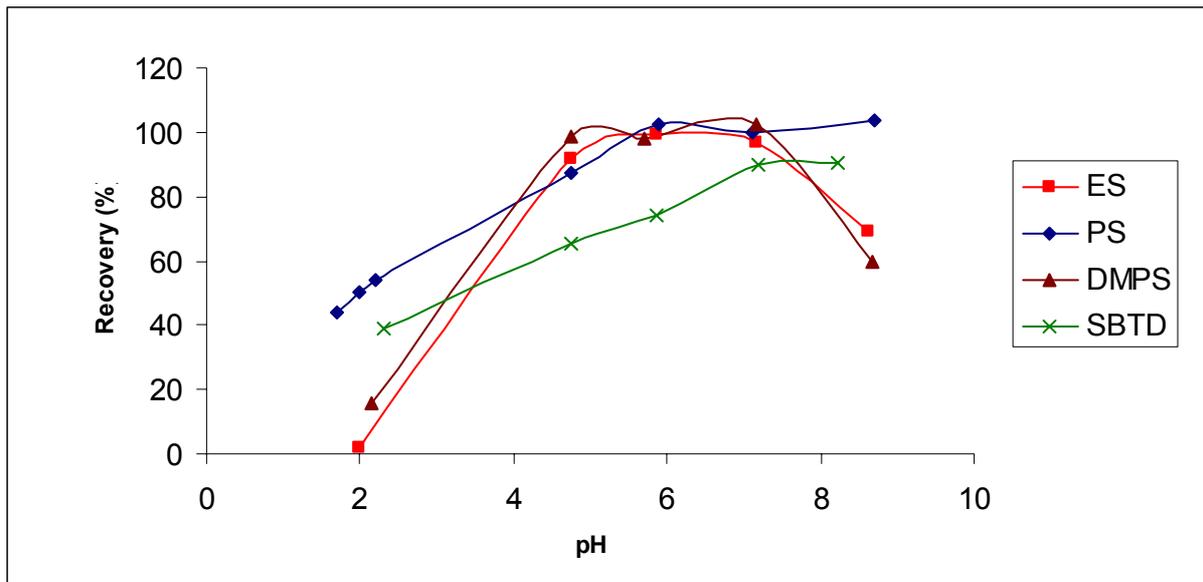


Figure 5-42 Influence of sample pH on the recovery of Zn(II) from Aminopropyl silica gel coated with Schiff's bases ligands.

The maximum recovery of Zn(II) ion was obtained at pH (4-6), the rapid increase in percentage uptake over a narrow pH range indicates that the uptake is accompanied by the net release of H^+ . The recovery was decreased in acidic solution because the hydroxyl protons of Schiff's bases were difficult to be dissociated. On the other side, the decrease of the recovery at higher pH was due to the formation of zinc hydroxide precipitate in the alkaline solution.

The contribution of the polar free sites on the solid support i.e. silanol groups or amine group in the interaction with Zn(II) ion is also expected in the adsorption process.

The feasibility of recommended sorbents for the pre-concentration of trace metals depends on the sorption capacity, selectivity, mechanical and chemical stability, practically complete insolubility in any solvent and the kinetic properties. However, the experimental conditions affecting the efficiency of the sorbent, i.e., pH, flow rate, etc., should also be considered.

The metal uptake by sorbents depends on the chemical form of the metal ions, specific surface properties of the sorbent, and the experimental conditions, such as pH, temperature and metal ion concentrations. The uptake process usually involves adsorption of the metal ions at the surface of the sorbent via interactions with various functional groups and ion exchange processes. During the sorption process, an equilibrium is established between the metal ions adsorbed on the sorbent's surface and the metal ions remaining in the solution. For low metal ion concentrations,

the mass of ions accumulated on the sorbent is usually proportional to the concentration of metal ions in the solution.

5.3.2 Effect of sample volume

From Figure 5-43, 5-44, and 5-45 the recovery values were quantitative up to 1000ml, the highest pre-concentration factor was found to be 100. Larger volumes have been avoided because of dynamic elution of Schiff bases by the sample is expected after 1000ml.

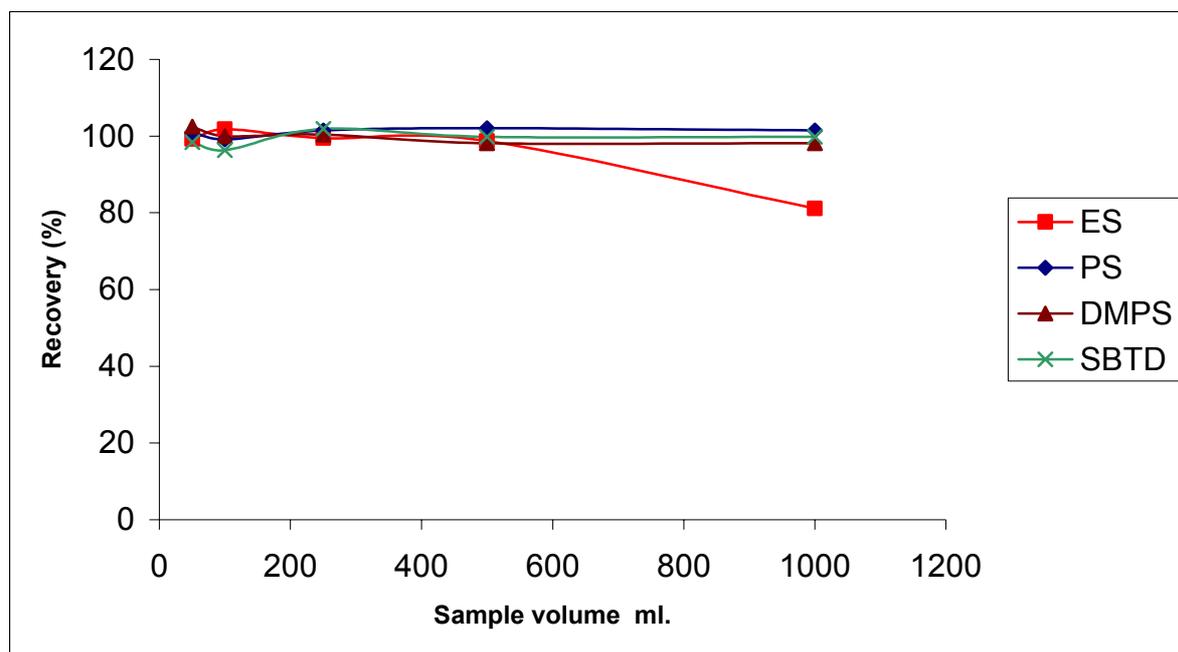


Figure 5-43 *Effect of sample volume on the recovery of Zn(II) ion from silica gel coated with Schiff's bases ligands.*

