CHEMICALLY MODIFIED POLYURETHANE FOAM FOR PRE-CONCENTRATION AND SEPARATION OF INORGANIC AND ORGANIC SPECIES

Vom

Fachbereich Chemie

der Universität Duisburg-Essen

Zur Erlangung des akademischen Grades eines

Dr. rer. nat

genehmigte Dissertation

Von

Sami Mohammed Abdel Azeem Abdel Salam

aus

Giza/Ägypten

Referent: Prof. Dr. Heinz-Martin Kuss

Koreferent: Prof. Dr. Mohamed Fathy El-Shahat

Tag der mündlichen Prüfung: 17. 09. 2008

AKNOWLEDGEMENTS

I would like to purely thank and appreciate my principal supervisor, **Prof. Dr. Heinz-Martin Kuβ**, for accepting me as a member of his research group, guideness, advising, and supporting throughout the duration of my project and about his agreement to have my PhD degree from Germany. I must not forget my Egyptian supervisor, **Prof. Dr. Mohamed Fathy El-Shahat**, who supported me during conduction of the experimental part in Egypt. Also, my grateful thanks to **Prof. Dr. Torsten Schmidt** the head of the instrumental analysis centre (IAC). My great thanks to Dr. Nagwa Burham, who assisted me a lot during performing my experiments at home university. I would like to acknowledge Priv. Doz. Dr. Ursula Telgheder, Prof. Dr. Karl Molt, Dr. Holger Krohn, Dr. Bernd Wermeckes, Dr. Mike Jochmann, Mr. Gerd Fischer, Mrs. Claudia Ullrich, Mrs. Gudrun Bechmann**,** Mr. Robert Knierim for their assistance during my research period. My friends Dr. Abdel Salam Asweisi, Nabil Bader, Rajab El-kailany, Kahled Elsherif, Ahmed Khalil, Fayaz Kondagula, Qian Yuan, Pinghua Li, Stefan Maisen, Dirk Steinmann, Manuel Stephan, Alexandra Jarocki and Holger Lutze are warmly acknowledged. I want also thank Prof. Dr. Alfred Golloch, Dr. Myint Sein for the friendly atmosphere during my work in this department. All co-workers in instrumental analysis institute are also acknowledged. Finally, I must thank my wife for her patience and taking care of my children over the time of doing this work.

CV

*Name***:** Sami Mohammed Abdel Azeem Abdel Salam
*Country***:** Egypt
Date of Birth: 15. 07. 1974
Social Status: Married

Position:

- Hired as Demonstrator at Chemistry Department, Faculty of Science-Fayoum Branch, Cairo University (1999).

- Assistant Lecturer in the same Department (2003) and till now.

Qualifications:

- BSC in Chemistry: Faculty of Science, Cairo University – Egypt (1997).

- MSc in Analytical Chemistry: Faculty of Science-Fayoum Branch, Cairo University (2003).

Publication List:

1-Separation and determination of trace amounts of zinc, lead, cadmium and mercury in tap and qaroun lake water using polyurethane foam functionalized with 4-hydroxytoluene and 4-hydroxyacetophenone, N. Burham; S.M.Abdel-Azeem; M. F. El-Shahat,Analytica Chimica Acta 579 (2006) 193–201.

2-Determination of lead and cadmium in tap water and apple leaves after preconcentration on a new acetylacetone bonded polyurethane foam sorbent, N. Burham; Sami M.Abdel-Azeem; Fathy El-Shahat, Intern J Environ Anal Chem 88 (2008) 775-789.

TABLE OF CONTENTS

LIST OF THE FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AAS: Atomic Absorption Spectrometry

APDC: Ammonium Pyrrolidine Dithiocarabamate

AP-PUF: 2-Aminophenol Functionalized Polyurethane Foam

BLAs: Beta-Lactam Antibiotics

5-BrPADAP: 2-(5-Bromo-2-Pyridylazo)-5-(Diethylamino)Phenol

BTAC: 2-Benzothiazolylazo-p-Cresol

C18: Octadecyl-Bonded Silica

CF: Concentration Factor

CL: Chemiluminescence

CMC: Critical Micelle Concentration

CTAB: Cetyltrimethylammonium Bromide

DAD: Diode Array Detectors

DDTC: Diethylammonium Diethyldithiocarbamate

DDTP: 0,0-Diethyl-dithiophosphate

DDW: Doubly Distilled Water

DMG: Dimethylglyoxime

DRS: Diffuse Reflectance Spectroscopy

ETAAS: Electrothermal Atomic Absorption Spectrometry

EU: European Union

FAAS: Flame Atomic Absorption Spectrometry

FIA: Flow Injection Analysis

Fl: Fluorescence

FT-IR: Fourier – Transform Infrared

GC: Gas Chromatography

HILIC: Hydrophilic Interaction Chromatography

HPAAA-PUF**:** Hydroxyphenylazoacetylacetone Functionalized Polyurethane Foam

HPANaph-PUF: Hydroxyphenylazonaphthol Functionalized Polyurethane Foam

HPAPyr-PUF: Hydroxyphenylazopyrazolone Functionalized Polyurethane Foam

HPLC: High Performance Liquid Chromatography

8-HQ: 8-Hydroxyquinoline

ICP – OES: Inductively Coupled Plasma – Optical Emission Spectrometry

LC: Liquid Chromatography

LOD: Limit of Detection

LOQ: Limit of Quantification

MBT: 2-Mercaptobenzothiazole

MDI: Methylene Diphenyl Diisocyanate

Me-BTABr: 2-[2´-(6-Methyl-Benzothiazolylazo)]-4-Bromophenol

Me-BTANC: 2-(6'-Methyl-2'-Benzothiazolylazo)Chromotropic acid

MNA: 2-Mercapto-*N*-2-Naphthylacetamide

MPSP: 3-Methyl-l-Phenyl-4-Stearoyl-5-Pyrazolone

NBDTFB: 4-Nitrobenzenediazonium Tetrafluoroborate

NMR: Nuclear Magnetic Resonance

NOM: Natural Organic Matter

PAH: Polyaromatic Hydrocarbons

PAN: l-(2-Pyridylazo)2-Naphthol

PAR: 4-(2-Pyridylazo)-Resorcinol

PMMA: Polymethyl Methacrylate

PS-DVB: Polystyrene-Divinylbenzene

PCTDD: Poly (N-Chloranil – N,N,N',N'–Tetramethylethylene-Diamine-Dichloride)

PUF: Polyurethane Foam

SPE: Solid – Phase Extraction

SPME: Solid – Phase Microextraction

TBP: Tributly Phosphate

TBT: Tributyltin

TDI: Toluene Diisocyanate

TLC: Thin Layer Chromatography

TPD: Temperature –Programmed Desorption

VOC: Volatile Organic Compounds

XRF: X-Ray-Fluorescence

TERMINOLOGY

Adsorption: accumulation in condensed form at the surface

Absorbents: substance capable to absorb

Sorbed: taken into and retained

Sorb: take up either by adsorption or by absorption

Sorbate: molecules or ions bounded to the surface

Solvate: combine with molecules or ions of the solute to form a compound

Solute: substance that is dissolved to form a solution

Adsorbate: a substance taken up on a surface by adsorption

Solvent: a substance, usually a liquid that dissolves

Adsorptive species: substance being adsorbed

Activity coefficient: a factor used in thermodynamics to account for deviations from ideal behavior in a mixture of chemical substances

ABSTRACT

In this work, polyurethane foam was chemically functionalized with different ligands to adsorb inorganic species (heavy metals) or organic compounds (antibiotics) and applying the off-line and on-line solid phase extraction procedure.

Chapter 2 includes a review about the adsorption process to solid surfaces and the factors that may affect this phenomenon. This has led us to discuss in some details the solid phase extraction technique and its utility in analytical applications with special reference to different kinds of solid sorbents that are commonly used. In addition, the superiority of polyurethane foam as sorbent over other materials is discussed because of its unique chemical and physical properties making it very attractive and a promising sorbent. Also the reasons why this material is different from other polymers are mentioned.

Furthermore, some basic information are explained about the material under investigation starting from the historical beginning of its manufacture, structure, physical and chemical properties, its application as sorbent in solid phase extraction methodology either in unloaded or loaded form and the applied procedures in this technique. Finally, various methods for modification of polyurethane foam which involves physical immobilization and chemical grafting or functionalization in order to further understanding into the early stages that have been done with attractive sorbent.

In chapter 3 we point out the aims of this study as short notes.

The experimental part which includes the instrumentations, chemicals and solutions, sample preparations and the recommended procedure is explained in details within chapter 4.

In the course of this work, chapter 5 concerned also with chemical modification of polyurethane foam since it was functionalized with different chemical compounds namely 2-aminophenol, 2-naphthol, acetylacetone and pyrazolone. The new sorbents were examined to preconcentration/separation of Cu, Zn, Pb, Cd and Ni. The sorbents have capacity within the range 11.9-18.7, 3.4-10.5, 3.5-6.1, 2.7-7.5 and 18.6-27.4 μmol/g for Cu, Zn, Pb, Cd and Ni respectively and preconcentration factor up to 500. Certified and natural samples were analysed and the results showed RSD from 1.3 to 8.2%.

Also in this chapter we investigated another kind of chemical treatment of polyurethane foam where we succeeded to immobilize a copolymer ligand on its surface. As far as I know, there is no available procedure in literature about the use of modified polyurethane foam for preconcentration and separation of antibiotics. The treated foam has the capability to adsorb anionic organic species such as β - lactam antibiotic compounds namely cefaclor, amoxicillin, ampicillin and cefotaxime in the pH 8-9. In this consequence, on-line flow injection analysis system with UV detection was set up. The developed procedure showed good preconcentration/separation performance. The proposed sorbent capacity varies from 0.60 up to 0.88 μmol/g, concentration factor from 21 to 39 at 2 min preconcentration time and sample frequency 12 h^{-1}. Moreover, the method was applied to analyse two spiked (≥ 100 μg/l) natural samples (human urine and cow milk) and pharmaceutical formulations with RSD within 0.1 – 9.7 %. The LOD of the method under investigation is found to be 11, 17, 24 and 13 μg/l for cefaclor, amoxicillin, ampicillin and cefotaxime, respectively. Finally, the sorbent showed excellent separation efficiency of the four examined antibiotics in synthetic and milk samples by the use of micellar mobile phase. It was carried out as comparable to liquid chromatographic technique under simple instrumentation setup.

INTRODUCTION

Despite the selectivity and sensitivity of analytical techniques such as FAAS, ETAAS, ICP-OES, and ICP-MS, LC-UV, LC - MS techniques, there is a critical need for the separation and preconcentration of trace analytes from matrices prior to their determination, due to their frequent presence at low concentrations in environmental samples and higher matrix interferences. Sample preparation processes including separation and preconcentration have a direct impact on trueness, precision and detection limits for many analytical methods. This process is also the rate determining step of the analytical method.

Analytical chemists continue to search for sample preparation procedures that are faster, easier, safer, and less expensive, to provide true and precise data with reasonable quantification limits. Among the separation/preconcentration methods, SPE has become the most frequently used technique for trace metal analysis. Chemically modified sorbents offer the opportunity to preconcentrate and separate the analyte under investigation with higher selectivity and sensitivity.

There are many solid materials available with different properties which are suitable for SPE applications. Among them, PUF, silica gel, inorganic oxides, activated carbon and cross-linked polystyrenes. Recently, PUF has attained considerable attention because it is one of the most interesting materials that have excellent hydrodynamic and chemical properties making it a promising sorbent in the area of SPE. This is also due to the wide variety in chelating reagents available to react chemically with the chemical groups on the surface of PUF.

In modern analytical chemistry, the general trend is towards the elaboration of simple, ecologically safe, sensitive, and selective methods for the determination of trace components combining previous concentration methods and further determination by physical or physico-chemical methods. Quantification at low concentration levels (µg/L) comprises one of the most considered targets in analytical chemistry. In recent years, trace concentrations have been determined in a great variety of environmental samples e. g. water, soil and food.

1 INTRODUCTION

The need to develop simple, less expensive preconcentration/separation procedures for analysis of natural samples is a great challenge especially in the developing countries. Researchers in these countries attempt to setup alternative simple techniques to the highly sophisticated one such as liquid or gas chromatography which is too expensive. So, one of the most important goals in this work is to achieve such simple preconcentration system that allows determination of the species at concentration within the µg/L range. Although there are several analytical systems capable to measure the compound at concentration less than µg/L level but we are interested to this range because the analytes under study exist around this concentration level in polluted environmental samples in my country. An important question that may be stated in this context, is it possible to achieve a simple, less expensive analytical procedure to preconcentrate/separate and determine the analyte with relevant accuracy at this concentration level without the need to highly sophisticated instruments?

THEORITICAL AND BACKGROUNG KNOWLEDGE

2.1 Theory of Adsorption at Solid Surfaces

Adsorption of analyte to solid surface depends on the interaction forces formed between the analyte in the sample and the properties of the solid sorbent. Many kinds of analytes can be retained on the solid material but in most cases their chemical properties can not be changed. Therefore, the analyte modification is not feasible and it is recognized independent variable in this process. Another factor is the solid material upon which the adsorption takes place. Indeed not the whole solid material participates in the adsorption process but mostly its surface since adsorption process is surface phenomenon. Solid surface is the interaction point between the dissolved analyte in the sample and the functional groups in the sorbent. Thus the best way to improve adsorption is to select suitable solid material that can be chemically changed in its surface so that it becomes able to strongly and/or selectively attract the analyte component from the sample.

Different processes can occur when the molecule approaches the surface. The molecule can be either reflected or adsorbed and the adsorption can either be as a molecule or as atoms in the case of molecular dissociation. Dissociation can occur either directly upon hitting the surface or proceed after molecular adsorption. In the last case the molecular adsorption acts as a precursor to dissociation. Once the molecule has been adsorbed it may desorb again if the energy supplied by the thermal bath as represented by the solid is sufficient for escaping from the solid surface into the liquid or gas phase. So far the possible procedures for dissociation are either described as a direct by hitting process or as a thermalised process by energy exchange with the surface which increase the internal degree of freedom in the molecule. It is very well possible that a continuous range of processes exists between the two extremes. What at first sight appears as a simple interaction proves to be a rather complicated process [1].

2.1.1 Sorption

Before discussion of the solid-phase extraction techniques, it is firstly necessary to understand the physical–chemical processes of sorption. *Absorption*, also referred to as partitioning, occurs when analyte pass into the bulk of the extracting phase and is retained. *Adsorption* is the attraction of an analyte to a solid that results in accumulation of the analyte at porous surfaces of the solid. Absorption results from weaker interactive forces than adsorption. Because adsorption and/or absorption processes are sometimes difficult to distinguish experimentally and often occur simultaneously, the general term *sorption* is used for both absorption and adsorption [2].

The term *sorbent* refers to the solid extracting phase, including certain solid-supported liquid phases. A process opposite to sorption is called *desorption* which is a phenomenon whereby a substance is released from or through a surface. To predict and optimize extraction it is important for the analyst to be aware of the nature of the sorbent used. Although different processes may dominate in different situations, it can be assumed that multiple steps occur during sorption of an organic compound from liquids ‘‘into’’ or ‘‘onto’’ a solid phase. Any of the steps may become a rate-limiting process in controlling sorption of an analyte.

The analyte may interact with a solid-phase sorbent in at least four ways: *Firstly***,** through absorption, the analyte may interact with the sorbent by penetrating its three-dimensional structure. Most sorption technologies act like a sponge or a filter, soaking up contaminants until they run out of surface area. Three-dimensional penetration into the sorbent is a particularly dominating process for solid-supported liquid phases. In the absorption process, analytes do not compete for sites; therefore, absorbents can have a high capacity for the analyte. *Secondly*, the analyte may interact two-dimensionally with the sorbent surface through adsorption due to intermolecular forces such as van der Waals or dipole–dipole interactions. In the adsorption process, analytes may compete for sites; therefore, adsorbents have limited capacity. *Thirdly*, if the compound is ionogenic (or ionizable) in aqueous solution, there may be an electrostatic attraction between the analyte and charged sites on the sorbent surface. Sorbents specifically designed to exploit these types of ionic interactions are referred to as ion-exchange (either anion- or cation-exchange) sorbents. *Finally*, it is possible that the analyte and the sorbent may be chemically reactive towards each other such that the analyte becomes covalently bonded to the solid-phase

sorbent. This type of sorption is generally detrimental to analytical recovery and may lead to slow or reduced recovery, also termed biphasic desorption. All of these interactions have the potential of operating simultaneously during sorption [2].