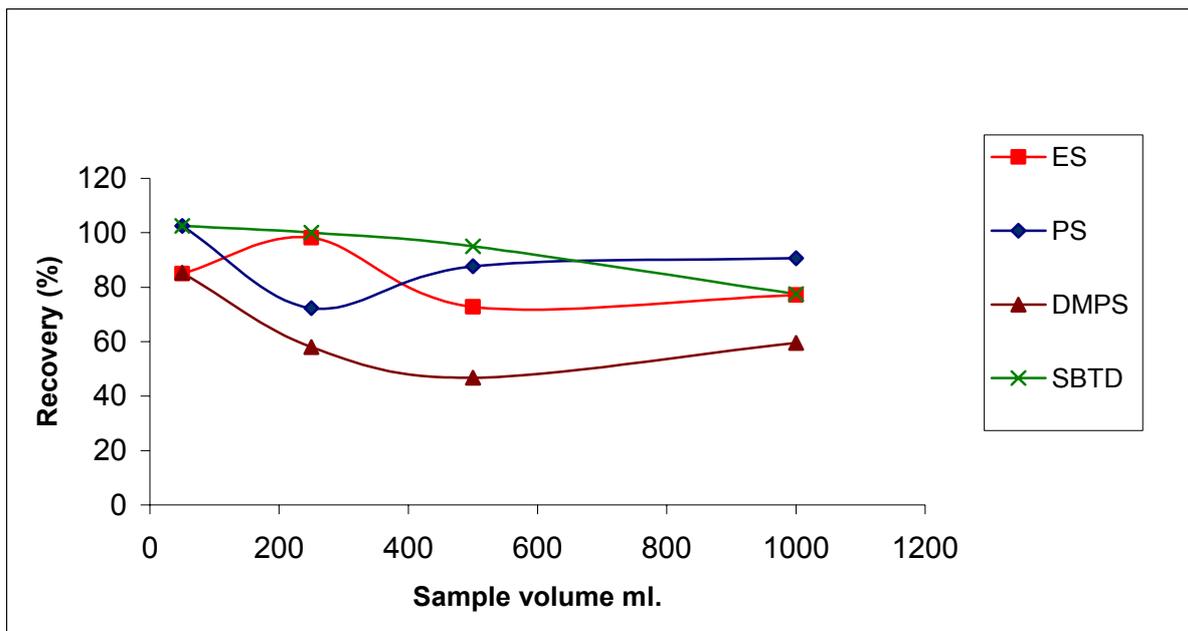


Figure 5-44 Effect of sample volume on the recovery of Zn(II) ion from C18 polar plus silica gel coated with Schiff's bases ligands.

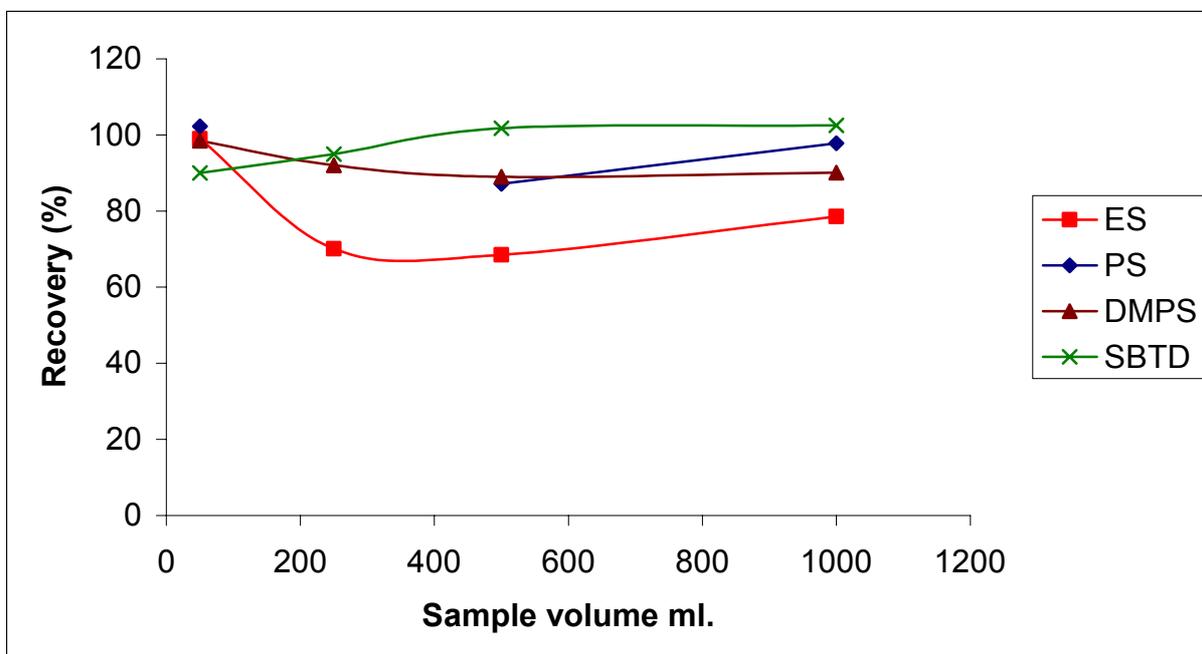


Figure 5-45 Effect of sample volume on the recovery of Zn(II) ion from Aminopropyl silica gel coated with Schiff's bases ligands.

Sorbents based on silica gel show high stability even at the large sample volumes leading to high pre-concentration factors, this is due to the strong interaction between the Schiff's bases and silanol groups on silica surface where is no steric hindrance to the interaction due to C18 chain or aminopropyl group in the other cases. The less recovery percent was by DMPS-C18 polar plus and ES-Aminopropyl sorbents. In case of C18 polar plus SBTD has more stability over the other Schiff's bases because of less polarity, the presence of extra methyl groups gives the priority to SBTD to interact with C18 aliphatic chain plus the interaction between imino group with the polar spot on the solid support. This is was also the case with aminopropyl with additional interaction between Zn(II) ion and the amine group of the solid support.

5.3.3 Selectivity

The selectivity of the sorbents toward Zn(II) ion has been calculated using Kurbatov plots (Figure 5-46, 5-47, and 5-48) using the equation $\log D = a + b (pH)$, Where a and b are empirical constants and D the distribution ratio defined as the ratio of the fraction of added metal that is adsorbed to the fraction of the added metal in the solution and is equal to $[\%Adsorbed/(100 - \%Adsorbed)]$. As discussed before the pH_{50} values are relative measures of the selectivity, at this value the half amount of the metal ion is adsorbed on the surface of the sorbents.

Symmetric Schiff's bases can act as tetra or hexa dentate ligands and can form a strong and week complex with metal ions. As mentioned earlier the nature and number of the N, O, S donor atoms and the semicavity size of the ligand are important in the formation of the stable selective complexes.

The factor governing the selectivity in this part of the study is the nature of the solid support. Selectivity values for sorbents calculated from Kurbatov plot are shown in Table 5-8. The selectivity sequence is variable from one sorbent to another.