2.1.1.1 Sorption of Nonpolar Compounds

Non-ionic compounds can be sorbed by inorganic solid extractors that contain polar groups such as hydroxyl groups on the surface of silica gel, clay, soil or sediments enable good extraction. It is hypothesized that the methylene groups of the aliphatic chain may form a kind of hydrogen bonding with the oxygen atom of the solid material of the type C–H…..O–Si. The degree of adsorption depends on the activity of the methylene groups and on the chain length in the compound. In conclusion, all these solids can sorb nonpolar compounds. Additionally, different types of bonds are involved in sorption of organic chemicals. Hydrophobic interactions are prevailing and the values of the distribution coefficient (K_d) depend linearly on the organic compounds content in the sorbent. Thus, sorption may be assimilated to a partitioning of the solute between an organic phase and an aqueous phase. Relationships between sorption coefficients of the organic compounds (log K_{oc}) by solid sorbent and partitioning into some organic solvents (log K_{ow}) were derived on this basis. Therefore, if the organic phase is the same for a series of sorbents, K_{oc} is expected to be the same; however, often this is not the case, and K_{oc} decreases by increasing the polar character of the organic matter [3].

Additionally, C18 is the most hydrophobic silica-based sorbent available. It is the most popular SPE sorbent because of its extreme retentive nature for non-polar compounds. C18 is generally regarded as the least selective silica based sorbent, since it retains most organic analytes from aqueous matrices - often a benefit when the compounds of interest vary widely in structure. The potential for polar interactions between the analyte and sorbent is less significant with C18 than with any other sorbent because of the predominant effect of the long hydrocarbon chain. When analyzing small to intermediate molecules, C18 can also be utilized for desalting aqueous matrices prior to ion exchange because salts pass through it unretained.

2.1.1.2 Sorption of Polar Compounds

Sorption of polar and ionizable compounds depends on various degrees of moisture content in sorbing system, the presence of exchangeable cations, electrolyte concentration and pH. Water solubility may also affect sorption. For example, many pharmaceuticals such as sulfonamides are both fairly water-soluble and polar compounds, which ionize depending on the pH of the matrix. Hydrophobic partitioning of these compounds may cause their sorption to soils via cation exchange, cation bridging, surface complexes, and hydrogen bonding. Accordingly, sorption of sulfonamides, such as sulfapyridine, varies between soils and is affected by the quantity, composition, and structure of soil colloids [4].

2.1.1.3 Hydration Effect on the Sorption of Organic Compounds

Changes in NOM upon hydration (e.g., swelling, increased flexibility, changes in ionization status of polar functional groups) can be expected to affect sorbate diffusion, sorption kinetics and the extent of organic compound interaction in a sorption domain. The extent of hydration effect correlates with compound ability to undergo interactions with NOM. In this conception, certain polar groups of dry NOM are unavailable for compound sorption due to strong intra- and intermolecular NOM interactions. Water molecules solvate these groups creating new sorption sites that are then available for compounds that successfully compete with water molecules for the new sites, resulting in a strong increase in sorption upon hydration. The studies demonstrate the importance of polar NOM non-covalent links in NOM aggregation and in the hydration effect on sorption of organic compounds. For many compounds with no or low ability to interact specifically with NOM, the changes in NOM sorbent structure that accompany hydration (increased flexibility, change in ionization status of polar functional groups, conformational reorientation of macromolecules), do not significantly affect their sorption, thus having important ramifications for understanding the role of sorbent rigidity for sorption [5].

2.1.2 Adsorption Processes

Adsorption is the process that takes place if the solute accumulates on the surface of solid or liquid (adsorbent) to form film of adsorbate. Adsorption phenomena are operative in most natural physical, biological, and chemical systems, and adsorption operations employing solids such as activated carbon and synthetic resins are used widely in industrial applications

and for purification of waters and wastewaters. The process of adsorption involves separation of a substance from one phase accompanied by its accumulation or concentration at the surface of another. Adsorption, either by ion exchange or chromatography, is a kind of sorption process where selective adsorption for certain species occurs by its transfer from the fluid phase (gas or liquid) to the surface of the insoluble or rigid particles (stationary phase) which are suspended in a vessel or packed in a column [6].

2.1.2.1 Origin of Adsorption Phenomena

Atoms or groups of atoms of the surface of a phase (solid adsorbent) differ fundamentally from those within the bulk phase. They cannot interact symmetrically with neighboring atoms and their effect is unbalanced. For this reason, they frequently exhibit unsaturated valences which are capable of forming a bond with foreign atoms or molecules on the surface (Fig. 2.1). This process, which is called adsorption, is of considerable interest for both fundamental and applied research. Adsorption influences all those phenomena which depend on the properties of the surfaces. They include, for example, electron emission caused by external energy transferred to the surface electrons or electrical resistance to contacts resulting from penetration of conduction electrons to the contact film. Adsorption constitutes the primary step in corrosion processes and it is a prerequisite for every catalytic reaction involving solid catalysts [7].

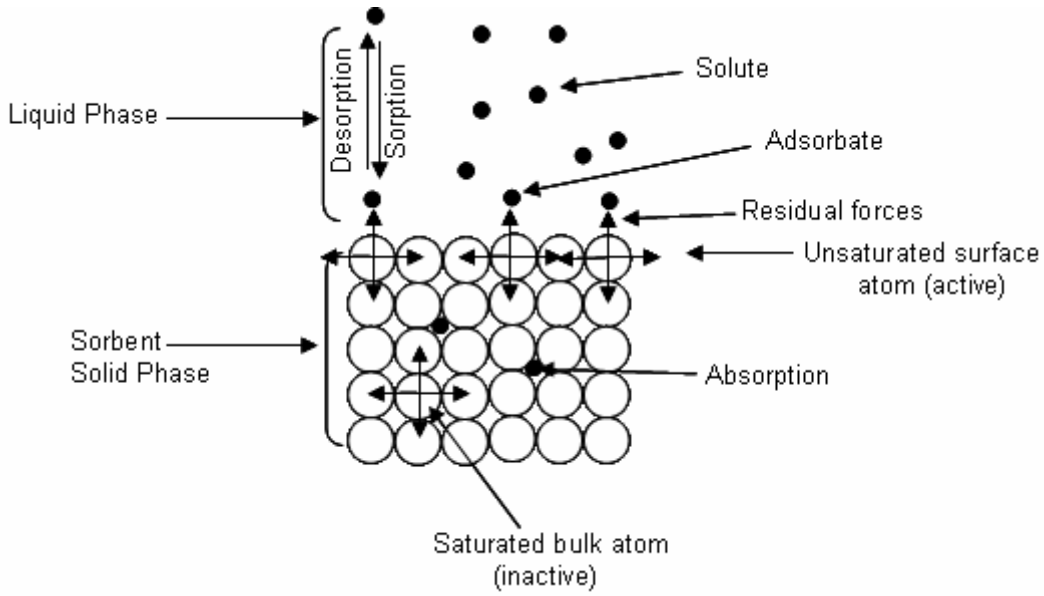


Fig. 2.1: Schematic diagram for the origin of adsorption process at solid surfaces

2.1.2.2 Adsorption versus Precipitation

Specific adsorption and precipitation processes are similar in many respects; the major difference being that adsorption is a two-dimensional process while precipitation is three dimensional. In natural systems, adsorption generally occurs on surfaces of minerals that are themselves precipitates. If an adsorbed species happens to be identical to one of the mineral components, adsorption will contribute to the crystal growth, making the adsorption reaction an integral part of the precipitation process such as the adsorption of iron species on the iron (III) hydroxide precipitate. If the adsorbed species is not a component of the mineral adsorbent, a number of reaction products are possible, depending on the chemical characteristics and concentration of the adsorbate. An example for this is the reaction of anions such as phosphate, arsenate or arsenite after its adsorption on the surface of hydrous oxides of iron or aluminum [8].

2.1.2.3 Factors Affecting Rate of Adsorption

2.1.2.3.1 Nature of Adsorbate

The properties of the adsorbate molecules strongly affect the adsorption process. For example, the degree of ionization of the solute molecules retards its sorption and this explains the lower tendency of extraction of the ionic salts by activated carbon. Similarly, the solubility of the solute in the solvent and branching in its geometric structure hinder the extraction by solid surfaces. On the other hand, increasing the molecular size and/or the chain length of the adsorbate species favors retention to the stationary solid phase. Additionally, the coiling of the molecule assists more adsorption to the solid phase because it is easily entrapped in the interstices [4].

2.1.2.3.2 Properties of the Adsorbent

2.1.2.3.2.1 Surface Energy

When a solid surface is exposed to an adsorptive species, the most energetic sites are occupied first. The heat of adsorption at a specific degree of surface coverage (loading) can be calculated using the Clausius-Clapeyron equation. The slope of an isostere plotted on a logarithmic scale (lnP vs $1/T)_n$, where n represents the degree of coverage associated with the isothere. The surface energy distribution as a function of coverage is described by a plot of similar points for different degrees of coverage. This information aids in predicting the activity of a catalyst towards a specific chemical reaction at a specific temperature. Adsorption energy also can be deduced from data obtained by the dynamic chemisorption technique, particularly TPD. The process by this method is in the opposite direction as that described for static volumetric technique. In the present case, heat (energy) is applied and, as temperature increases, molecules are liberated in order of weakest bonding. The desorbed molecules are swept away and no re-adsorption is allowed to occur. The rate of change of surface coverage or loading is related to the rate of change in temperature [10].

2.1.2.3.2.2 Surface Composition

The surface chemical composition of the sorbent material plays an important role in the retention process of the solute substance. The careful selection of the type of the surface functional groups is critical point in the ion exchange or chromatographic analysis. The choice of solid sorbent is highly dependent upon the analyte of interest and the sorbent system to be used. For example, sorbents immobilized with sulphur containing ligands are strongly recommended for the adsorption of Hg and Ag ions while amino and imine groups have strong affinity towards Zn ions. Obviously the number of active sites available on the sorbent should not be exceeded by the number of molecules of analyte otherwise breakthrough will occur. Therefore, it is important to assess the capacity of the sorbent for its intended application.

2.1.2.3.2.3 Surface Area

The surface area is a limiting factor which distinguishes between various rates of sorption. By increasing the available area for sorption on the surface of the solid material (the powdered form is larger surface area than in the granular form), the number of the adsorbate molecules will increase and the speed of extraction is higher. It was found that the rate of adsorption is inversely proportional to the diameter of the nonporous sorbent particles. Adsorption is a surface phenomenon that is directly related to surface area. Increase the surface area, and specific adsorption will increase. Often however, sorption is reported as a bulk property or a per gram weight basis. With these units, some materials which have higher surface area such as clays have a much higher sorption capacity than other minerals do. Sorption should always be reported on an area basis. The definition of surface area, however, is hazy, and often difficult to measure unambiguously. Micro pores and molecular porosity increase the sorptive capacity, but are not necessarily measured [11].

2.1.2.3.3 Surface Tension of the Solvent

Substances which decrease the surface tension will be concentrated on the surface layer of the solvent which dissolve this substance (e.g. organic compounds in aqueous solvent). This will decrease the opportunity of contact between the solute molecules and the solid phase and accordingly the extent of sorption. Controversially, the solute molecules which raise the surface tension of the solvent will be concentrated in the bulk of the solution and become more close contact to the sorbent surface. Accordingly, higher sorption of the solute molecules takes place. Inorganic ion (heavy elements) in water is a good example.

The following Gibbs equation represents the effect of the variation of the surface tension of the solvent with the addition of solute substance:

$$\Gamma = -\frac{1}{RT}\left(\frac{\partial\gamma}{\partial \ln C}\right)_{T,P}$$

Where Γ (mol/m^2) called the surface concentration, which represents excess of solute per unit area of the surface over what would be present if the bulk concentration prevailed all

the way to the surface. γ (N/m^2) is the surface tension of the solvent; C is the concentration of solute in the bulk of the solution. R, T and P are the gas constant, absolute temperature and the pressure respectively.

2.1.2.3.4 Effect of Cosolvent and Dissolved Organic Matters

Sorption coefficient (K_{sorp}, ratio of the substance concentration in the solid to that in solution) for organic compounds is normally measured in aqueous systems. Therefore, it may not be always applicable to the determination of these compounds (prediction of contamination) in water sample due to the presence of waste disposal or treatment sites so that it alters the solvent composition. In these situations often pollutants are in solution of water and various water-miscible organic solvents. It is therefore necessary to develop a more general approach for describing sorption of these compounds from aqueous and mixed solvent systems [12]. Rao *et al.* [13] applied the ‘‘solvophobic theory’’ for predicting sorption of halogenated organic compounds by soils from both water and aqueous–organic solvent mixtures. This approach was already adopted for predicting the solubility of nonpolar and polar solutes in polar solvent mixtures.

Co-solvents are water soluble organic solvents, such as methanol or acetone. These may be present in the aquifer as a part of a mixed waste. Co-solvents can decrease the K_{sorp} by increasing the apparent solubility. The pyrene K_{sorp}, for example, decreases by 30% in 10% methanol-water mixture. The presence of dissolved organic matter commonly reduces the adsorption of a chemical. This may be due to the increased solubility of the chemical in such a solution, or to competitive adsorption to the solid sorbent.

2.1.2.3.5 Salinity

An increase in salinity can significantly lower the sorption coefficient of cations, probably due to replacement/exchange by alkali cations. The adsorption of some acid herbicides increases with greater salinity at pH values above the pKa of the acid. Therefore, pH influences the effects of salinity. Neutral molecules are generally less affected by salinity, but often show an increased adsorption with increasing salt concentration, probably due to the increase in the activity coefficient of neutral molecules and resulting decrease in aqueous solubility, i.e. salting out. For example, pyrene sorption increased 15% with an increase of

salinity from 0 to 0.34 M sodium chloride. Increased salinity may also change the interlayer spacing of layer solids, as well as the morphology of the sorbent and organic matter [14].

2.1.2.3.6 Temperature Effect

Sorption of a substance on a solid sorbent occurs when the free energy of the sorption exchange is negative according to the equation

$$\Delta G = \Delta H - T\Delta S$$

Where ΔG is the change of the Gibbs free energy (kJ mol^{-1}); ΔH is the change in enthalpy (kJ mol^{-1}) and ΔS is the change in entropy (kJ mol^{-1} K^{-1}) and T is absolute temperature (K). ΔH represents the difference in binding energies between the sorbent and the sorbate and between the solvent and the solute. Thus, sorption may occur as the result of two types of forces: enthalpy-related and entropy-related forces. Hydrophobic bonding is an example of an entropy-driven process; it is due to a combination of London dispersion forces (instantaneous dipole-induced dipole) associated with large entropy changes resulting from the removal of the sorbate from the solution.

For polar compounds, the enthalpy-related forces are greater, due to the additional contribution of electrostatic interactions. Generally sorption coefficients decrease with increasing temperature. However, some examples of increasing equilibrium sorption with increasing temperature and of no effect of temperature on sorption equilibrium were also found [3].

Since adsorption is an exothermic process, values of K_{sorp} usually decrease with increasing temperature. A 10% decrease in K_{sorp} would occur with a temperature rise from 20 to 30 ^{o}C. The probable control is the aqueous activity coefficient with the variation in γ depending on the magnitude of ΔH [15] as given by the following relation:

$$\ln \gamma_w = \frac{\Delta Hs}{RT} + C$$

$$\Delta H_s = -RT\ln X_w + T\Delta S$$

$$\Delta H_s = \Delta h_{ads} + \Delta h_{abs}$$

Where:

γ_w: Surface tension of the solvent, ΔH_s: Heat of adsorption (J/mol), C : Concentration of the added solute in the bulk (mol/l), X_w: Mole fraction of the solute, ΔS: Entropy change (J/K/mol), Δh_{ads}: Heat of adsorption (J/mol), Δh_{abs}: Heat of absorption (J/mol), R: Gas constant and T: Absolute temperature. It was found that Δh_{ads} for large polar compounds is about a 25 KJ/mol [11]. Moreover, Chiou *et al.* [16] observed that an inverse relationship exists for organic compounds between sorption coefficients and solubilities. Lower K_{sorp} values are found at higher temperatures for most organic compounds for which solubility increases with temperature, while increased sorption at higher temperatures can be expected for compounds for which solubility decreases with temperature. Therefore, due to the dependence of both sorption coefficients and solubility on temperature, the measured effect of temperature on sorption isotherms is the result of combined sorption and solubility contributions.

2.1.2.4 Forces in Adsorption at Solid Surfaces

In adsorption processes, the forces involved are of two main kinds, physical and chemical and accordingly the adsorption is either physical (physisorption) or chemical (chemisorption). In physical adsorption the bonding is by weak Van der Waals - type forces. There is no significant redistribution of electron density in either the molecule or at the sorbent surface. In chemisorption, a chemical bond, involving substantial rearrangement of electron density, is formed between the adsorbate and substrate. The nature of this bond may lie anywhere between the extremes of virtually complete ionic or complete covalent character.

2.1.2.4.1 Physical Adsorption

Physical adsorption (physisorption) is defined as an adsorption process in which the forces involved are intermolecular forces (van der Waals forces) of the same kind as those responsible for the imperfection of real gases and the condensation of vapours, and which do not involve a significant change in the electronic orbital patterns of the species involved. The term van der Waals adsorption is synonymous with physical adsorption, but its use is not recommended [17]. Physisorption is characterized by low ambient temperature, always under the critical temperature of the adsorbate; type of interaction: intermolecular forces (van der Waals forces); low enthalpy: $\Delta H < 20$ kJ/mol; adsorption takes place in multilayers; low activation energy; the energy state of adsorbate is not altered; and finally it is a reversible process.

2.1.2.4.2 Chemical Adsorption

Adsorption process which results from chemical bond formation (strong interaction) between the adsorbent and the adsorbate in a monolayer on the surface [17]. Chemisorption differs from physisorption since it is accompanied with evolution of higher temperature; strong forces of interaction; bond between adsorbate and surface is covalent; the enthalpy of adsorption is high (50 kJ/mol $<\Delta H<$ 800 kJ/mol); adsorption takes place only in a monolayer; high activation energy; increase in electron density in the adsorbent-adsorbate interface and finally; the process is reversible only at high temperature.

2.2 Solid - Phase Extraction

SPE is one of various techniques available to an analyst to bridge the gap that exist between the sample collection and the analysis step. It is seldom used without other sample preparation steps, such as dilution or pH adjustment. However, the action of performing SPE often simultaneously completes several other preparation goals. Moreover, SPE has been coupled with an analytical technique or another preparation method to enhance the benefits of each separate technique [18]. The technique refers to the nonequilibrium, exhaustive removal of chemical constituents from a flowing liquid sample via retention on a contained solid sorbent and subsequent recovery of selected constituents by elution from the sorbent. The affinity, which is strong enough to be analytically useful for sorbents inexpensive enough to be economically feasible, is useful in both pharmaceutical and environmental applications. SPE is sometimes referred to as digital chromatography, indicating the all-or-nothing extremes in the sorptive nature of these sorbents, caused by the strong attraction for the analyte by the sorbent [5]. SPE is a convenient approach for sample preparation in the chromatographic analysis of various samples which sometimes require a small amount of the test sample, low volumes of organic solvents; whereas the treatment is accomplished in a greatly shorter period of time than in other techniques [19].

2.2.1 Basic Principles of SPE

The method of SPE always consists of three to four successive steps. *Firstly*, the preconditioning of the sorbent which is done by using an appropriate solvent (the same solvent in the sample). This enables the wetting of the packing material and solvation of the functional groups, removal of the possible impurities initially contained in the sorbent or the packaging, getting rid off the air bubbles present in the column and filling the void volume with solvent. *Secondly*, the sample is percolated through the sorbent. The sample may be applied to the column by gravity, pumping, aspirated by vacuum or by an automated system. The sample flow-rate through the sorbent should be low enough to enable efficient retention of the analytes, and high enough to avoid excessive duration. *Thirdly*, an optional step which is the washing of the solid sorbent with an appropriate solvent that have low elution strength in order to eliminate matrix components that have been retained by the solid sorbent, without displacing the analytes. *Finally*, the analyte is eluted by an appropriate solvent, without

removing the retained matrix components. The eluent volume should be adjusted so that quantitative recovery of the analyte is achieved with subsequent low dilution [20].

2.2.3 The Objectives of SPE

There are three objectives for applying SPE procedures. The first goal is the analyte concentration in order to be able to measure the quantity of a compound at higher concentration than in the sample. This will ensure the largest response from the detection system and will minimize error in precision caused by background noise. The second aim of SPE is sample clean up. Since concentration of the analyte is pointless if we cannot measure the analyte in a final concentrated solution. The extracted sample contains interfering compounds. Among these components, some contaminates that mask the analyte during analysis. The third target is sample matrix removal and solvent exchange. Many analytical instruments (e.g. GC or LC, NMR and IR) require the sample to be analyzed in a specific environment. In such case, we need to remove the sample matrix and convert it into a form compatible with the instrument to be used [18].

2.2.4 Mechanisms of SPE

Sorbents in SPE can be divided into three classes, normal phase, reversed phase and ion-exchange sorbents. Normal phase sorbents have polar functional groups, e.g. cyano, amino and diol. These sorbents are characterized by their polar nature which is more likely that polar compounds, e.g. phenol, will be retained [21]. Reversed phase types have non-polar functional groups, e.g. octadecyl, octyl and methyl, and conversely are more likely to retain non-polar compounds, e.g. polycyclic aromatic hydrocarbons. Retention of organic analytes from polar solutions (e.g. water) onto these sorbents is due to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the solid surface [22]. The ion exchange sorbents have either cationic or anionic functional groups. In the ionized form it attracts compounds of the opposite charge. These sorbents separate analytes based on electrostatic interactions between the analyte of interest and the positively charged groups on the stationary phase. For ion exchange to occur, both the stationary phase and sample must be at a pH where both are charged [23].

2.2.5 Retention Forces

Because the solute molecules interact more strongly with the stationary phase molecules than they do with the mobile phase molecules, solutes are retained in the stationary phase (regardless of its volume). Interaction between solute and solvent molecules results from three basic types of intermolecular force, all of which are electrical in nature. These types of interactive forces are dispersive, polar and ionic. Polar forces have been further divided into sub groups ranging from 'strong dipole-dipole interactions' (hydrogen bonding) to 'weak dipole-dipole interactions ((π)-(π) interactions). Division into the two groups is appropriate, as it describes two physically different types of polar interaction that is very pertinent to chromatographic retention. Most molecular interactions consist of a mixture of at least two different types of interaction; the only type of interaction that can occur in isolation is dispersive [24].

2.2.6 SPE Procedures

In solid – phase extraction technique, there are two main procedures commonly used for determination of organic or inorganic species, the off – line SPE and on – line SPE modes.

2.2.6.1 Off – Line SPE

In the off – line SPE mode the sample preparation and the detection steps are carried out separately. Firstly, the sample is treated with the proper solid phase extraction procedure (column, batch, cartridge or disc) to make sample cleanup, matrix removal and analyte preconcentration. After this, the analyte in the solid phase is eluted with suitable solvent and become compatible for detection and is indirectly (manually or automatically) transferred to the separation and/or detection instrument.

2.2.6.2 On – Line Automatic SPE

On-line automatic SPE procedure forms part of the so-called 'column switching 'or 'coupled column' techniques. This allows an on-line sample clean-up before the main chromatographic separation. The most common arrangement is made in which the loop of the chromatographic injection valve is replaced by the precolumn. When the injector is in the load

position, sample is pumped through the pre-column where analytes are retained. Simultaneously, the mobile phase passes directly through the analytical column. When the preconcentration step is finished the injection valve is turned to the inject position and the mobile phase then passes through the pre-column and removes the analytes to the main column. SPE has usually been coupled on-line with HPLC systems with UV-visible detectors (SPE-LC-UV) but fluorescence detectors, electrochemical detectors, diode array detectors (SPE-LC-DAD) and mass spectrometers (SPE-LC-MS) have also been used [25].

2.2.6.3 Off – Line Versus On – Line SPE

On-line flow-injection preconcentration with solid adsorbents has several advantages over the corresponding off-line procedures. Sensitivity is enhanced and consumption of sample and reagents is reduced. A very special characteristic of the on-line procedure is the easy combination with conventional analytical techniques, e.g. FAAS or ETAAS. Furthermore, on-line methods are advantageous as compared with the off-line batch systems, because they are automated; make use of simpler apparatus with easier operation, much cheaper equipment and lower running costs [26, 27]. Moreover, procedures by batch using adsorption process are very efficient, but these involve tedious and delayed steps. On the other hand, flow injection procedures with solid phase extraction are preferred due advantages obtained, such as: high sample throughput, high enrichment efficiencies, low sample and reagent consumption, high reproducibility and very limited laboratory bench space and utensils required [28].

2.3 Classification of SPE Sorbents

2.3.1 Inorganic Based Sorbents

Inorganic based sorbents are mainly made of silica gel even though other inorganic oxides may be used. Silica gel based sorbents present the advantages of mechanical, thermal and chemical stability under various conditions. They frequently offer a high selectivity towards a given metal ion. However, all silica-based sorbents suffer from different chemical limitations, namely the presence of residual surface silanol groups (even after an end-capping treatment) and a narrow pH stability range.

2.3.1.1 Silica gel

Silica gel is an amorphous, highly porous, partially hydrated form of *silica,* a substance made from *silicon* and *oxygen,* the two most abundant elements in the earth's crust. Actually, more than 55%w/w of the earth's surface consists of either silica (silicon dioxide) or silicates (metallic silicate salts made from silica combined with metal oxides). The majority of silica (as opposed to silicates) found *naturally* is not significantly hydrated and, although it can exist in both crystalline and amorphous forms, it usually occurs naturally as quartz, cristobalite or tridymite crystals. There are basically two forms of interaction that can take place between a solute and the silica gel surface. Firstly, the solute molecule can interact with the adsorbed solvent layer and rest on the top of it. This type of interaction is called sorption interaction and occurs when the molecular forces between the solute and the silica are relatively weak compared with the forces between the solvent molecules and the silica. Secondly, the solute molecules can displace the solvent molecules from the surface and interact directly with the silica gel itself, for example, the silanol groups. This type of interaction is called displacement interaction and occurs when the interactive forces between the solute molecules and the silica surface are much stronger than those between the solvent molecules and the silica surface [29].

Silica gel can be used as a very successful adsorbing agent, as it does not swell or strain, has good mechanical strength and can undergo heat treatment. In addition, chelating agents can be easily loaded on silica gel with high stability, or be bound chemically to the support, affording a higher stability. The surface of silica gel is characterized by the presence of silanol groups, which are known to be weak ion-exchangers, causing low interaction,

binding and extraction of ionic species. In particular, silica gel presents high sorption capacity for metal ions, such as Cu, Ni, Co, Zn or Fe. Retention is highly dependent on sample pH with quantitative retention requiring pH values over 7.5–8, as under acidic conditions silanol groups are protonated and the ion-exchange capacity of the silica gel is greatly reduced or even reduced to zero at low pHs. In addition, this sorbent has a very low selectivity, and is prone to hydrolysis at basic pH. Consequently, modification of the silica gel surface has been performed to obtain solid sorbents with greater selectivity. 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. Impregnating reagents are ion-exchangers or chelating compounds. Numerous reagents have been investigated for impregnation of silica gel as a means of increasing retention capacity and selectivity of the sorbent for trace elements, namely thionalide, MNA, MBT, 8-HQ, MPSP, salicylaldoxime, DMG, 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) [20]. Additionally, silica gel packed column was also empolyed to retain organic compounds. Among these applications, it was used in separation and preconcentration of certain organic compounds such as of benzylpenicillin, levomycetin (chloramphenicol), and tetracycline in food products followed by high-performance liquid chromatography [30].

2.3.1.2 C_{18}-bonded silica gel

Despite the large variety of bonded phases available, octadecyl-bonded silica has currently become the most popular phase used. Numerous applications has been reported the use of C_{18}-silica, In particular, organometallic compounds (e.g. TBT, alkylselenides) can be retained on this sorbent due to possible hydrophobic interaction [31, 32]. Bare C_{18}-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, C_{18}-silica is not well suited for retention of trace element species, as the latter are often polar or ionic. Retention on C_{18}-silica may be improved by addition of a ligand reagent to the sample before its percolation through the sorbent. An alternative approach is to form the complex by passing the sample through a C_{18}-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. Despite their broad application to trace element preconcentration, bonded silica phases (either C_{18}-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. As a consequence, polymeric sorbents may be preferred [20].

A CL micro-flow system combined with on-line solid phase extraction (SPE) was presented based on the use of C_{18} bonded silica for determination of some β-lactam antibiotics (penicillin, cefradine, cefadroxil, cefalexin) in milk. It is based on the enhancement effect of β-lactam antibiotics on the luminol-$K_3[Fe(CN)_6]$ signal in CL system. The micro-flow system was fabricated from two PMMA plates (50 mm × 40 mm × 5 mm) with the microchannels of 200 μm wide and 150 μm deep. C_{18}-modified silica gel was packed into the microchannel (length: 10 mm; width: 1 mm; depth: 500 μm) to serve as SPE device [33].

2.3.1.3 Other inorganic oxides

Away from silica sorbents, other inorganic oxides have been tested for the adsorption of organic and inorganic compounds. The acidic oxides (such as SiO_2), due to its acidic properties, is expected to adsorb only cations, while basic oxides (such as magnesia MgO) should adsorb only anions. In fact, adsorption of ions on oxide surfaces is believed to proceed with participation of hydroxyl groups. These groups are negatively charged (deprotonated) under basic conditions, thereby retaining cations and positively charged (protonated) under acidic conditions, thereby retaining anions. Consequently, on amphoteric oxides (namely titania TiO_2, alumina Al_2O_3, zirconia ZrO_2), cations are adsorbed under basic conditions while anions are adsorbed under acidic conditions. The concurrent adsorption of H^+ is responsible for the absence of retention of cationic species at very low pHs. So it may be preferred to find a suitable sorbent for retaining the targeted species with subsequent selective elution for

further speciation studies. The preparation technique is of prime importance, as the adsorption properties of many oxides strongly depend on the characteristics of the solid, namely crystal structure, morphology, defects, specific surface area, hydroxyl coverage, surface impurities and modifiers.

Not only the inorganic ions can be adsorbed by the inorganic oxides but also some of them can adsorb organic matters. For example, stannic oxide was used with simple mobile phases for separation of cephalosporins. It has been studied for the first time by Nabi *et al.* [34]. They described TLC procedure that enables simple and rapid separation and detection of different spontaneous, chemical, and enzymatic degradation products of the cephalosporins. Suitable combination of mobile phase and spray/detection reagent enables identification of these products in aqueous preparations and in biological fluids and micro-biological culture broths.

2.3.2 Organic Based Sorbents

Organic based sorbents may be divided into polymeric and non-polymeric sorbents, Polymeric sorbents have been used for trace analyte preconcentration having the advantage over bonded silica in that they can be used over the entire pH range. Their disadvantage is that the conditioning step is more time consuming as they require extensive cleaning before use [35]. This section summarizes the most frequently used organic based sorbents, as well as the more recently reported ones. In most applications, new sorbents have been synthesized by chemically bonding chelating groups to polymeric cross-linked chains and characterizing their ability to selectively adsorb trace elements or compounds. Most of the chelating groups reported have low water solubility to avoid their leaching from the sorbent, as most applications deal with aqueous samples. At the same time, a too hydrophobic group will hinder wet ability of the sorbent by the aqueous sample, resulting in poor retention efficiency. A compromise is thus necessary. In addition to the functional group, the efficiency of polymeric sorbents depends on various physico-chemical parameters, such as particle size, surface area, pore diameter, pore volume, degree of crosslinking and particle size distribution.