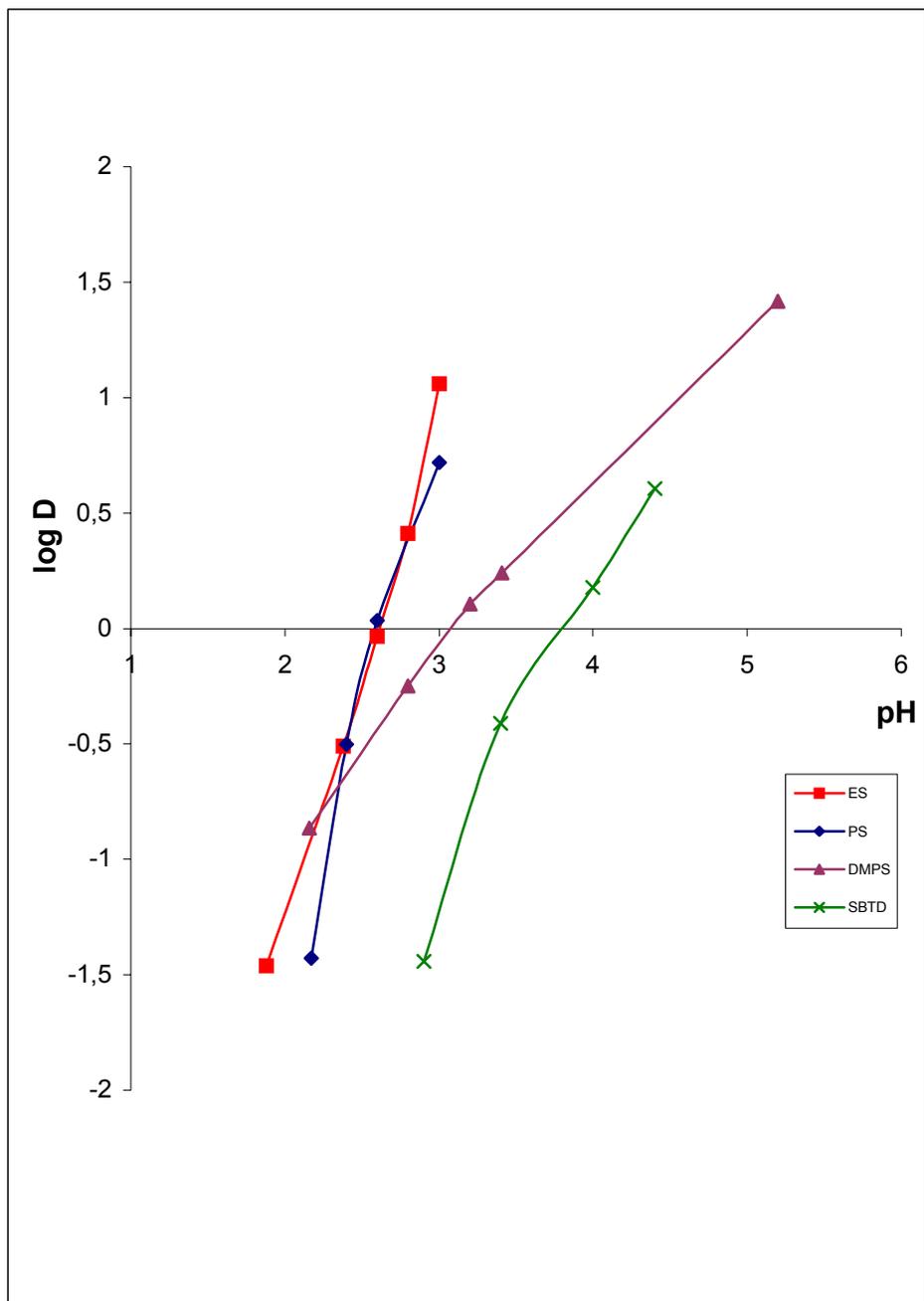


Figure 5-46 Kurbatov plot for Zn adsorption on silica gel coated with Schiff's bases.

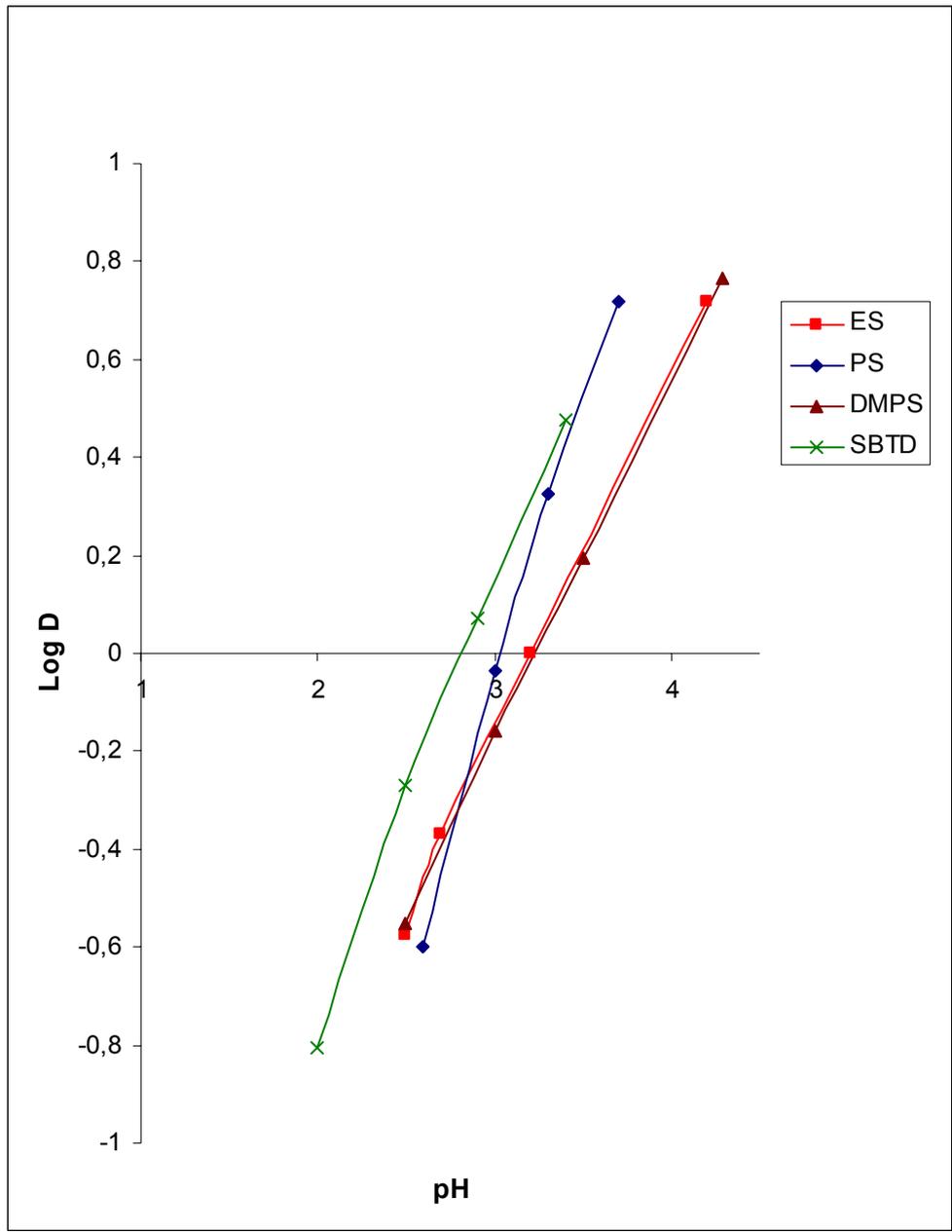


Figure 5-47 Kurbatov plot for Zn adsorption on C18 polar plus silica gel coated with Schiff's bases.