2.3.2.1 Polystyrene-Divinylbenzcne Based Sorbents

Amberlite XAD series are macroporous hydrophobic resins are considered as good supports for developing chelating matrices. Amberlite XAD-1, XAD-2, XAD-4 and XAD-16 are PS-DVB resins with a high hydrophobic character and no ion-exchange capacity. In addition to the hydrophobic interaction that also occurs with C_{18}-silica, such sorbents allow π–π interactions with aromatic analytes. Due to the hydrophobic character of PS-DVB, retention of trace elements on such sorbents requires the addition of a ligand to the sample. Inorganic ligands may be used [36], but organic ligands are preferred, such as APDC [37], or 8-HQ [38]. Alternatively, ligands may be attached to the PS-DVB by physical adsorption such as dithizone [39], PAN [40] or 5-BrPADAP [41].

However, in practice, the resins prepared by impregnation of the ligand are difficult to reuse, due to partial leaching of the ligand (thus resulting in poor repeatability). To overcome this problem, the resin may be chemically functionalized. The ligands are generally coupled to a methylene or an azo spacer on the matrix. Among ligands, one can cite Alizarin Red-S [42], salicylic acid [43], thiosalicylic acid [44], pyrocatechol violet [45], chromotropic acid [46], pyrocatechol [47]. Of great interest are also the sulfonated PS-DVB resins, as they show excellent hydrophilicity and high extraction efficiencies for polar organic compounds [48]. Serrano et al. [49] investigated the separation and determination of β-lactam antibiotics (ampicillin, amoxicillin, cephradine, and cephalexin) in environmental water samples. The samples were enriched by SPE by passage through PS-DVB based sorbent (weak base anion exchange Amberlite® IRA-93) column followed by liquid chromatographic separation and determination.

Cephalosporin C adsorption on the non-ionic polymer Amberlite XAD-2 was investigated by Lee et al. [50]. The results showed that the adsorption capacity increased with temperature and pH. The axial dispersion coefficient and the film mass transfer coefficient were estimated from experimental conditions by correlations given in literature. The surface diffusion coefficient was obtained from experimental breakthrough data. In this adsorption system, cephalosporin, the intraparticle diffusion is highly the rate controlling step. The adsorption model, which employs the surface diffusion for intraparticle mass transfer combined with the Freundlich equation for the single species equilibrium, successfully simulates the adsorption breakthrough curves under various experimental conditions.

2.3.2.2 Divinylbenzene-Vinylpyrrolidone Copolymers

Sorbents made of divinylbenzene-vinylpyrrolidone copolymers have recently been developed, such as Oasis HLB. The hydrophilic *N*-vinylpyrrolidine affords good wettability of the resin, while the hydrophobic divinylbenzene provides reversed-phase retention of analytes. This sorbent has been successfully applied to the determination of polar organic compounds in water samples. It is more convenient to use, compared to classical sorbents, as it can dry out during the extraction procedure without reducing its ability to retain analytes. In addition, it is stable over the entire pH range. However, until now, no application related to the preconcentration of trace elements has been reported [20].

A sorbent cartridge based on this copolymer has been applied for the simultaneous determination of BLAs (penicillin G, amoxicillin, ampicillin, penicillin V, oxacillin, cloxacillin, dicloxacillin and nafcillin) in wastewater. The method is based on SPE and high performance liquid chromatography with UV-DAD. The SPE cartridge (Oasis MAX, vinylpyrrolidone and divinylbenzene polymer) have been used for sample clean up and preconcentration [51].

2.3.2.3 Polyacrylate Polymers

The resins Amberlite XAD-7 and XAD-8 are ethylene-dimethacrylate copolymers. They possess very low ion-exchange capacity since they are non-aromatic in character. Because of the polarity of acrylates, such resins are able to retain polar compounds. However, many reagents are added to increase retention due to the quite moderate polarity of these materials. Sometimes, direct addition of the reagent to the sample is performed. As an example, Cu (II) forms a complex with 8-hydroxyquinoline-5-sulfonic acid, which can be further retained on Amberlite XAD-8 as an ion-pair with CTAB [52]. Yet, several chelating reagents have been loaded on such resins, mainly Amberlite XAD-7, to increase their retention capacity and/or their selectivity. Such loaded sorbents are stable for several months and can be reused. For a higher stability, chemical binding of the chelating group may be performed.

In the extraction of polar organic compounds, surfactant material is added to the extraction solution. For example, acrylic polymer XAD-7 HP was utilized in the extraction and determination of avoparcin (avoparcin is a mixture of the two polar glycopeptide antibiotics) during the sample preparation step in pressurized hot water extracts from kidney samples. In situ sample clean-up was achieved by using matrix solid-phase dispersion utilizing the acrylic polymer XAD-7 HP, and by adding triethylammonium phosphate to the extraction solvent. The aqueous extracts were concentrated by solid-phase extraction (SPE) on the HILIC material polyhydroxyethyl aspartamide [53].

2.3.2.4 Polyurethane Polymers

Due to its sorption capacity for several trace elements polyurethane foam has been tested for use in SPE. In many cases, complexing reagents are added to enhance the sorption capacity. As example, PUF coated with DMG, or hexamethylenedithiocarbamate was found to be efficient in retaining trace elements. The chelating reagent can also be directly added to the sample, and the metal chelates further retained on PUF, as observed with thiocyanate complexes [54], and (0,0-Diethyl-dithiophosphate) complexes [55]. Very recently, the immobilization of an enzyme (alkaline phosphatase) has been reported on PUF with further application as an enzymatic procedure for Pb(II) determination [56]. Additionally, PUF can also adsorb variety of organic compounds such as aromatic phenols, amines and polycyclic and heterocyclic compounds.

2.3.2.5 Polyethylene Polymers

Polyethylene is an attractive sorbent material in SPE since this support is able to adsorb several metals when complexed with hydrophobic ligands. Additionally, the adsorbed complexes can be eluted with a small volume of organic solvents which allow achievement of high enrichment factors. This material can also be used in strongly acidic and basic media. Polyethylene polymer shows some inert properties towards organic compounds. Accordingly, it is widely used as porous frits as stopper for sorbent packing in cartridges. Yet, it is not known the use of such polymeric material as sorbent for organic compounds.

2.3.2.6 Polytetrafluoroethylene Polymers

The polytetrafluoroethylene polymer membranes are highly durable and resistant to a broad range of temperatures and chemicals. This hydrophobic material can be used for the filtration of air samples, aggressive solvents, and as the backing for AcroWell 96 filter plates. It can retain trace elements after addition of a chelating reagent to the sample such as dithizone[57]. PTFE may also be precoated with a suitable ligand, like 2-methyl-8-hydroxyquinoline [58]. The sorbent can be used as turnings, beads or as tubing in a knotted reactor [59]. Additionally, the material is widely used as membrane filters in the sample preparation step in antibiotic analysis.

2.3.2.7 Polystyrene Polymers

Polystyrene polymers are considered an interesting and alternative material over the common sorbents such as Amberlites XAD-2 and XAD-8 or C_{18}-silica when they have high cross-link structure. In some cases, this material is employed when addition of a reagent to the sample is required to form complexes that are further retained on the hydrophobic sorbent [60]. Moreover, Dmitryenko et al have studied the sorption of Oxytetracycline and Chlorotetracycline by sulphonated polystyrene resins. They investigated effect of the structure of the ion-exchange resin on the sorption process. Evidence has been obtained for the aggregation of these ions in the resin. It was found that the variations in the degree of cross-linking of the resin structure and the concentration of the functional groups have significant effect on the selective behaviour of the resin towards these antibiotics [61].

2.3.2.8 Polyamide Polymers

Rare earth elements have been retained with polyamide polymers by addition of chelating reagent to the sample for complexing the trace elements. The reagent Thorin (*o*-[3,6-disulfo-2-hydroxy-l-naphthylazo]benzenearsonic acid) was applied to enable interaction of the elements with the sorbent through electrostatic forces and non-hydrophobic interaction [62].

The direct interaction between the polyamide polymer and antibiotic is a promising means of providing infection resistance textile fabrics. Natural polyamide polymers (such as wool or chitosn) can extract some antibiotics to obtain antimicrobial materials. Ionic interaction between cationic reactive groups (antibiotic) and carboxylic groups in wool, wool/polyamide, wool/polyester, and polyamide was used as a tool to develop desirable, durable antimicrobial fabrics. It is important to carefully adjust the finishing conditions such as pH, temperature, and time. The results revealed that pH of the finishing bath and the antibiotic concentration as well as finishing temperature is very critical parameters in affecting exhaustion of the antibiotic by the fabric along with the extent of ionic interactions [63].

2.3.2.9 Iminodiacetate-Type Chelating Resins

Polymers containing iminodiacetate groups as active sites have been widely used for the retention purposes. Iminodiacetate functional groups are chemically linked to several polymeric sorbents, such as polystyrene (Chelex-100). The length of the spacer arm has pronounced effect on the complex formation with metal ions in the chelating resin [57]. Limitation of such IDA sorbents is due to the protonation of the weak acid functional group since it critically affects the ability of the resin to retain metal cations. Hence, for Chelex-100, complete protonation of the carboxylates and the donor N atom are reported to be at pH 2.21 and complete deprotonation is reached at pH 12.30. Additionally, such sorbents are non-selective, which may reduce retention of trace elements due to retention of major ions (namely Ca(II) and Mg(II)). Besides, the presence of ligands in the sample may prevent trace element retention on the sorbent due to their complexation as observed in real waters due to the presence of organic matter. In case of organic compounds, it was reported that this sorbents (chelex-100) are able to adsorb ionic species in presence of metal ions. MacKay et al. [64] have demonstrated the application of chelex-100 in the sorption of Oxytetracycline by Metal-Bridging. Oxytetracycline could be adsorbed to Ca- and Cu-loaded on Chelex-100 resin. The extraction is increased with increasing metal/sorbate ratio at pH 7.6.

2.3.2.10 Propylenediaminetetraacetate-Type Chelating Resins

It was reported recently by Kumagai *et al.* [65] the synthesis of macroporous polymer-based propylenediaminetetraacetic acid type resin in a fine-particle form. The sorbent has very similar structure to that of ethylene diamine tetraacetic acid with a spacer arm enabling the retention of several rare earth elements upon chelation.

2.3.2.11Polyacrylonitrile Based Resins

Polyacrylonitrile polymers have been functionalized with various chelating agents to obtain ion-exchange chelating sorbents. It was linked to aminophosphonic, dithiocarbamate or aminothiourea groups. However, an alternative is to coat the polyacrylonitrile fiber with a proper reagent for further trace element retention such as 8-HQ [66] since the functionalization process is time consuming. Furthermore, polyacrylonitrile polymers are capable to adsorb some antibiotic compounds. Haemofilters made from this material was tested for the extraction of Levofloxacin. The adsorption process was found to be concentration dependent and reversible in vitro which suggest that adsorption by polyacrylonitrile haemofilters is unlikely to affect levofloxacin pharmacokinetics significantly in vivo [67].

2.3.2.12 Ring-Opening Polymerisation-Based Polymers

Carboxylic acid-functionalized resin having high capacity has been prepared using ring-opening metathesis polymerisation. Electron microscopy revealed that the obtained material consists of irregularly shaped, agglomerated particles having a non-porous structure with diameter and specific surface area dependent on the polymerisation sequence and the stoichiometries. The material is pH stable and could be reused. The sorbent has excellent hydrophilic character due to the presence of carboxylic groups ensuring a high wettability of the sorbent by water. Also, significant reversed-phase character is provided by the polyunsaturation of the carrier chain, as well as the entire backbone. The sorbent enable to retain rare earth elements by the weak coordination sites from the carboxylic acid groups.

Similarly, dipyridyl amide-functionalized resins have been reported to allow the extraction of 'soft' metals such as Pd(II) and Hg(II) [68].

2.3.2.13 Carbon Sorbents

Activated carbon is prepared by low-temperature oxidation of vegetable charcoals. Because of their large surface areas (300–1000 m^2/g), these sorbents are well-recognized for their very strong sorption both for trace organic compounds and trace elements. There is evidence of types of adsorption sites on activated carbons are reported. Firstly, adsorption through van der Waals forces enabled by graphite-like basal planes, especially π-electron interactions. Secondly, ionic interaction of hydrogen bonding that may take place through polar groups like carbonyls, hydroxyls and carboxyls. Consequently, trace elements may be directly adsorbed on activated carbon. The major drawback when utilizing activated carbons is their heterogeneous surface with active functional groups those often lead to low reproducibility. Fortunately, along with the development of polymer materials and bonded phases, a new generation of carbon sorbents appeared in the 1970s and 1980s with a more homogeneous structure and more reproducible properties.

Another kind of carbon sorbents is the graphitized carbon blacks which are obtained by heating carbon blacks at 2700–3000 °C in an inert atmosphere. These sorbents are non-specific and non-porous (surface area about 100 m^2/g). Also, due to the presence of positively charged chemical heterogeneities on their surface, they are considered to be both reversed-phase sorbents and anion-exchangers [20].

Carbon sorbents have been extensively used in the past few years for the SPE of polar organic pollutants from water samples [35], while in trace elements SPE is still rare. The main limitations are possible irreversible retention of analytes, which may be overcome by elution in the backflush mode, and poor mechanical stability.

Already many years ago removal of antibiotics from water samples by activated carbon is well known. One of theses conventional drinking water treatment processes by activated carbon were evaluated under typical water treatment plant conditions to determine their effectiveness in the removal of some common antibiotics. Carbadox, sulfachlorpyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfathiazole, and

trimethoprim were exmined. The study shows that these antibiotics could be effectively removed using processes already in use in many water treatment plants. However, additional work is needed on the by-product formation and the removal of other classes of antibiotics [69].

2.3.2.14 Cellulose

Cellulose was found to be an effective material in retaining trace elements that exist in water samples either directly or by the addition of a chelating agent to the sample. Also, functionalization of cellulose is possible to increase the SPE selectivity. For organic constituents, it was investigated by the study of adsorption of gentamicin and other related aminoglycoside antibiotics to cellulose. According to work done by Wagman *et al.* [70] on five types of aminoglycosides indicated that, about 30 to 100% of these antibiotics were adsorbed to cellulose depending on the ratio of antibiotic to adsorbent, and the total quantity could not be removed by acidification.

2.3.2.15 Naphthalene Based Sorbents

Microcrystalline naphthalene shows feasible retention of trace elements, either by addition of a ligand to the sample, or after functionalization of the solid to ensure better adsorption characteristics. However, the use of this solid support is rather uncommon. In addition, until now the sorbent has been applied only to batch experiments.

2.3.2.16 Molecularly Imprinted Polymers

By the appearance of molecular imprinting, highly stable synthetic polymers that possess selective molecular recognition properties have been obtained. Recognition sites within the polymer matrix are complementary to the analyte in the shape and positioning of functional groups. Molecularly imprinted polymers are made by synthesizing highly crosslinked polymers in the presence of a template molecule. After removal of this molecule,

the polymer can be used as a selective binding medium for the template (analyte) or structurally related compounds.

The mechanisms, by which these polymers specifically bind the template and related ligands, are attributed to the formation of functional groups in a specific arrangement within the polymer. They correspond to the template and the presence of shape-selective cavities. Some of these polymers have high selectivities and affinity constants, comparable with naturally occurring recognition systems such as monoclonal antibodies, which make them especially suitable as constituents in chemical (biomimetic) sensors for analytical chemistry or simply for enhancing the selectivity in SPE. There are already a few applications of these polymers [35].

An inorganic based sorbent obtained from silica gel-bound molecularly imprinted polymer to oxazolone(s) exclusively derived from certain cephalosporins (cefaclor) and penicillins (amoxicillin and ampicillin) was prepared using ethylacetate as a porogen. This was used as a packing material for solid phase extraction in column chromatography [71].

An oxacillin imprinted polymer with excelling a BLAs, was prepared by the use of 4-vinylpyridine as functional monomer and trifluoromethyl acrylic acid as crosslinking agent. The sorbent was applied to the separation of a mixture of penicillin V, penicillin G and the print molecule on an MIP utilizing an aqueous mobile phase could be achieved. Baseline separation of the two penicillins from oxacillin was achieved when employing the molecularly imprinted polymer [72].

2.4 Polyurethanes are Different from Currently Used Polymers

The majority of the polymers manufactured in industry have a fairly simple chemical structure since they are synthesized from one or two monomers, therefore leading to the formation of homopolymers or copolymers. Examples of these polymers are poly(ethyleneterephthalate), poly(tetrafluoroethylene), poly(styrene), poly(ethylene), poly(propylene), poly(butadiene), etc. On the other hand, polyurethanes possess more complex chemical structures that typically comprise three monomers: a diisocyanate, a macroglycol (which is an oligomeric macromonomer) and a chain extender. Accordingly, the nature of the polyurethane composition implies a wide diversity of surface characteristics, which in turn, are of prime importance when dealing with an eventual use of PUFs [73].

Basically, PUF is a sorbent that has been used quite frequently in recent years for the determination of trace amounts of different components. It has been used in the unloaded and also the loaded forms with complexing reagents. The structural form of PUF allows the easy use of this sorbent in automatic and on-line pre-concentration systems. In this context, it has advantages over other sorbents, such as active carbon, alumina and silica. The use of PUF and classical complexing agents also makes possible several procedures for speciation analysis. A comparison between PUF and other sorbents reveals that this solid phase has obvious advantages and limitations. PUF cannot adsorb metal ions without prior complexation, whereas, for example, activated carbon and alumina can. The sorption capacity of PUF is generally lower than that of activated carbon; however, for on-line systems this property is actually a disadvantage. Analyte elution from activated carbon as a consequence requires drastic conditions, including the use of concentrated acids. The great advantage of PUF is in on-line systems, as the back-pressure presented by minicolumn is very low compared with other sorbents, such as activated carbon, silica or alumina.

In conclusion, based on the above mentioned characteristics of PUF material, it seems to us that this is a very attractive sorbent to deal with in this work. One of the most important parameter that motivates the use of PUF is its easier application in analytical methods.

2.5 Polyurethane foam: Structure, Properties and Analytical Applications

2.5.1 Historical Overview of PUF

In 1937 Otto Bayer and coworkers were the pioneering worker on polyurethane polymers that was conducted at the laboratories of I.G. Farben at Leverkusen in Germany [74]. By recognizing that, use of the polyaddition principle to produce polyurethanes from liquid diisocyanates and liquid polyether or polyester diols seemed to point to special opportunities, especially when compared to already existing plastics that were made by polymerizing olefins, or by polycondensation. The new monomer combination also circumvented existing patents obtained by Wallace Car others on polyesters. Initially, work focused on the production of fibres and flexible foams. With development constrained by World War II, the PUFs were applied on a limited scale as aircraft coating.

It was not until 1952 that polyisocyanates became commercially available, commercial production of flexible polyurethane foam began in 1954, based on TDI and polyester polyols. The invention of these foams was thanks to water accidentally introduced in the reaction mixture. These materials were also used to produce rigid foams, gum rubber, and elastomers. Linear fibres were produced from hexamethylene diisocyanate and 1,4-butanediol. The first polyether polyol was commercially available, is poly(tetramethylene ether) glycol), that was introduced by DuPont in 1956 by polymerizing tetrahydrofuran. The following year 1957, BASF and Dow Chemical introduced less expensive less expensive polyalkylene glycols. These polyether polyols offered technical and commercial advantages such as low cost, ease of handling, and better hydrolytic stability; and quickly supplanted polyester polyols in the manufacture of polyurethane goods. Another early pioneer in PUFs was the Mobay coorporation [75]. In 1960 more than 45,000 tons of flexible polyurethane foams were produced. As the decade progressed, the availability of chlorofluoroalkane blowing agents, inexpensive polyether polyols, and MDI heralded the development and use of polyurethane rigid foams as high performance insulation materials. Rigid foams based on polymeric MDI offered better thermal stability and combustion characteristics than those based on TDI. In 1967, urethane modified polyisocyanurate rigid foams were introduced, offering even better thermal stability and flammability resistance to low density insulation products. Also during the 1960s, automotive interior safety components such as

instrument and door panels were produced by back-filling thermoplastic skins with semi-rigid foam. In 1969, Bayer AG exhibited an all plastic car in Dusseldorf, Germany. Parts of this car were manufactured using a new process called RIM, Reaction Injection Molding. RIM technology uses high-pressure impingement of liquid components followed by the rapid flow of the reaction mixture into a mold cavity. Large parts, such as automotive fascia and body panels, can be molded in this manner. Polyurethane RIM evolved into a number of different products and processes. Using diamine chain extenders and trimerization technology gave poly(urethane urea), poly(urethane isocyanurate), and polyurea RIM. The addition of fillers, such as milled glass, mica, and processed mineral fibres gave arise to reinforced RIM, which provided improvements in flexural modulus (stiffness) and thermal stability. This technology allowed production of the first plastic-body automobile in the United Sates, the Pontiac Fiero, in 1983. Starting in the early 1980s, water-blown microcellular flexible foam was used to mold gaskets for panel and radial seal air filters in the automotive industry. Since then, increasing energy prices and the desire to eliminate PVC plastisol from automotive applications have greatly increased market share. Highly filled polyurethane elastomers, and more recently unfilled polyurethane foams are now used in high-temperature oil filter applications. PUF (including foam rubber) is often made by adding small amounts of volatile materials, so-called blowing agents, to the reaction mixture. These simple volatile chemicals yield important performance characteristics, primarily thermal insulation. In the early 1990s, because of their impact on ozone depletion, the Montreal Protocol led to the greatly reduced use of many chlorine-containing blowing agents, such as trichlorofluoromethane. Other haloalkanes, such as the hydrochlorofluorocarbon 1,1-dichloro-1-fluoroethane, were used as interim replacements until their phase out under the directive on greenhouse gases in 1994 and by the VOC directive of the EU in 1997. By the late 1990s, the use of blowing agents such as carbon dioxide, pentane, 1,1,1,2-tetrafluoroethane and 1,1,1,3,3-pentafluoropropane became more widespread in North America and the EU, although chlorinated blowing agents remained in use in many developing countries [76].

Building on existing polyurethane spray coating technology and polyetheramine chemistry, extensive development of two-component polyurea spray elastomers took place in the 1990s. Their fast reactivity and relative insensitivity to moisture make them useful coatings for large surface area projects, such as secondary containment, manhole and tunnel coatings, and tank liners. Excellent adhesion to concrete and steel is obtained with the proper primer and

surface treatment. During the same period, new two-component polyurethane and hybrid polyurethane-polyurea elastomer technology was used to enter the marketplace of spray-in-place load bed liners. This technique for coating pickup truck beds and other cargo bays creates a durable, abrasion resistant composite with the metal substrate, and eliminates corrosion and brittleness associated with drop-in thermoplastic bed liners. The use of polyols derived from vegetable oils to make polyurethane products began garnishing attention beginning around 2004, partly due to the rising costs of petrochemical feedstocks and partially due to an enhanced public desire for environmentally friendly green products [77].

2.5.2 Foam Structure and Properties

The structural unit of PUF is the single cell. The size, shape, and construction of these cells determine thermal conductivity, strength, and other physical properties. The main cell shape of PUF is an irregular polyhedron whose shape is dependent upon foaming conditions [78]. If we regard the fine structure of PUF, it is a microcellular polymer, which is produced by the internal generation or liberation of a gas in a fluid medium that simultaneously polymerizes while expanding its volume. The final polymer is either open-, closed- or mixed- cell foam. The mixed-cell structure contains proportion of the closed-cell PUF.

The structure of open-cell PUF provides better acoustic insulation, permeability of water vapour and gases. Open-cell PUFs are suitable as pre-concentrating and separating agents, because they possess, owing to their membrane-like structure, outstanding sorption and mass-transfer properties; these enable absorption and extraction processes to take place in solid - liquid, solid - gas and liquid - liquid phases. Their excellent hydrodynamic and effective mass transfer properties allow the use of relatively high flow-rates in column separations without impairment of the separation efficiency. Resilient open-cell polyurethane foams can be used as conventional static and pulsed column beds in the form of cylindrical shaped packing material, which can further broaden the applicability of these foams for separations. A squeezing technique as a version of a pulsed polyurethane foam column has been developed for trace metal analysis of bulky water samples [79].

The closed cell foam has better thermal isolation and higher resistance to external factors. It is frequently used in industrial applications especially in the insulation purposes. It is among the most efficient insulating materials commercially available, like adhesives, sprays and paints.

Basically, PUFs are based on polyether- or polyester urethanes. The polyether type is resistant to hydrolysis and chemical treatment; however it is not resistant to the oxidation process. Polyester urethanes have a high mechanical stability, but have no resistance to chemical reagents. According to their types, PUF are classified into flexible, semi-flexible and rigid foams. The flexible foam show relatively low-bearing properties with high recovery properties similar to a coiled metal spring, while the rigid foam displays high load-bearing properties and a subsequent cellular collapse and lack of recovery. Also, the flexible foam is unstable to UV radiation [80]. The semiflexible foam possesses a mixture of these characteristics. This kind of PUF is produced by using suitable combinations of polyesters and isocyanates. This foam is somewhat thermoplastic and do not melt, but it becomes notably softer with a moderate increase in the temperature, however this foam does not distort under its own weight below 90 °C, and hence can be used at higher temperatures if not under stress. Since it is composed of open cells, water can be mechanically absorbed. Acoustic insulation is one of the advantages of the open-cellular structure [81].

Any cellular product that falls between the values for the rigid and flexible foam curves can be classified as semiflexible. Microcellular urethanes are high-density elastomers of cellular composition, which has a linear load deformation. They are designed for heavy-duty mechanical applications. The flexible PUF yields open-cell materials that allow a free movement of air throughout the materials when flexed. They remain flexible down to –18 °C. In general, the flexible foam is based on polyether (the most important are the polyoxypropylene derivatives and polyester polyols). The latter is less resilient and less stable to hydrolysis but exhibit higher strength and elongation. The working temperature of these foams varies from 50 to 100 °C, depending on the application [82].

Rigid foam has a high strength and low weight, good resistance properties, an excellent adhesion to metal, wood, and ceramics. The percentage of the closed cells in the rigid foam depends on the degree of cross-linking and the surfactant used during the foaming as well as on

the polyol equivalent weight. The strength of the rigid PUF increases with the increasing the density and decreases with the increasing temperature, particularly at higher densities [80].

2.5.3 Physical and Chemical Properties of PUF

PUFs are plastic materials in which a proportion of the solid phase is occupied by gas in the form of large number of small bubbles (cells). From the geometrical point of view, these cells may be spherical in shape if the gas bubbles occupy less than 76% of the total volume, while they will be distorted into quasispherical polyhedra if they occupy a volume larger than 76% [83].

The material has been prepared in soft, flexible and rigid forms using a variety of polyesters and polyethers. The two most important reactions in the preparation of urethane foams are those between isocyanate and hydroxyl compounds (polyester or polyether polyols) and those between isocyanate and water. The physical and chemical properties of PUF are a direct function of the preparation process. Figure 2.2 shows the typical structure of PUF in its original form i.e., in the form in which it is used for sorption purposes. Unloaded PUF only adsorbs metal ions after complex formation. Organic and inorganic ligands can be used for this operation.

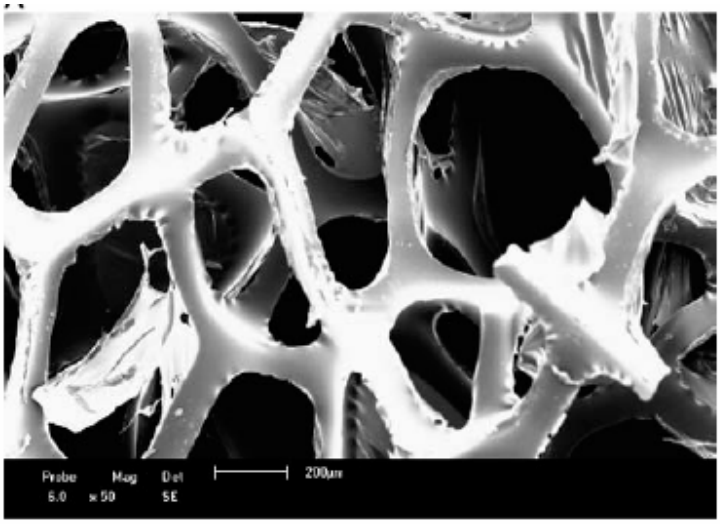


Fig. 2.2: Scanning electron micrographs of PUF (abstracted from [87])

Various factors determine the chemical and physical properties of PUF: functional groups, bond strength, crosslink density and flexibility, the effect of isocyanates, polyol, and their ratio. The stability of the foams derived from aliphatic diisocyanates is greater than of their aromatic counterparts. The regulation of the crosslink density allows the urethane polymer molecule to vary from the uncrosslinked linear polymers (fibers and thermoplastics) to a moderate crosslinking (elastomers, flexible coatings and foams) and finally to the very highly crosslinked structures (rigid thermosetting resins and foams). Cross-linking can be controlled by: Molecular weight and functionality of the polyol component; Functionality of the isocyanate component and Structure and functionality of a chain extender [84].

Based on the type of diisocyanate or polyol compound the PUF could be obtained in soft, flexible, semiflexible or rigid form. In case of the flexible PUF, the most commonly used isocyanate is TDI in various 2,4- and 2,6-isomer molar ratios. MDI in various forms has been found to be increasingly used, particularly in high-resiliency flexible and semiflexible foam. While in production of rigid PUF, modified TDI, MDI or polymethylene polyphenyl diisocyanate are utilized. The Diisocyanates are mostly used for the synthesis of PUF and it constitutes about 75% of the final product. By mixing commercial prepolymers several kinds of PUFs can be produced which contain different amounts of active isocyanate groups and diol-containing commercial product. Moreover, it is possible to use a commercial hydrophilic foamable PUF prepolymer which is a water-activated derivative. Polyols are the origin of hydroxyl groups in the foam material for almost all commercial uses of urethane polymers such as polyethers, polyesters and naturally-occuring hydroxyl-bearing oils like castor oil.

According to the equivalent weight, functionality, and the degree of rigidity or flexibility in the chain units in the polyols, the PUF is rigid or flexible, brittle or non-brittle, permeable of gas and moisture or not. Flexible foam is manufactured from polyols of a moderately high molecular weight and a low degree of branching, while the rigid foam is prepared from a lower-molecular-weight and highly branched polyols. The functionality of polyols has a substantial effect on the properties of the rigid foam. In case of higher functionality polyol, greater heat resistance and dimensional stability foam is obtained. Generally, at higher ratio of NCO/OH, more brittle foam is prepared with high compressive strength [84].

Chemically, PUF shows relevant stability towards thermo-oxidative destruction where it can withstand rising temperature up to 220 ^{o}C. The polymer is resistant to common acids such as hydrochloric acid up to 6 mol/l, sulfuric or nitric acid up to 2 mol/l. Also, basic solutions such as sodium or ammonium hydroxide up to concentration 2 mol/l cannot alter the PUF material. Additionally, it shows enhanced stability against some organic solvents such as alcohols, acetone, benzene, chloroform, carbon tetrachloride, ethylacetate, diethylether and hexane. Nevertheless, it dissolves in concentrated sulfuric acid and hot arsenic (III) chloride, oxidized by concentrated nitric acid and decomposed by alkaline potassium permanganate [85].

2.5.4 Synthesis of PUF

PUF are obtained by a reaction of isocyanates (di-, tri-, and other) with glycols (polyglycols, polyester polyols, or polyether polyols). However, urethane foams contain a large number of functional groups other than urethane linkages. For example, in addition to ester and ether groups, urea, biuret, allophanate, and imide groups may be found in these polymers. The preparation of PUF is by two main reactions. The first one is between the isocyanate compound, usually 2,4-TDI and a polyol compound (polyethylene or polypropylene glycol and propylene oxide adduct are frequently used). The second reaction is which is responsible for the foaming process due to liberation of carbon dioxide (in situ blowing agent) coming from the reaction between water molecules and the isocyanate groups [82].