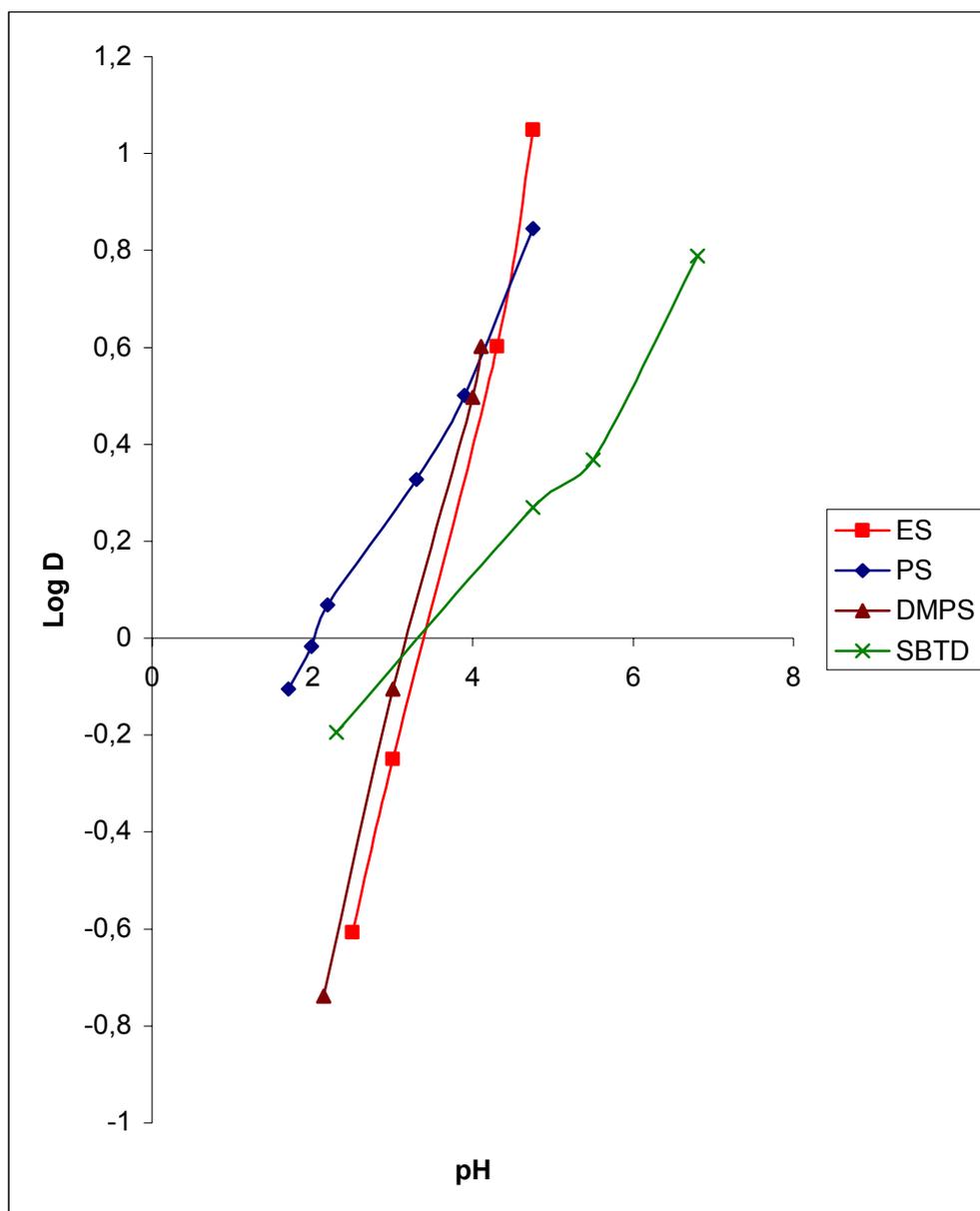


Figure 5-48 Kurbatov plot for Zn adsorption on Aminopropyl silica gel coated with Schiff's bases.

The selectivity in case of silica gel was in the following sequence PS > ES > DMPS > SBTD, (Table 5-6) this sequence is expected because of the ability of PS to form stable complexes with Zn ions, it has a proper semicavity forming six membered ring which is more stable than the five membered one formed by ES. The formation of the complex with DMPS is hindered by the methyl groups on the NN bridge. The hindrance is increased in case of SBTD due to the presence of more methyl groups attached to the imine groups.

Table 5-8 Selectivity of the sorbents toward Zn(II) ion.

Metal/ Ligand	PS	ES	DMPS	SBTD
Silica gel	2.62	2.64	3.05	3.80
C18 polar plus	3.02	3.20	3.22	2.83
Aminopropyl	2.00	3.76	3.30	3.32

In case of C18 polar plus SBTD has the highest selectivity, this may attributed to the lower polarity of the solid support which allows SBTD to be adsorbed more easily by Van der Waals forces. There was no significant difference between ES and DMPS.

In case of aminopropyl sorbent the sequence was PS>> DMPS > SBTD> ES. The solid support itself is involved in retaining process the Zn ion. The moderate length of the aliphatic chain allows Zn ion to make bonds with one or two amino groups on the surface.

In case of silica gel, PS and ES selectivity values are very close, and in the case of C18 polar plus ES and DMPS are very close, while with aminopropyl DMPS and SBTD are close to each other, this could be attributed to the polarity of the solid support since silica is polar and make it easy to attract PS and ES molecules. The polarity of C18 polar plus silica is low therefore, SBTD is more selective and ES and DMPS are close to each other but less than PS which forms more stable complex Zn ion. Aminopropyl with PS has the high selectivity this is because of the combination between the efficiency of the solid support itself and the stability of the complex formed between PS and Zn. Aminopropyl silica has both polar and non polar properties therefore it can share in the adsorption of Zn ion by the amine group at the end of alkyl group.

5.3.4 Capacity of the sorbents

The sorption capacity is defined as the maximal amount of metal ion adsorbed per unit mass of sorbent. The capacities of the sorbents under study toward Zn(II) ion are shown in Table 5-9

Table 5-9 Sorbents capacities toward Zn ion ($\mu\text{g/g}$ sorbent).

METAL/LIGAND	ES	PS	DMPS	SBTD
Silica gel	445	473	418	302
C18 polar plus	123	134	142	146
Aminopropyl	1771	1910	2006	1779

Though its high capacity, aminopropyl is not preferred to be used in SPE after coating with Schiff's bases because it can be used only one time and is not reusable also it is relatively high expensive comparing with C18 or silica gel.

Silica gel is preferred over C18 because of its stability, resistance to solvents and needs less steps for conditioning. Silica support has great resistance to organic solvents and very high thermal resistance, Moreover Silica gel has high mass exchange characteristics and no swelling and finally it is the first commercially available high specific surface area substrate with constant composition.

5.3.5 Analytical performance

The final calibration curves for all metal ions used with all sorbents are presented in figures (5-49, 50, and 51). The calibration curves were constructed with 50ml standard solutions containing 0.2 – 2 $\mu\text{g/ml}$ of Zn(II) ion using silica gel or C18 polar plus loaded with Schiff's bases and containing 0.2 – 5 $\mu\text{g/ml}$ in case of aminopropyl silica gel loaded with Schiff's bases according to the general procedure. The linearly ranges, regression equation and correlation coefficient were obtained by the method of least squares. The limits of detection (LOD) and the limits of quantification (LOQ) were evaluated as the concentration corresponding to three times and ten times the standard deviation of the blank signal, respectively. The results are presented in Table 5-10

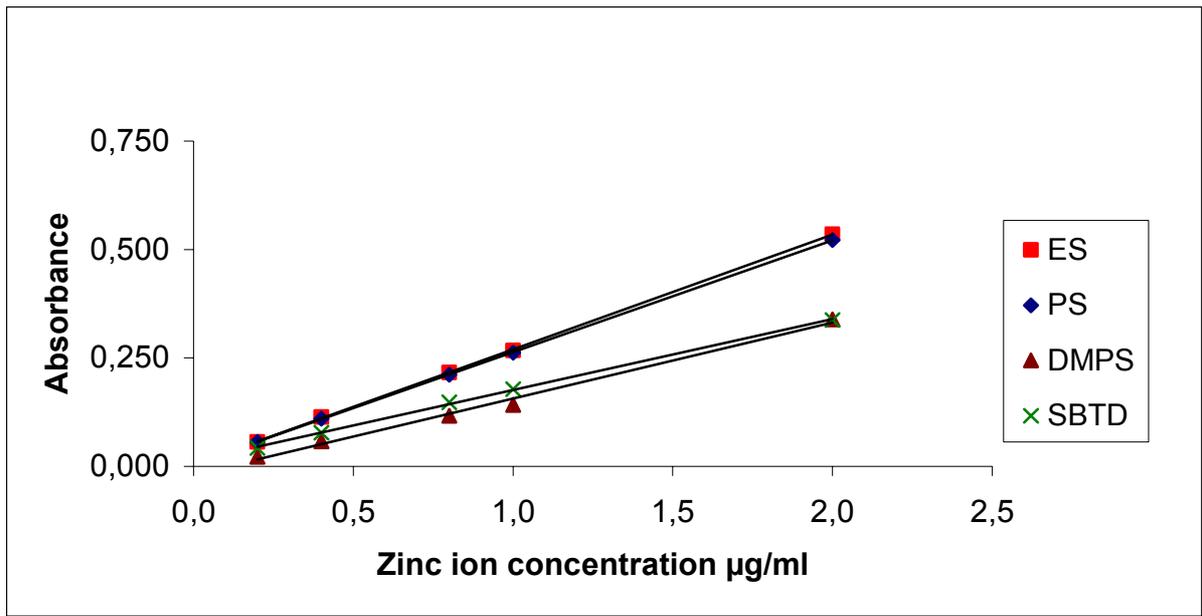


Figure 5-49 Calibration curves of Zn(II) ion using silica gel coated by Schiff's bases.

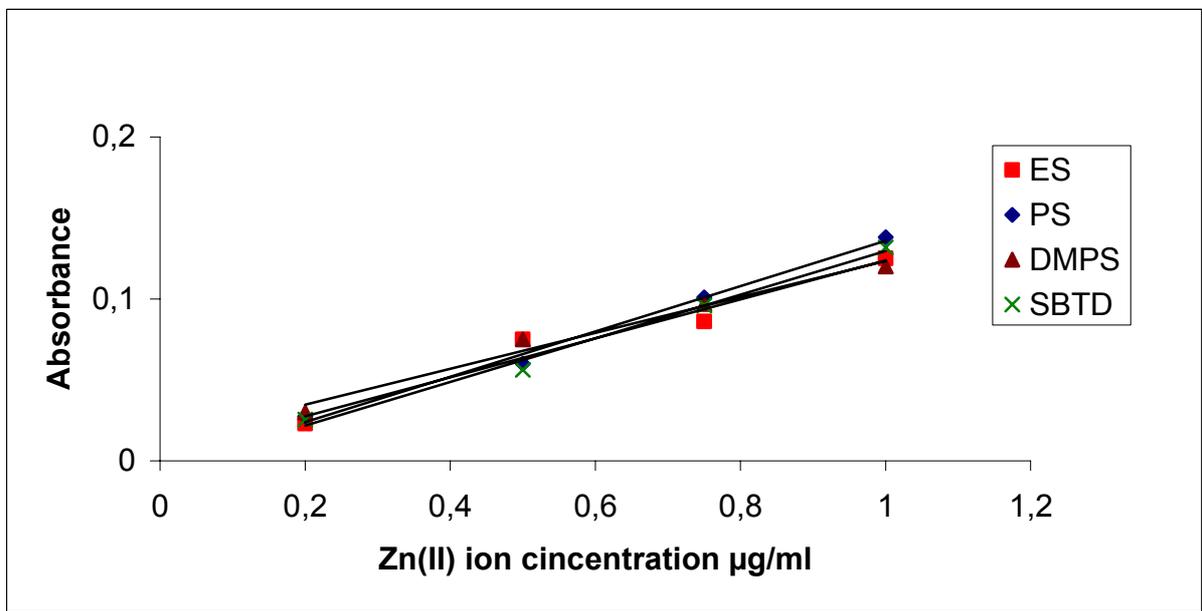


Figure 5-50 Calibration curves of Zn(II) ion using C18 polar plus silica gel coated by Schiff's bases

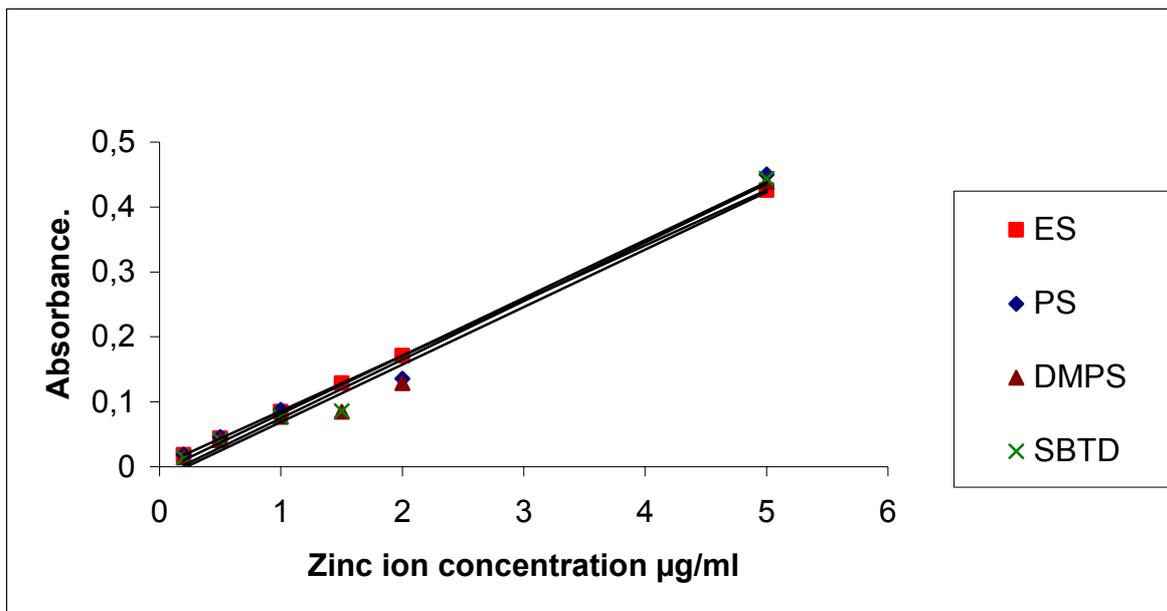


Figure 5-51 Calibration curves of Zn(II) ion using aminopropyl silica gel coated by Schiff's bases.

Table 5-10 Analytical limits of the Zn(II) ion.