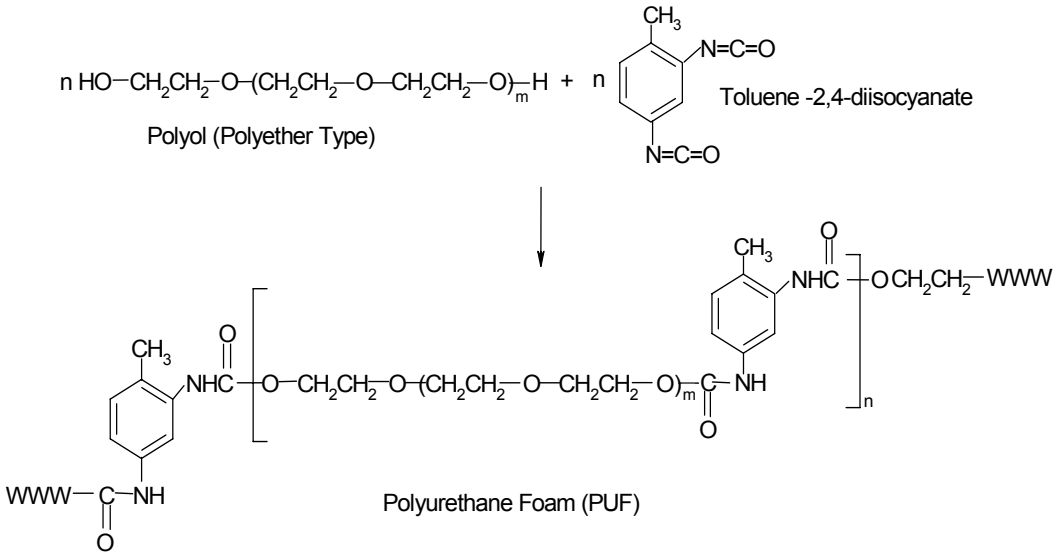


Fig. 2.3: Chemical structure of polyether-type PUF

During foam formation, the polymer is distributed by gas generation and constitutes the walls to gas bubbles and strands where the gas bubbles intersect. The bubbles are called quasi-spherical cells, and the walls are the factual membranes. In open-cell flexible polyurethane foam, at least two factual membranes in each cell must be ruptured to form windows through which fluid can pass freely. For this purpose, it is necessary for each window to be shared by two cells, and each strand to be shared by three cells. The cell structure, i.e. presence or absence of windows in the cell or the number of windows per cell, is a function of the process by which the foam is made. Reticulated foam, which is the most recommended form of PUF in analytical applications, is that one where the structure made up of only strands in which all the factual membranes have been ruptured [86].

2.5.5 Surface Modification of PUF

Although the ability of PUF to extract variety of chemical species, the aim of many surface modification studies therefore has been to maintain the sorptive properties of PUFs while providing a surface with improved selectivity and capacity. Other studies have aimed to improve cell colonization or degradation resistance by surface modification approaches. The properties and composition of polyurethane surfaces play an important role in chemical interactions, such as faster rate of sorption, and therefore in the suitability of this class of materials for use in analytical devices. In the absence of chemical constituents and with appropriate shape, the PUF surface has a large influence on the outcomes of the analytical responses to an analyte. Hence, it is of great practical interest to analyze, understand, and control the surface composition of polyurethanes, and thereby interpret and improve their utility. The application of surface analytical techniques to the elucidation of the chemical composition of polyurethane surfaces is very important to investigate the possible mechanisms by which this material can adsorb the chemical constituent. The surface modification of polymers has become a very attractive route towards expanding the use of existing polymers into applications for which they possess suitable bulk properties but inadequate surface properties. Surface modification approaches have thus assumed increasing importance in SPE research over the last decade. It is often difficult for the synthetic polymer chemist to design and develop polyurethane that meets all the requirements needed for specific analytical application.

2.5.6 PUF as SPE Sorbent

Considerable attention have been received in recent years on cellular and foamed plastic materials such as PUF to their use after special treatment with a suitable reagent for simple and fast separation and preconcentration procedures. Chelating agents supported on PUF have many advantages since they have powerful potential for separations due to the homogeneous distribution of the organic reagent on the relatively high surface area of the foam which allows reasonable, adequate contact between the analyte compound in solution and the chelating agent on the foam. This was confirmed by the fast and quantitative collection of trace amounts of constituents in large volumes of aqueous solution on reagent foams by means of static or dynamic techniques.

Furthermore, PUF is a good sorbent material in SPE, and it has been the subject of several review articles. The first paper reporting sorption and recovery of some inorganic and organic compounds from aqueous solution using this sorbent was published in 1970. One year later, untreated PUF was proposed for sorption of organic contaminants from water using a batch technique. Also the first application of PUF for chromatographic separation was investigated during this year. These studies resulted in a number of publications focusing on the use of unloaded and loaded PUF (polyether and polyester type) in separation and preconcentration procedures for the determination of inorganic and organic species using different analytical techniques [87].

2.5.7 Advantages of PUF as Sorbent

The foam material has several advantages over other solid phase sorbents. Among those, it is commercially available, easy to prepare and handle. PUF is an excellent sorbent material due to high available surface area, cellular structure and extremely low cost. In addition, it is stable in acids (except concentrated nitric and sulfuric acids), bases and organic solvents and also, it will not change its structure when heated up to about 180 ^{o}C**.** Moreover**,** the use of PUFs have been used in column techniques in off – line or flow injection preconcentration system is advantageous because it shows low resistance to passage of fluids and did not show any overpressure nor swelling as commonly occur when using other sorbents [88].

2.5.8 Types of PUF Sorbents

2.5.8.1 Untreated PUF

Unloaded PUF only adsorbs metal ions after complex formation. Organic and inorganic ligands can be used for this purpose. Several batch and on-line procedures have been established this way. In a batch method, for example, molybdenum (VI) ions were quantitatively extracted with unloaded PUF after formation of thiocyanate complexes [89]. Several metal ions and organic compounds were determined by using the unloaded PUF. Table (2.1) summarizes some of the applications of PUF in metal ion preconcentration and separation.

A lot of work has been done on the extraction of inorganic compounds by the foam, but only a few reviews have investigated the nature of extraction mechanism. It was concluded that, the surface adsorption is not the reason for extraction due to the relatively high capacity measurement (it varies from 0.5 to l.8 mmole/g PUF). Since the compounds extractable by the foam are also extractable by diethyl ether, a solvent extraction mechanism was suggested. This idea was extended to recognize the foam as a polymeric analogue of diethyl ether. This mechanism has been widely accepted by many workers to describe the extraction of various species, e.g., $GaCl_4^-$, $FeCl_4^-$, $SnCl_5^-$, $SbCl_4^-$, and $Rb(SCN)_6^{3-}$. Additionally, a cation chelation mechanism was proposed to account for the extraction of anionic metal complexes. Based on to this mechanism, the efficient complexation of the cation with the crown-ether type of configuration in the foam facilitates the extraction of anionic metal complexes. Polyether- and polyester-type PUFs are referred to as polyether and polyester foams, respectively. In the polyether foam, the poly(ethylene oxide) portion forms a helical structure with inwardly-directed oxygen atoms, is considered to be responsible for the specific interaction between the cation and the PUF. It was observed that polyether PUF demonstrates similar cation selectivity to 18-crown-6. On the other hand, polyester PUF does not readily assume such a helical structure, and accordingly the no strong interaction with the cation compared to polyether PUF. Therefore, polyether PUF is a better extractor than polyester type. Accordingly, several extraction systems involving inorganic compounds have been explained by this mechanism of extraction [90].

Table 2.1: Extraction of metal ions by unloaded PUF

Element	Sample	Extraction medium	Analysis Technique	LOD	Ref.
Ni	Silicates alloys	SCN	Spectrophotometry	77 ng/ml	[54]
Zn	Cd matrices	SCN	Spectrophotometry	-	[91]
Cu	Mussel tissue	APDC	FAAS	0.2 µg/l	[27]
Pb	Mussel tissue	APDC	FAAS	1.8 µg/l	[27]
Cr	Waste Water	APDC	FAAS	2.0 µg/l	[27]
Hg	Ore samples	Cu concentrates	X-ray fluorescence	2.7 µg	[92]
Ga	Al Alloys	6 M HCl	X-ray fluorescence	60 ng/ml	[93]
Mn	Silicates/Glass	SCN/Dithizone/ Hexamine/NH_2OH	Spectrophotometry	0.1 µg/ml	[94]
W	Standard samples	8-9 M HCl	Spectrophotometry	0.1µg/ml	[95]
V	Ceramics	Salicylhydroxamic	Spectrophotometry	0.5µg/ml	[96]
Fe	Glass/ ceramics	Phenanthroline	Spectrophotometry	0.05µg/ml	[97]
Mo,	Sea water	SCN/HCl	Radiotracer	0.2 µg	[98]
W	Sea water	SCN/HCl	Radiotracer	0.2 µg	[98]
Tc	Sea water	SCN/HCl	Radiotracer	0.2 µg	[98]
Co	-	1M KSCN/3MNH_4Cl	X-ray fluorescence	0.05 mg/l	[99]
Co	Steel Alloys	BTAC/Triton -X100	Spectrophotometry	1.6µg/ml	[100]
Zn	Al Alloys	SCN	ICP-AES	0.02 µg/l	[101]
Sb	Glass	Iodid/SCN/H_2SO_4	Spectrophotometry	0.1 µg	[102]
Pt	Glass	HCl/$SnCl_2$/Dithizone	Spectrophotometry	0.1µg/ml	[103]
U	Mine drainage	Salicylate	X-ray fluorescence	5.5 µg/l	[104]

There are numerous applications of the untreated PUF published as sorbent for different organic compounds in particular those recognized as environmental pollutants in water and air. Enrichment of those compounds is very important before their determination since they present at very low levels in natural samples. Accordingly, it is necessary to percolate large sample volumes through PUF packed columns to achieve the analyte at relevant concentration suitable for accurate detection. After preconcentration on the PUF sorbent, the constituent is recovered by either simple elution by soxhelt extraction with organic solvent such as hexane or acetone. Then, an appreciate volume reduction is achieved and the recovered amount is analyzed with suitable analytical method such as GC, spectrophotometry or LC [105].

Over the years, PUF has been successfully applied to concentrate various organic contaminants from water such as chlorinated organic compounds and aromatic hydrocarbons (PAH). In 1971, Gesser et al. [106] used PUF packed in a column for the concentration of polychlorinated biphenyls. PUFs were also used as an alternative to activated carbon for the determination of trace organic contaminants in water by Gesser et al. [107]. Afghan et al. [108] reported that PUF is capable of concentrating PAH at parts per trillion levels in natural waters and has a concentrating ability similar to other sorbents such as XAD-2 and silica bonded phase packing. Musty and Nickless [109] have investigated the quantitative extraction of chlorinated insecticides by PUF at pH between 6 and 9. According to the results obtained, they concluded that the foam is superior to activated carbon since the material recovered from activated charcoal is often different from the original form due to catalytic effects. Retention and recovery of bifunctional aromatic compounds such as phthalate esters by the foam have been reported by Gough and Gesser [110].

Recently, Schumack and Chow [111] studied the mechanism of the extraction of simple aromatic compounds by PUF. The extraction with PUF has been compared to identical extractions into diethyl ether. Salting-out effect was observed to be similar which indicates an ether-like solvent extraction mechanism in the PUF extraction process. It was observed that the presence of phenolic or carboxylic group in the aromatic compound increase its distribution coefficients due to the possibility of hydrogen bonding with the foam. Nevertheless, the presence of a strong electron-donor group *ortho* to the hydrogen bonding group prevents the formation of such bonding as in the case of 2-nitrophenol or salicylaldehyde where intramolecular hydrogen bonding can take place. Additionally, the strength of hydrogen bonding in case of polyether foam is greater relative to polyester foam. Organic dyes extraction by PUF was investigated by Chow et al. [112]. They studied the extraction from many from aqueous and 50% aqueous methanol solutions. It was revealed that the neutral dyes are extracted according to a simple solvent extraction mechanism, while the anionic dyes could be explained with the cation chelation mechanism. Generally, it can be stated that, many organic compounds are extracted by an ether-like solvent extraction mechanism; while those involving inorganic complex anions can be explained by the cation chelation mechanism [94]. Table 2.2 indicates some organic compounds that could be extracted by the untreated PUF sorbent.

Table 2.2: SPE for determination of some organic compounds using untreated PUF.

ompound	Sample	Medium	Analysis Technique	R (%)	Ref.
Carbaryl	Spiked water	Water	UV Spectrophototometry	102	[113]
Benzo(a)pyrene	Natural Water	Water	GC – Chromatography	96	[114]
Aniline	Water	CTAB/Na_2CO_3	DRS	-	[115]
Phenol	Water	CTAB/Na_2CO_3	DRS	-	[115]
Salicylic acid	Water	pH 3.15	UV - Spectrophotometry	-	[90]
Cinnamic acid	Water	pH 3.9	UV - Spectrophotometry	-	[90]
Diazinon	Water	pH 6-8	UV - Spectrophotometry	95	[116]
Cypermthrin	Tomato/Parsley	pH 1.5	UV - Spectrophotometry	96	[117]
Parathion	Tomato/Parsley	pH 1.5	UV - Spectrophotometry	96	[117]
Malathion	Tomato/Parsley	pH 1.5	UV - Spectrophotometry	99	[117]
Dicofol	Tomato/Parsley	pH ≤3	UV - Spectrophotometry	95	[117]
Bromopropylate	Tomato/Parsley	pH ≤3	UV - Spectrophotometry	95	[117]
Phenol	Tap Water	pH < 3	UV - Spectrophotometry	96	[118]
O,m-Cresol	Tap Water	pH < 3	UV - Spectrophotometry	97	[118]
o-Nitophenol	Tap Water	pH < 3	UV - Spectrophotometry	95	[118]
Salicyladhyde	Tap Water	pH < 3	UV - Spectrophotometry	97	[118]
Naphthalene	water	Water	HPLC-flourecence	83	[119]
Acenaphthalene	water	Water	HPLC-flourecence	92	[119]
Flourene	water	Water	HPLC-flourecence	96	[119]
Phenanthrene	water	Water	HPLC-flourecence	97	[119]
Anthracene	water	water	HPLC-flourecence	98	[119]
Pyrene	Water	water	HPLC-flourecence	99	[119]
Benzopyrene	water	water	HPLC-flourecence	99	[119]
Gallic acid	Beverages	NBDTFB	DRS	-	[120]
Dimethoate	Tap water	pH 7	UV – Spectrophotometry	94	[121]
Azodrine	Tap water	pH 7	UV - Spectrophotometry	98	[121]
Lannate	Tap water	pH 10	VIS-Spectrophotometry	90	[121]

2.5.8.2 Modified PUF

Owing to the versatile physico-chemical structure and properties of PUF, there are several ways to modify the surface of this attractive material in order to satisfy the purpose of its intended analytical application. The properties of the final PUF sorbent depend on the method utilized in the modification step. PUF can be applied without any treatment (unloaded PUF) or after reagent loading. The reagent loaded PUF can be done either physically (usually termed as loaded, impregnated or immobilized PUF) or chemically (reacted PUF). The chemically loaded PUF is obtained in two ways. The first method is by plactization (grafting) of the reagent into the PUF backbone and it is called grafted PUF. The second method depends on chemical linking of

the reagent to the terminal groups of the readily existent polymer and the resulting material known as functionalized PUF.

2.5.8.2 1 Physical Treatment of PUF: immobilized PUF

The capability of PUF to adsorb variety organic reagents on its surface provides the possibility of using this material as high surface area support in the sorbent preparation. There are numerous number of literature reviews have been published about the utilization of physically loaded PUF for preconcentration and separation of different species. Moreover, loading process offer a wider field of application of PUF than the unloaded one since it improves the selectivity and the capacity of the sorbent. The reagent to be loaded should not be water soluble since the immobilization process takes place by dissolving the reagent in volatile organic solvent such as acetone. The solvent evaporates and leave the organic substance adsorbed on the PUF surface which resists washing out by the aqueous solvents. One of the obvious drawbacks of this kind of sorbents, is that it is not liable for extraction from non aqueouse media due to leaching of the reagent from the support surface. Although, the polymer seems to be used as support for the reagent but there are also different kinds of interaction between the analyte and the functional groups of the PUF. These subsidiary functional groups play important role in the extraction process since it may promote or hider the binding of the analyte to the sorbent.

It was found that PUF is able to retain a considerable amount of various organic solvents by swelling; where the organic solvents are firmly retained in the produced swollen foam. Accordingly, the material could be immobilized with various organic reagents simply by dissolving these organic reagents in the hydrophobic organic solvent before allowing the foam material to swell in the latter. By this way, it was possible to immobilize chloroform or carbon tetrachloride solutions of such as dithizone on or in PUF. Although this method was successful in the preparation of several reagent foams, yet the rapid volatilization of the solvent (carbon tetrachloride or chloroform) affected the homogeneity of the loaded foam, because of precipitation of the solid reagent on the external surfaces of the foam material, Obviously, this would have serious limitations in application of the reagent foams, particularly in semiquantitative analysis [122].