Sorbent	Regression equation	Corr.	LOD ng/ml	LOD (Ave.) ng/ml	Linearity range (µg/ml)
ES-silica	Abs. = 0.2662C + 0.0029	$R^2 = 0.9992$	7.60	7.00	0.0253 – 2
PS-silica	Abs. = 0.2589C + 0.004	$R^2 = 0.9997$	7.30		0.0242 – 2
DMPS-silica	Abs. = 0.1699C - 0.0116	$R^2 = 0.9913$	13.40		0.0448 – 2
SBTD-silica	Abs. = 0.1661C + 0.0090	$R^2 = 0.9985$	11.70		0.0391 – 2
ES-C18 P.P.	Abs. = 0.1204C + 0.0035	$R^2 = 0.9603$	1.90	1.60	0.0063 – 1
PS-C18 P.P.	Abs. = 0.1401C - 0.0042	$R^2 = 0.9924$	1.90		0.0063 – 1
DMPS-C18 P.P.	Abs. = 0.1111C + 0.0124	$R^2 = 0.9805$	1.90		0.0063 – 1
SBTD-C18 P.P.	Abs. = 0.1346C - 0.0051	$R^2 = 0.9915$	0.70		0.0022 – 1
ES-A.P.	Abs. = 0.085C + 0.0010	$R^2 = 1.000$	1.50	2.37	0.0051 – 5
PS-A.P.	Abs. = 0.0892C - 0.0076	$R^2 = 0.9872$	0.30		0.0102 – 5
DMPS-A.P.	Abs. = 0.0886C - 0.0198	$R^2 = 0.9798$	4.90		0.0164 – 5
SBTD-A.P.	Abs. = 0.0903C - 0.0156	$R^2 = 0.9878$	2.80		0.0095 – 5

The LOD in case of silica gel is relatively higher than those of C18 polar plus and aminopropyl silica, but in general all LOD values are much lower than FAAS limit of detection which is about 84ng/ml for Zn metal.

The limits of detection achieved with the present method are lower than the detection limits of FAAS without SPE. Moreover taking into account 100 times pre-concentration factor in the most cases (see 5.3.2) we could achieve lower detection limits 100 times lower than the tabulated values, which means 0.007, 0.016, and 0.0237ng/ml for silica gel, C18 polar plus silica, and aminopropyl silica respectively.

5.4 Analysis of trace elements in real samples

5.4.1 Determination of zinc in drinking water

The proposed method was applied to determination of Zinc in water samples. 50ml samples of drinking water from the market were taken as synthetic matrix to test the ability of the sorbents to separate Zn ions. Results are given in

Table 5-11

Table 5-11 Analysis results of water samples.

Schiff's base	Zn(II) added $\mu\text{g/ml}$	Found, $\mu\text{g/ml}$
ES	0.00	N.D.
	0.10	0.094 ± 0.0128
	0.30	0.310 ± 0.0656
	0.50	0.512 ± 0.0242
PS	0.00	N.D.
	0.10	0.103 ± 0.0148
	0.30	0.300 ± 0.0139
	0.50	0.499 ± 0.0154
DMPS	0.00	N.D.
	0.10	0.101 ± 0.0180
	0.30	0.284 ± 0.0270
	0.50	0.485 ± 0.0450
SBTD	0.00	N.D.
	0.10	0.119 ± 0.0170
	0.30	0.314 ± 0.0280
	0.50	0.489 ± 0.0454

N.D. = not detected

As is seen from the results, good agreement is observed between the added and the found amounts of test ion. These results prove the accuracy of the procedure.

Also 500ml samples of bottled drinking water from the market were spiked by $50\mu\text{g}$ Zinc. The amount of Zinc has been determined by the proposed method and the results were $50.21 \pm 1.58 \mu\text{g}$, $49.41 \pm 1.68 \mu\text{g}$, $54.615 \pm 2.713 \mu\text{g}$ and $43.236 \pm 2.333 \mu\text{g}$ for ES, PS, DMPS and SBTD respectively.

5.4.2 Zn in Zinc and multivitamins tablets

The sample (1 capsule) has been dissolved in a small portion of concentrated nitric acid and heated, then diluted to 500 ml with MQ-water. Many aliquots were prepared by transferring 2ml from the solution into 50ml volumetric flask and diluted with MQ-water. The general procedure was applied using the all the sorbents, the final concentration determined by FAAS. The described amount of Zn in the tablets was 5mg/capsules. The results shown in Table 5-12

Table 5-12 Analysis results of Zinc in Multivitamin tablets.

Schiff's base	Zn (mg/tablet)	RSD%
ES	5.1204 ± 0.2299	2.44
PS	5.0912 ± 0.1073	1.14
DMPS	4.9695 ± 0.0733	0.80
SBTD	4.9712 ± 0.0468	0.51

5.4.3 Analysis of Hair sample

The proposed method was applied for the determination of zinc in human hair certified sample, which contains 19.9 μ g zinc. 1.00g of sample has been dissolved in a small amount of concentrated HNO₃ then diluted to 50ml by MQ-water then passed through separation column contains silica gel loaded with PS Schiff's base. The result obtained ($22.8 \pm 0.667 \mu$ g for n=3) without using any masking agent was in reasonable agreement with the certified value, showing that it is possible to use the proposed system for the determination of zinc, even in complex sample.

5.4.4 Determination of Cadmium in drinking water

The method was applied to the separation and recovery of cadmium ion from water samples spiked with certain amount of cadmium ions. 50ml samples of drinking water from the market were taken as synthetic matrix to test the ability of the sorbents to separate Cd ions. Double columns have been used each contains 1g of sorbent. The results are shown in Table 5-13

Table 5-13 Analysis results of drinking water spiked with Cd.

Cd μg Added	PS	SBTD
0.00	0.00	0.00
7.50	7.528 ± 0.34	7.11 ± 0.63
12.50	11.87 ± 0.25	11.705 ± 0.24

6 Summary

The proposed analytical method is based on the separation/pre-concentration of metal ions using solid sorbents coated with Schiff's bases. Four Schiff's bases have been used in the study of the three environmental related and important elements Cd, Cu, and Zn.

The developed method for solid – liquid extraction is convenient, simple, sensitive and of low costs. The preparation of the Schiff's bases used and the solid adsorbent is very simple.

Different factors, including the pH of the sample solution, the sample volume, and the amount of the Schiff's base were examined to optimize the conditions for the pre-concentration of these metal ions in aqueous solutions. The pre-concentration of the metal ions is effective and the elution is quantitative in small volume of 2M HNO₃ solution. The metal ions in the eluent were determined by flame atomic absorption spectrometer (FAAS).

A pre-concentration factor of 100 was obtained, considering the relation between final small volume and initial large volume of sample. Zn and Cd have high recovery until 1000ml sample volume.

The interference effects of the additional ions in the solution have been studied, metal ions of Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Ag⁺, Pd²⁺, V⁴⁺, and V⁵⁺ and anions, Cl⁻, CO₃²⁻, SO₄²⁻, and acetate. There was no significant effect in case of Zn ion. Cd and Cu were affected by the ions, it was clear that the sorbents are selective for Zn ions in solution.

The adsorption behaviour of the sorbents used has been studied. All sorbents undergo Langmuir isotherm.

The limit of detections of SPE-FAAS have been calculated. The improved LODs are 0.007, 0.362, and 0.102 ng/ml for Zn, Cd, and Cu respectively. The improved LODs are much lower than the LODs of FAAS and approaching the LODs of GFAAS.

The system has been used for ultra trace element analysis of Cd using GFAAS as a detection system. Pre-concentration factor of 1334 has been achieved which means a LOD of 0.005ng/L, this value is lower than the LOD of ICP-MS.