In the separation and preconcentration of metal ions, there are huge numbers of organic ligands that already immobilized and utilized for this purpose. Among those used in the determination of heavy lements are: DDTC for the determination of As, Co, Se, Cr, Pb, Zn, Cu, Mn, Cd, Sb, and Sn in water sample [123], APDC for the determination of Cu, Pb and Cr in natural water [27], DMG for the determination of nickel [124], PAR for Zn [125], Hg in acetate medium [126], Cd in drinking water [127] and Pb in drinking water and oil refinery [128], BTAC for Pb in wine [83] Cd in environmental and biological samples [129], Me-BTANC for Cu in food [130] and Cd in black tea and spinach leaves [131], Me-BTABr for Zn in natural water [132], DDTP for Cd [133] and Pb [134] in natural water samples. Alexandrova et al. [135] used hexamethyleneammonium hexamethylenedithiocarbamate immobilized on PUF mixed with methyltri-octylammonium chloride immobilized on filter paper as well as the solid sorbent PUF was applied to the preconcentration of traces of Cd, Co, Cu, Hg, Ni and Pb in analytical-reagent grade oxalic acid prior to their measurements by AAS.

Alkaline earth elements could be also separated from complex matrices such as trace calcium ions in glass and ceramics for its spectrophotometric determination using tributyl phosphate loaded PUF. The sorbent shows excellent selectivity of extraction for Ca ions in sodium hydroxide solution which is eluted with hydrochloric acid for visible spectrophotometric analysis using calcon reagent at pH 12 [136].

Organic compounds in environmental samples could be extracted by loaded PUF material. The extraction of phenol and 1-naphthol with equimolar mixtures of ethyl acetate and hexane using TBP impregnated into PUF under batch and dynamic conditions was studied by Sukhanov *et al.* [137]. The TBP – loaded PUF was also applied in similar procedure for determining phenol and 1-naphthol in aqueous samples and elution with a 0.1 M NaCl solution of pH 12.5–13.0. Additionally, tri-n-octylamine immobilized PUF was applied to the preconcentration of various pesticides, pyrethroid and acaricide residues in agricultural products such as tomato and parsley plants and in water at ppb level using polyether type PUF [117]. Dmitrienko et al. [138] developed an analytical procedure for the determination of ascorbic acid based on the reduction of molybdosilicic heteropolyacid immobilized on PUF by ascorbic acid in a microwave oven. A blue-colored product of reduction of molybdosilicic heteropolyacid was determined immediately in the PUF using diffuse reflectance spectroscopy.

Furthermore, loaded PUF found some biochemical applications in the preparation of biosorbents by immobilizing some biological substances such as enzymes then applied to the determination of different chemical species. In this context, an alkaline phosphatase enzyme immobilized in *N*-phthalylchitosan on PUF in *p*-nitrophenylphosphate hydrolysis was proposed by Irina et al [56]. The inhibitory effect of lead (II) towards the activity of the enzyme has been used for developing a visual test procedure for the determination of Pb(II). The procedure was successfully applied to the determination of the element in different soil samples. Also, Baker's yeast (*Saccharomyces cerevisiae*) immobilized on PUF could be applied to the determination of toxic elements such as Sb in river water. The immobilized material was used for the spciation analysis of Sb (III) and Sb (V) by the determination of Sb(III) and total Sb using hydride generation inductively coupled plasma optical emission [139].

Moreover, horseradish peroxidase immobilized in chitosan on PUF in the o-phenylenediamine oxidation reaction has been used for developing a visual test procedure for the determination of Hg(II). The inhibitory effect of Hg (II) ions on the catalytic activity of horseradish peroxidase that is immobilized in the natural polymer such as chitosan in the oxidation of aromatic amines could be used as indicator [140]. Additionally, a biomass from the seaweed *Ascophyllum nodosum* was incorporated into an inert PUF carrier matrix during the foam formation process. The immobilized material was used compare the uptake capacity and adsorption rate of a known heavy metal on biosorbent immobilised in PUF and applied to the determination of Cu ions in natural samples [141].

2.5.8.2.2 Chemical Treatment of PUF: Reacted PUF

Modification of PUF cannot only be done physically but also chemically. In this case, the reagent or chelating ligand is binded to the backbone of the polymer via chemical bond. Two possible methods are available for changing the chemical properties of the PUF. Firstly, the reagent is incorporated as building unit in the polymer chain and this is called grafted PUF. Secondly, the reagent is linked to a side group in a readily prepared PUF through chemical reaction and this is called functionalized PUF.

2.5.8.2.2.1 Grafted PUF

Grafting is proposed to increase either the selectivity or the capacity of PUF. It is well known that PUF can extract broad spectrum of metal ions as thiocyante, chloride or iodide complex. Therefore, in order to enhance the sensitivity and the capacity of the material, special reagents with greater number are needed to be added within the polymer chain in order to increase the number of chelating sites. In this context, the reagent is added during the manufacturing process of the PUF so that it can add as monomer and participate in the polymerization reaction. Accordingly, the resulting polymer chains contain huge number of the reagent molecules. For this, the reagent should be carefully selected so that it should contain side groups necessary for polymerization and chelation and it must be soluble in the polymerization reaction mixture. Sometimes, grafting could be done via radical polymerization, where a free radical from the reagent is liberated via radiation effect which in turn attacks the polymer chain.

A novel method has been described for the incorporation of the basic dye *Nile blue A* into polyurethane foam matrix [142]. The material was found to be very suitable for the extraction of metal ions from aqueous solutions. It was applied for the separation and preconcentration of iron (III), zinc (II), cadmium (II) and mercury (II) from waste water as thiocyanate complexes in acidic medium. Also, polyether polyol, toluene diisocyanate and basic dyestuff (Methylene blue, Rhodamine B and Brilliant green) were used to in the synthesis of grafted PUF materials and characterized using UV/Vis, IR and TGA. The adsorption properties and chromatographic behaviour of these new adsorbents for preconcentration and separation of uranium (VI) ions at low concentrations from aqueous thiocyanate media were investigated [143].

Radical copolymerization was successfully used to introduce peroxide groups onto the surface of PUF through one atmospheric pressure plasma treatment and sequentially grafted poly(acrylic acid) on the surface of PUF. By using plasma treatment which can generate large amount of peroxides on the surface of PUF that act as initiators for further grafting of poly(acrylic acid) in the monomer solution. The surface of the modified PUF was qualitatively and quantitatively analyzed through the use of FT-IR and weight measurement, respectively. SEM and photo analysis was also utilized investigate the surface change before and after plasma induced graft co-polymerization [144].

Gamma irradiation was applied to modify PUF by grafting a mixture of two monomers acrylonitrile/acrylic acid. Effects of various parameters such as, concentration of co-monomer composition, grafting yield, gamma irradiation doses, dose rates and pH of adsorbent on the adsorption capacity were studied. Characterizations of the grafted polyurethane were investigated using IR spectroscopic analysis, X-ray diffraction and scanning electron microscope. Moreover, the adsorption capacities of some heavy metals such as, Zn(II), Fe(II), Ca(II), Ni(II), Cu(II) and Pb(II) onto the prepared grafted PUF were studied [145].

2.5.8.2.2.2 Functionalized PUF

The second type of reacted PUF is obtained by covalently linking chemical reagent to the side group in the prepared PUF material. There are different functional groups in the polymer chain that can be utilized in this purpose. The terminal amino and isocyante in the toluene diisocyante moiety and carbonyl and imino of the urethane group in addition to the hydroxyl groups in the polyol residues are available for chemical reactions. The only known modification till now is that performed by using the terminal amino groups. Dmitrienko et al. [146] have studied the chemical reactions of the functional groups in PUF by use of DRS and IR spectroscopy. It was found, that the functional groups are highly reactive towards diazotization by sodium nitrite, azo coupling with 4-nitrophenyldiazonium tetrafluoroborate, and oxidation by active chlorine, and condensation with formaldehyde, resulting in the formation of intensely colored products. Heterogeneous chemical reactions of PUF with these compounds in aqueous solution proceed rapidly at room temperature and at low solute concentrations.

Modification by azo coupling of the terminal amino group to different coupling compounds was investigated by Moawed et al. who have prepared chemically modified PUF by covalently linking 8-HQ [147] and 1-Naphthol [148] Alizarine red S [149] through the azo coupling of the phenolic compounds to the diazonium ion derived from the terminal amino group in the PUF. Additionally, azo derivatives of the PUF were also achieved by reaction of PUF to p-cresol or p-hydroxyacetophenon and were applied to the preconcentration and separation of heavy metal ions in lake water [150].

Another kind of functionalized PUF was done by condensation reaction between the terminal amino groups in the PUF with aromatic aldhyde. Polyurethane foam functionalized with pyrocatechol by covalent coupling through –N=C– group, is applied to an online pre-concentration system for cobalt, copper and nickel determination in food samples. The preparation of the packing material involved the reaction of 2,3-dihydroxybenzaldehyde with the amino groups of polyurethane foam. Metal ions were sorbed in the minicolumn, from which it could be eluted with acid solutions directly to the nebulizer-burner system of the FAAS [151].

2.5.9 Analytical Applications of PUF in SPE

2.5.9.1 Off – Line Separation and Preconcentration Procedure

In the off – line procedure, the SPE system is not directly connected to the detection unit. The first step of the analysis is enrichment of the target analyte and matrix removal by using the solid sorbent in either batch (using PUF pellets or cubes) or PUF packed column mode. This step eliminates the interfering coexisting ions and makes the analyte in the detectable concentration range. Finally, the determination step can be made using the suitable measuring technique. Although this method is simple and fast, but the opportunity for the contamination is rather high due to the manual handling in-between the two analysis steps.

2.5.9.2 On – Line Separation and Preconcentration Procedure

De Jesus et al. [152] were the first to employ the polyether-type PUF for on-line SPE preconcentration and determination of zinc. Since it, this material has been established for continuous flow analysis and many literature reviews have been published for on-line determination of various metal ions. Unloaded PUF was recently introduced in on-line separation and preconcentration systems. Application of PUF in these produces smaller resistance for fluid passage than materials often employed for this proposal. Thus, it results low overpressure in the system reducing risk of leakage. Since the on-line pre-concentration systems utilizing PUF have been introduced only recently; they are predominantly used with loaded PUF, although unloaded PUF has been applied as well. These systems have been coupled with analytical techniques such as FAAS and ICP-OES. With these procedures, the main experimental parameters that have been optimized are the pH, sampling flow rate, elution flow rate and type and concentration of eluent. The importance of pH investigation because of the complexation reaction between the species

and ligands involved in the process is a critical factor which affects the retention properties. The sampling flow rate determines the sorption kinetics of the solid phase (PUF). Although, a higher sampling flow rate implies a lower phase transfer factor, however it causes problems only if this factor is different for samples and standard solutions.

An on-line SPE pre-concentration method for the determination of lead in saline effluents and drinking water using a minicolumn of PUF loaded with PAR was proposed. The sampling flow rate was applied at 11 mL min^{-1} and the phase transfer factor was found to be 73 [153]. Usually, the choice of the eluent is made in the same way as in batch procedures. For this purpose, most procedures use hydrochloric acid in the concentration range 0.50–3.0 mol /l. Because it can destroy the PUF, nitric acid is normally avoided [154]. Organic solvents as eluent have also been reported. Stratis and co-workers [27] have used isobutylmethylketone for the determination of copper, lead and chromium (VI) in natural waters and biological samples by FAAS. Also, ascorbic acid was used as the eluent for iron (III) retained as thiocyanate complex on PUF in an on-line method for quantitation of iron in biological matrices [155]. Lemos and Ferreira [28] have compared the performance of two on-line pre-concentration systems for the determination of lead using a minicolumn of amberlite XAD-2 loaded with BTAC and another one of PUF loaded with the same reagent. The PUF minicolumn proved to be the pre-concentration system with higher sorption capacity, enrichment factor and limit of detection.

Continuous flow analysis of organic compounds using PUF minicolumn was examined by Cassella et al. [113]. They reported the retention of carbaryl by polyether type polyurethane foam in on-line solid phase extraction mode. Several parameters that can influence the extraction of carbaryl from water samples were investigated such as concentration of carbaryl, mass of sorbent in the column, sample pH, saline concentration, solvent washing and sample flow rate. Results show that it is possible to achieve quantitative extractions when the sample flow rate is maintained up to 2.4 mL /min, a mass of foam of 300 mg and a sample volume of 30 mL in 0.5 NaCl are employed. They also indicate that there is an ether-like solvent extraction mechanism controlling the sorption of the substance by the foam. Some recovery tests were performed in spiked water samples and recoveries between 93 and 102% were obtained.

The setup of the on-line preconcentration system varies according to the compound to be analysed but there are three basic components in such systems. These components are the FI peristaltic pump fitted with Tygon tubes for pumping the sample, eluent or reagent, and a minicolumn packed with PUF, coupled to the detection system which is either spectrophotometric deterctor equipped with flow cell or FAAS. The flow system operates in a time based mode. Two basic steps are carried out in every analysis cycle. Firstly, the Preconcentration step. In it, a solution (Sample) containing of the analyte is kept at optimum pH with addition of buffer solution and pumped at suitable carrier flow rate to percolate through a minicolumn that retained the analyte. Then, the compoumnd under investigation are retained by chemical sorption as complex and the remaining solution was discharged to the waste. Secondly, the elution step which is made by switching the injection valve, a stream of the eluent displaces the analyte ions. This eluate is taken direct, after mixing with the necessary reagent in the reaction coil RC, to either the flow cell in the spectrophotometer or the nebulizer-burner system of the FAAS. Signals were measured as peak height by using instrument software. It is necessary to recondition the minicolumn at the end of each cycle if the samples were not buffered before preconcentration step.

2.5.9.3 Application of PUF in Speciation Analysis

Lemos et al. [88] developed a method for the direct determination of free available lead(II) and total lead content in wine samples based on the chemical sorption of lead(II) from solutions buffered at pH 7 on a minicolumn of PUF loaded with BTAC. Lead was directly eluted into an air-acetylene flame with 0.1 mol L^{-1} hydrochloric acid and determined by FAAS. Free available lead(II) was determined by direct on-line pre-concentration of the untreated sample, whereas total lead was determined after sample digestion with nitric acid and hydrogen peroxide.

Speciation analysis of phenylmercury, methylmercury and inorganic mercury in water was established by Braun et al. [156]. They used DDTC-loaded PUF disks and radioisotope-induced XRF as analytical technique used was. El-wakil et al. [157] proposed a specific procedure for the determination of chromium(VI) in natural water using PUF loaded with TBP and molecular absorption spectrophotometry. Anthemidis et al. [27] also developed an on-line Preconcentration system for the determination of chromium(VI) in environmental samples where the APDC complex was sorbed on the unloaded PUF.

2.5.9.4 Other Applications

Heteropoly acids are capable to be adsorbed on PUF. Accordingly, arsenate and phosphate have been determined by the quantitative sorption of their heteropoly blue species of the corresponding arsenomolybdic and phosphomolybdic using solid phase spectrophotometry [158]. Moreover, PUF has also been applied for enhancing the selectivity of analytical systems. In a study for the determination of aluminum by FI spectrophotometry with methyl thymol blue, interferents were retained on PUF as thiocyanate complexes and the aluminium ions pass through for the subsequent detection [86]. Filters from PUF, joined to ultrasonic nebulization of ICP - OES, were successfully employed by Martinez and co-workers in the determination of cadmium in commercial tea samples [159] and lead in Ilex paraguariensis samples [160]. A separation procedure, to overcome the interference of aluminum in the determination of zinc in aluminum matrices, was proposed by Ferreira and co-workers [101]. The PUF was utilized as sorbent to retain zinc ions as thiocyante complex which is then eluted and determined by ICP - OES.