The effect of the structure of the solid support on the adsorption of Zn ion has been also studied. Aminopropyl silica and C18 polar plus were taken as solid supports to make a comparable study with silica gel on the effect of the solid support on the adsorption of Zn in aqueous solutions. The capacity sequence was as following Aminopropyl silica.> Silica gel> C18 polar plus silica.

Silica gel has more advantages over the other supports because its reusability and resistance. The improved detection limits of SPE-FAAS for Zn using those sorbents have been found to be 0.007, 0.016, and 0.0237 ng/ml for silica gel, C18 polar plus silica, and aminopropyl silica respectively

By the combination of solid phase extraction system with flame atomic spectroscopy, the detection limit of ICP-AES and GFAAS can be achieved, and the limit of detection of the combination with GFAAS lies in the detection limit range of ICP-MS.

The newly developed procedure SPE-FAAS has been found to be successful in separating the complex matrices (hair, vitamin tablets, and water) and pre-concentrating Zn and Cd ions from relatively large sample volume.

7 References

- ¹ James S. Fritz Analytical Solid Phase Extraction, John Wiley and Sons 1999, 8
- ² A. G. Howard and P.J. Statham, Inorganic Trace Analysis Philosophy and Practice, John Wiley and Sons Ltd., 1993, 139
- ³ K. Studlar, Collect. *Czech. Chem. Commun.* 31, 1966, 1999
- ⁴ A. R. Byrne and D. Gorene, *Anal. Chim. Acta*, 59, 1972, 81
- ⁵ A. A. Blokhim, N. N. Lapytko, V. P. Taushankov, E. A. Andromov, M. A. Vasil'ev, M. I. Semenov and A. A. Nazarov. *Zavodisk*, 45, 1979, 693.
- ⁶ Z. Marczenko, Separation and Spectrophotometric determination of elements", Ellis Harwood, Chichester, 1986.
- ⁷ E. M. Donaldson, *Talanta*, 23, 1976, 411.
- ⁸ Z. Marezenko" Separation and Spectrophotometric determination of elements", Ellis Harwood, Chichester, 1996.
- ⁹ E. Jackwerth, J. Lohmar and G. Wittler, *Z. Anal. Chem.* 266, 1973, 1
- ¹⁰ E. Jackwerth and J. Messersmidt, *Anal. Chim. Acta*, 107, 1979, 177
- ¹¹ J. A. Rodriguez – Vasquez, *Anal. Chim. Acta*, 73, 1974, 1
- ¹² D. Kealey & P. J. Haines. Analytical Chemistry-instant notes, BIOS Scientific Publishers Limited, 2002, 115
- ¹³ V. Camel, *Spectrochimica Acta Part B* 58 (2003) 1177–1233
- ¹⁴ J. F. Biernat, P. Konieczka, B. Tarbet, J. S. Bradshaw, and R. M. Izatt *Separation and purification reviews*, 23(2), 1994, 77-348
- ¹⁵ R. M. Izzat, J. S. Bradshaw, and R. L. Bruening, *Pure & Appl. Chem.*, 68, No.6, 1996

, 1237-1241

¹⁶ M.M.Osman, S. A. Kholeif, N. A. Abou Al-Maaty, and M. E. Mahmoud. *Microchim. Acta* 143, 2003, 25-31

¹⁷ P.K. Jal, S. Patel, B.K. Mishra, *Talanta*, 62, 2004, 1005–1028

¹⁸ V. Ponec, Z. Knor, S. Cerny., ADSORPTION ON SOLIDS, Butterworth, 1974

¹⁹ L.B. Bjorklund, G.M. Morrison. *Anal. Chim. Acta* 343, 1997, 259

²⁰ A.B. Tawali, G. Schwedt. *Fresenius J. Anal. Chem.* 357, 1997, 50

²¹ Mojtaba Shamsipur, Armen Avanes , Mohammad Kazem Rofouei , Hashem Sharghi, Ghasem Aghapour. *Talanta* 54, 2001, 863–869

²² M. Mashhadizadeh, M. Pesteh, Mahzad Talakesh, I. Sheikhshoaie, M. Ardakani, M.Karimi. *Spectrochimica Acta Part B* 63, 2008, 885–888

²³ M. Shamsipur, A.R. Ghiasvand, H. Sharghi, H. Naeimi. *Anal. Chim. Acta* 408, 2000, 271

²⁴ Rydberg, C. Musicas, and G. R. Choppin, *Principle and Practices of Solvent Extraction*, Marcel Dekker, Inc., New York, 1992

²⁵ Baird, and C. H. Mollabar, *Handbook of Solvent Extraction* , Krieger Publ. Comp., 1991

²⁶ I. P. Alimarin, O. M. Petrukhin, and V. V. Bagreev, “*Theory and Application of Solvent Extraction Methods* , Nauka, Moscow , 1985

²⁷ Yu. E. Pazukhina, N. V. Isakova, V. Nagy, and O. M. Petrukhin, *Solvent Extr. Ion Exch.*, 15, 1997, 777.

²⁸ O. M. Petrukhin, T. V. Shipulo, V. Nagy, Yu. E. Pazukhina, and J. Narbutt, *Solvent Extr. Ion Exch.*, 1997, 15, 757.

²⁹ B. Ya. Spivakov, Galina I. Malofeeva, and O. M. Petrukhin *ANALYTICAL SCIENCES APRIL*, 22, 2006, 507

³⁰ Mortimer. Physical chemistry, second edition, Academic press, London, UK , 2000

-
- ³¹ Wedler. Chemosorption: an experimental approach, Butterwoth publishers, London UK 1976
- ³² Roman Rodriguez., PhD Thesis, IAC, Duisburg Essen University 2005
- ³³ Pecsok, Shields, Cairns and Mcwilliam. Modern methods of chemical analysis., John Wiley and Sons Inc. Toronto, Canada.1976
- ³⁴ P. G. Cozzi, *Chem. Soc. Rev.*, 33, 2004, 410-421
- ³⁵ Xiang-Dong Du, Xian-Da Yu., *J. Pol. Sci.: Part A: Polymer Chemistry*, 35, 1997, 3249-3254
- ³⁶ Minna Raeisaenen Ph D thesis Laboratory of Inorganic Chemistry Department of Chemistry Faculty of Science University of Helsinki Finland
- ³⁷ Gyo In, Young-Sang Kim, and Jong-Moon Choi, *Bull. Korean Chem. Soc.*, 29(5), 2008, 969
- ³⁸ Eisch, J. J.; Sanchez, R. *J. Org. Chem.* 51, 1986, 1848–1852.
- ³⁹ Cho, B.R.; Oh, Y. C.; Lee, S. H.; Park, Y. J. *J. Org. Chem.*, 61, 1996, 5656–5658.
- ⁴⁰ Bbadbhade, M. M.; Srinivas, D. *Inorg. Chem.*, 32, 1993, 6122–6130.
- ⁴¹ Glaser, T.; Heidemeier, M.; Grimme, S.; Bill, E. *Inorg. Chem.*, 43, 2004, 5192–5194.
- ⁴² Li, Z.; Jablonski, C. *Inorg. Chem.*, 39, 2000, 2456–2461.
- ⁴³ Ervin Jungreis and Samir Thabet Marcell Dekker Analytical application of Schiff bases- 1969 New York
- ⁴⁴ Gladiola Tantar, V. Dorneanu, Maria Stan., *J. Pharm. Biomed. Anal.* 27, 2002, 827–832
- ⁴⁵ Holzbecher, Z., *Chem. Listy*, 52, 1958, 425
- ⁴⁶ Holzbecher, Z., *Chem. Listy*, 52, 1958, 430
- ⁴⁷ M. Ganjali, J. Ravanshad, M. Hosseini, M. M.Niasiri, M. Pourjaved, M. Baezzat., *Electroanalysis*, 21, 2004, 16
- ⁴⁸ M. Ganjali, P. Norouzi, M. Golmohammadi, M. Rezapour, M.Niasiri., *Electroanalysis*, 11, 2004, 16

-
- ⁴⁹ Young-Sang Kim, Gyo In, Mi-Hyun Kim, and Jong-Moon Choi *Bull. Korean Chem. Soc.*, 27 (11), 2006, 1757
- ⁵⁰ Eun-Jook Kim, Young-Sang Kim, and Jong-Moon Choi. *Bull. Korean Chem. Soc.*, 29(1) 2008, 99
- ⁵¹ M. Ganjali, M. Pourjavid and L. Babaei, *Quim. Nova*, 27(29), 2004, 213-217
- ⁵² F. Shemirani, A. Alsatat, M. Niasiri and R. Kozani, *Journal of Analytical Chemistry*, 59(3), 2004, 228–233.
- ⁵³ M. Shamsipur, A. Soleymanpour, M. Akhond, H. Sharghi, M. H. Sarvarib, *Electroanalysis* 17(9) 2005, 776-782
- ⁵⁴ D. Budziak, E. Luiz da Silva, S. Denofre da Campos, and Eduardo Carasek *Microchimica Acta* 141, 2003, 169-174
- ⁵⁵ M. H. Mashhadizade, M. Sadat Azimi, M. Pesteh, I. Sheikhshoei, M. M. Ardakani, M. Ali Karimi. *Spectrochimica Acta Part B* 63, 2008, 889–892
- ⁵⁶ S. Dadfarnia, A. M. Shabani, A. Ali Jafari, Z. Saadat, F. Tamaddon *Canadian Journal of Analytical Sciences and Spectroscopy* 51(6), 2006
- ⁵⁷ A. P. Jadid and H. Eskandari, *E-Journal of Chemistry* 5(4), 2008, 878-883
- ⁵⁸ 1M. Shabany, 1A. M. Haji Shabani, 1S. Dadfarnia, 1A. Gorji and 2S. H. Ahmadi, *Ecl. Quím., São Paulo*, 33(2), 2008, 61-66
- ⁵⁹ M. Minceva, R. Fajgar, L. Markovska, V. Meshko. *Separation Science and Technology*, 43, 2008, 2117–2143
- ⁶⁰ K N. UPADHAYA. A text book of Inorganic Chemistry, third edition, New Delhi, 2004
- ⁶¹ G. Bortoleto, G. Macarowscha and S. Cadore, *J. Brazil. Chem. Soc.*, 15, (2), 2004, 313-317
- ⁶² M. A. Taher, *Analyst*, 125, 2000, 1865-1868
- ⁶³ Ö. Dalman, S. Karaböcek, A. Demirak, M. Tüfecki, *Turk. J. Chem.* 27, 2003, 649-656
- ⁶⁴ Skoog, Holler and Nieman principles of Instrumental Analysis 5th ed. 1998

-
- ⁶⁵ L. G Harris Analytical Chemistry – principles and techniques Printice-Hall, Inc 1988
- ⁶⁶ Atakol O., Hasan Nazir, Cengiz Arici, Sefa Durmus, Ingrid Svoboda and Hartmut Fuess., *Inorganica Chimica Acta.*, 342(10), 2003, 295-300
- ⁶⁷ Farnoush Faridbod, Mohammad Reza Ganjali, Rassoul Dinarvand, Parviz Norouzi and Siavash Riahi *Sensors*, 8, 2008, 1645-1703
- ⁶⁸ Cotton, Wilkinson, Murillo, and Bochmann, Advanced Inorganic Chemistry, 6th edition John Wiley and Sons, 1999
- ⁶⁹ David Kinniburgh and Marion L. Jackson, Cation adsorption by hydrous metal oxides and clay. Chapter 3 In “Adsorption of inorganics at solid-liquid interface” edited by Marc A. Anderson and Alan J. Rubin, ANN ARBOR CSIENCE Michigan, 1981
- ⁷⁰ John Burgess Ions in solution – Basic principles of chemical interaction, Ellis Horwood, 1988
- ⁷¹ Jolante Hermann, Dinah Schumacher, and Andrea Erxleben *Eur. J. Inorg. Chem.* 2002, 2276-2280
- ⁷² Anssi Haikarainen, Laboratory of Organic Chemistry Department of Chemistry University of Helsinki Finland Academic Dissertation
- ⁷³ I. L. Alcantara, P. S. Roldan, G. R. Castro, F. V. Moraes, F. A. Silva, C. F. Padilha, J. D. Oliveira, and P. M. Padilha, *ANALYTICAL SCIENCE*, 20, 2004, 1029-1032
- ⁷⁴ R. A. Alieva, S. Z. Gamidov, F.M. Chyragov, and A.A. Azizov, *J. Anal. Chem.* 60 (12) 2005, 1114-1117
- ⁷⁵ Anupama Goswami, Ajai K. Singh, B. Venkataramani. *Talanta* 60, 2003, 1141-1154
- ⁷⁶ Bbadbhade, M. M.; Srinivas, D. *Inorg. Chem.*, 32, 1993, 6122–6130.
- ⁷⁷ Glaser, T.; Heidemeier, M.; Grimme, S.; Bill, E. *Inorg. Chem.*, 43, 2004, 5192–5194.
- ⁷⁸ Li, Z.; Jablonski, C. *Inorg. Chem.*, 39, 2000, 2456–2461.
- ⁷⁹ K. Zih-Pere'nyi, A. La'sztity, Zs. Horva'th, A'. Le'vai. *Talanta* 47, 1998, 673–679

⁸⁰ M. R. Ganjali, P. Norouzi, F. Faridbod, S. Riahi, J. Ravanshad, J. Tashkhourian, M. Salavati-Niasari, and M. Javaheri *SENSORS JOURNAL*, 7(4), APRIL 2007

⁸¹ Benjamin, M.M. *Water Chemistry* McGraw-Hill, Singapore 2002

⁸² U. K. Saha, S. Taniguchi, and K. Sakurai. *SOIL. SCI. SOC. AM. J.*, 65, May-June 2001

⁸³ Schindler, Surface complexes at oxide water interfaces, Chapter 1 In “Adsorption of inorganics at solid-liquid interface” edited by Marc A. Anderson and Alan J. Rubin, ANN ARBOR CSIENCE Michigan, 1981

⁸⁴ Marc Anderson, C. Bauer, D. Hansmann, N. Loux and R. Stanforth. Expectations and limitations for aqueous adsorption chemistry. Chapter 9 In Adsorption of inorganics at solid-liquid interface edited by Marc A. Anderson and Alan J. Rubin 1981 ANN ARBOR CSIENCE Michigan

⁸⁵ Eugène Papirer., *Adsorption on Silica Surfaces.*, CRC Press, 2000