AIM OF THE WORK

In this study we planned to develop certain sorbents by modification of the same support so that it can be utilized in extraction of inorganic and organic environmental pollutants. If we look to other commonly used materials such as silica gel or resins, the modification either takes longer time in case of silica gel or the hindrance in entering the ligands due to cross-linking in case of resin. PUF provides the opportunity to make the necessary modifications with fast and simple reactions. Furthermore, in this work, we intended to apply the newly prepared sorbents in both the off-line and on-line SPE modes. Therefore, the choice of PUF facilitates the some problems like column overpressure that may come from the use of less porous materials since we need to some experiments at higher flow rate.

An additional factor standing behind the selection of PUF is economic. In my countries, due to the lack of highly sophisticated instruments which permit working under high column pressure such as HPLC, we tried to design simple preconcentration and separation system to help the analysis of organic compounds. In this system, we intended to avoid the high pressure inside the analytical column and the most popular and highly porous material for such purpose is the open-cell PUF. Therefore we can summarize the aims as follows:

- Preconcentration and matrix separation for usage of simple instrumental systems.

- Application for determination of both heavy metals and antibiotics in water and environmental samples.

- Synthetic and natural water samples used as aqueous extraction.

- Heavy metals can be distinguished by the analytical detection systems but antibiotics not. For antibiotics separation procedure should be developed.

- Simple experimental set up be developed. It includes simple instrumentation and modified PUF as sorption system.

EXPERIMENTAL

4.1 Preconcentration/Separation and Determination of Heavy Metals

4.1.1 Instrumentation

Atomic absorption spectrometer AAS5 FL, (Carl Zeiss Technology 1995, Germany), was used for the determination of Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) under the conditions recommended by the manufacturer (Table 4.1). Infrared (IR) analysis (4000 – 400 cm^{-1}) was recorded on a Nicolet 5DX Fourier transform IR spectrometer (USA). A Perkin Elmer elemental analyzer, model 240 C, was used for elemental analyses. The pH adjustments were carried out using the microprocessor pH meter BT 500 BOECO (Hamburg, Germany), which was calibrated against two standard buffer solutions at pH 4 and 9. A mechanical shaker (SL 350 NU¨ve san Malz ml Ve Tic.A. Akyurt Ankara-Turky) with maximum speed up to 200 rpm, was employed to shake the samples. Doubly distilled water (DDW) was obtained from two successive distillations using Hamilton laboratory glass instrument (Europe House, Sandwich Industrial Estate, Sandwich Kent, UK).

Table 4.1: Operational conditions for measurement of Cu(II), Zn(II), Pb(II), Cd(II) and Ni (II) with FAAS

Parameters	Metal ion				
	Copper (II)	Zinc (II)	Lead (II)	Cadmium (II)	Nickel (II)
HC lamp current (mA)	3.0	4.0	3.0	2.0	6.0
Slit width (nm)	1.2	0.5	1.2	1.2	1.2
Wavelength (nm)	324.8	213.9	217.0	228.8	232.0
Fuel flow (NL/h)	50	50	65	50	55
Burner height (mm)	4-10	4-10	5-10	4-12	5-12

The obtained linear equations along with regression (R^2) are: Zn (II): A = 0.10919 C+ -0.00082, R^2 = 0.99202); Cu (II): A = 0.03351C + 0.00198, R^2 =0.99905; Pb (II): A = 0.16111 C +0.000062, R^2 = 0.99971; Cd (II): A = 0.12272 C + 0.0017, R^2 = 0.99995 and Ni (II): A = 0.02688 C + 0.00079, R^2 =0.99692. Where A is the peak height absorbance and C is concentration in μg ml^{-1}

4.1.2 Chemicals and solutions

Unless otherwise stated, all reagents used were of analytical grade and all solutions were prepared DDW. Lab ware and glassware were used throughout and repeatedly cleaned with nitric acid and rinsed with DDW. The pH adjustments were made with from pH 2 to pH 10 using nitric acid or sodium hydroxide solution. The utilized chemical reagents are listed in Table 4.2.

Table 4.2: Standard chemicals and materials

Material	Company	Purity
2-Aminophenol	Aldrich - USA	99.0 %
2-Naphthol	Merck - Germany	98.0 %
Acetylacetone	Fluka - Switzerland	98.0 %
3-methyl-1-phenyl-2-pyrazolin-5-one	Fluka - Switzerland	98.0 %
$CuSO_4$.$5H_2O$	Aldrich - USA	99.0%
Zn SO_4 . $7H_2O$	Redel - de Haén- Germany	99.9 %
$NiSO_4$. $6H_2O$	Fluka - Switzerland	≥ 98%
$Pb(NO_3)_2$	Aldrich - USA	99.9 %
$Cd(NO_3)_2.4H_2O$	Panreac – Spain	98.0 %
H_2SO_4	Panreac – Spain	98.0 %
HNO_3	Merck - Germany	65 %
NaOH	Winlab – UK	99.0%
$NaNO_2$	Fluka - Switzerland	99.9 %
Ethanol	Adwic - Egypt	95 %
Open-cell Polyether PUF	Commercial PUF - Egypt	31 kg/m^3

Standard solutions (1.0 mg ml^{-1}) of copper, zinc, lead, cadmium and nickel and were prepared by dissolving appropriate amounts of analytical grade reagents: 3.928 g. copper sulphate, 4.390 g zinc sulphate or 4.478 g nickel (II) Sulfate, respectively, in 10 ml of concentrated H_2SO_4 and diluting the solution to 1 l with DDW. Lead (II) nitrate 1.598g or 2.7445g Cadmium nitrate is dissolved in concentrated nitric acid (10 ml) and finally were made up to 1l. Working solutions were prepared immediately before use.

4.1.3 Synthesis of PUF the Sorbents for Heavy Metals:

Coupling of the PUF to 2-aminophenol was carried out according to the method reported [148]. Twenty grams PUF cubes was soaked in a 5 mol l^{-1} hydrochloric acid solution for 1.0 h in order to liberate the maximum number of amino groups by the hydrolysis of the free isocyanate groups in the PUF. After this, PUF is diazotized by gradual addition of 1 mol l^{-1} sodium nitrite solution to the continuously stirred PUF cubes in 0.1 mol l^{-1} hydrochloric acid at 3 ^{o}C. The mixture is left overnight in a fridge and the resulting brown colored AP-PUF is washed with acetone, DDW, and dried in air. Additionally, the amino group of 2-aminophenol in the prepared AP-PUF was similarly diazotized and coupled to 2-naphthol (3.60 g in 100 ml 1mol L^{-1} NaOH), 2.50 g Acetylacetone or 3.35 g Pyrazolone (each in 100 ml ethanol containing 20 g sodium acetate) and left for 24h at 3 ^{o}C. The products are washed with ethanol, DDW and dried in air. The dark red, yellow and orange solid materials of HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF respectively are kept in dark bottles for further use.

4.1.4 Sample Preparation

4.1.4.1 Reference material

Certified reference material NIST-SRM 1577b Bovine liver was analyzed: Decomposition of these materials was carried out according to modification of the method [28]: Accurately weighed 0.4 g of material was treated with 4.0 ml of 1:1 (v/v) nitric acid solution and kept in clean glass vessel. Afterwards, the vessel was closed and left for digestion for 72 h. The thermal heating was carried out in a stove at 170◦ C for 16 h. After cooling at room temperature, the residue was diluted with Millipore water and adjusted to pH 7.0 with a 10% (w/v) sodium hydroxide solution. Finally, the volume was made up to 25 ml.

4.1.4.2 Tap water

Drinking water was collected from our research laboratory in Faculty of Science at Fayoum City. One - liter of the sample is adjusted to pH 7.5 and passed at relevant flow rate through the modified PUF column. After this, the column was eluted by 25 ml nitric acid solutions with suitable concentration. The metal ions Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) in the collected eluate are measured by FAAS and the recovery percentage and RSD for three repetitions are evaluated.

4.1.4.3 Synthetic seawater

Synthetic sea water was prepared by mixing 25.40 g of sodium chloride, 10.84 g of magnesium chloride hexahydrate, 1.104 g calcium chloride, 0.722 g of potassium chloride, 0.026 g of boric acid, and 0.341 g of strontium chloride hexahydrate in a volumetric flask and made up to 1l with DDW [161]. One liter of this sample was spiked with 20 µg l^{-1} each of Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II). Each 250 ml sample was passed through the column at the optimum pH and flow rate and the retained metal ions are eluted from the column by nitric acid solution for FAAS determination.

4.1.4.4 Apple leaves

Fresh apple leaves were obtained from Fayoum City, Egypt. It were washed by DDW and dried overnight at 105 ^{o}C. The dry samples were grinded in a mortar and dried again at 120 ^{o}C for 6 h till constant weight and the sample was digested according to the procedure [162]. An accurately weighed dried sample (0.100 g) was digested in 40.0 ml of concentrated (14.0 mol l^{-1}) nitric acid until nearly dryness and diluted up to 25.0 ml with DDW after adjusting to pH 7.5 with 10 % (w/v) sodium hydroxide solution and passed through modified PUF column. The retained metal ions were eluted with suitable stripping eluent (nitric acid) and measured by FAAS. The results for triplicate experiments are used to calculate the recovery (%) and the RSD%.

4.1.5 Recommended SPE Procedures

4.1.5.1 Batch Procedure

The capacity of the modified PUF sorbents toward Cu (II), Zn (II), Pb(II), Cd(II) or Ni (II) at different pHs was determined under static conditions in the batch mode. For this purpose, 100 mg of the sorbent was added to 10 ml, 1 µg mL^{-1} metal ion solution at varying pH values. The mixture was mechanically shaken for 60 min in polyethylene bottles at room temperature to attain equilibrium. The sorbent was separated, and the unextracted metal ion in the filtrate was determined by FAAS. The maximum exchange capacity of the modified PUF was determined by shaking of 20 ml (100µg ml^{-1} each element) solution with 100 mg modified sorbent for 1.0 h at the optimum pH. The sorbent was separated and the amount of the sorbed metal ion was determined by FAAS. The effect of shaking time on metal exchange capacity was determined under the same batch conditions at different shaking periods (2, 5, 10, 15, 20 and 30 min.) at the selected optimum pH of maximum extraction. The optimum

condition (pH and shaking time) were applied during the study of the interfering effects of different foreign ions on the efficiency of separation and to examine the selectivity of the proposed sorbents.

4.1.5.2 Column Procedure

Glass column (100 mm long, and 10 mm i.d) having a stopcock was used for the preconcentration and application steps. One gram PUF sorbent was packed by gentile pressure with glass rod on the foam plugs while the column is kept filled with DDW to avoid any air bubbles and glass beads was added on the top of foam bed to fix it and prevent the upward flotation. The bed height of the PUF in the column is about 60 mm. The column was treated with 0.1 mol l^{-1} nitric acid solution and washed by DDW until the effluent is free from any acid. A suitable aliquot of the sample solution containing Cu (II), Zn(II), Pb(II), Cd(II) or NI (II) in the concentration range of 5 – 25 µg l^{-1} was passed through the column after adjusting its pH to an optimum value and at a flow rate of 3 ml min^{-1}. The column was washed with DDW to remove unbounded metal ions and the sorbed metal ions were stripped off from the PUF column with 0.1 mol L^{-1} nitric acid. The recovered amounts of the metal ions in the eluate were determined with FAAS.

4.2 Preconcentration/Separation and Determination of BLAs

4.2.1 Instrumentations

Peristaltic FI pump, FIAS – 400 pump (Perkin Elmer, USA) up to 120 rpm, with two filling and two injection ports, the sample loops are from 100 – 1000 µl. The pump is controlled by a computerized program to obtain the desired flow rate and the injected sample volume. Tygon tubes of 1.52 mm i.d were used in all connections between the sample, eluant and carrier to the solution and from the pump to the foam column, the flow cell and finally to the waste. The on-line determination of the antibiotics was made using UV-VIS spectrophotometer with Helma flow cell. UV 1650PC spectrophotometer (Shimadzu), with absorbance-time recorder and UV2.10 probe software. The pH adjustment was done by pH meter 780 Metrohm, (Herisau, Switzerland). Millipore water obtained from Elix Millipore water purification system was used in all preparations and deionised water for washing glassware. The standard chemicals utilized in this work are listed in Table 4.3.

Table 4.3: The utilized standard chemicals along with their source and purity in BLAs study

Material	Company	Purity
Cefaclor	Sigma - USA	99 %
Amoxicillin	Fluka - Switzerland	≥ 97 %
Ampicillin	Fluka - Switzerland	≥ 98 %
Cefotaxime	Fluka - Switzerland	≥ 98 %
Chloranil	Fluka - Switzerland	99 %
N,N,N',N'-tetramethylethylenediamine	Fluka - Switzerland	≥ 97 %
Dry Toluene	Aldrich - USA	≥ 98 %
Ethanol	KMF - Germany	≥ 99 %
HCl	KMF - Germany	≥ 98 %
NH_4OH	Riedel de Haen - Germany	99.8 %
NH_4Cl	KMF - Germany	99.8 %
Acetic acid	KMF - Germany	37%
CTAB	Aldrich - USA	99.5 %
Acetylsalicylic acid	Fluka - Switzerland	25 %
Hippuric acid	Fluka - Switzerland	99.8 %
Glycin	Riedel de Haen - Germany	> 99 %
Valin	Fluka - Switzerland	99 %
Tyrosine	Fluka - Switzerland	97 %
Tryptophan	Fluka - Switzerland	99 %
Glucose	Fluka - Switzerland	99.5 %
Glutamic acid	Acros - USA	99 %
Caffeine	Fluka - Switzerland	99.5 %
Paracetamol	Sigma - USA	99 %
Oxalic acid	Riedel de Haen - Germany	99 %
Sodium Citrate	Riedel de Haen - Germany	99 %
Aspartic acid	Fluka - Switzerland	97 %
Barbturic acid	Aldrich	≥ 99 %
Open – Cell Polyether PUF	Contipas – Germany	99.5 %
Cefaclor acis, Filmtablette	Arzneimittel GmbH, Germany	99.5 %
Amoxicillin acis, Filmtablette	Arzneimittel GmbH, Germany	99 %
Ampicillin Capsule	Adwic, Egypt	55 kg/m^3
Bacticlor, Oral Suspension (Cefaclor)	Ranbaxy, Egypt	500 mg
Dorecef, Oral Suspension (Cefaclor)	Rameda, Egypt	500 mg

4.2.2 Synthesis of PCTDD – PUF sorbent

PCTDD – PUF sorbent was used for the extraction of β - lactam antibiotics. The copolymer, poly[*N*-chloranil *N*,*N*,*N*',*N*'-tetramethylethylene diammonium dichloride], was prepared according to the work done by Gupta et al. [163]. An equimolar amounts of *N*,*N*, *N*',*N*'-tetramethylethylene diamine (1.23 ml in 10 ml dry toluene) and chloranil (2.0 g in 50 ml dry toluene) are refluxed together for 4 hours and kept overnight. The resulting dark brown product is washed with toluene and dried in vacuum, then characterised by elemental analysis. For loading the PCTDD to the PUF, 0.5 g of the copolymer (dissolved in the least amount of DMF) added to 2.0 g PUF plugs in dry toluene solution and stirred for 72 hours at 90 ^{o}C. The brown PUF plugs are washed with dry toluene solution then ethanol and dried.

4.2.3 Developed Procedures

The flow system was operated in a time-based mode, in which a sample solution of the antibiotic compound (250 ng/ml) adjusted at the optimized pH value, pumped at 3.0 ml/min percolated through the minicolumn containing the solid sorbent for suitable preconcentration time. Then, antibiotics are retained by chemical sorption as ion associate complex and the remaining solution was discharged to waste. By switching the injection valve, a hydrochloric acid (eluent) was injected at suitable flow rate to displace the analyte. This eluate was directly taken to the flow cell of the spectrophotometer. All the measurements were made in peak height, which were proportional to the analyte concentration in sample. Signal height was measured by the instrument software. It was read three times and averaged. It was not necessary to recondition the minicolumn at the end of each cycle since samples were buffered before preconcentration. Different flow and chemical variables are investigated to optimize the performance of the on-line system. The carrier flow rate, sample pH, injected sample volume, sample flow rate, eluent concentration, eluent flow rate, preconcentration time, all of them were studied and optimized under the current system setup.

4.2.4 Sample Preparation

Three samples were subjected to analysis by the proposed procedure namely cow milk, human urine and pharmaceutical formulations. Firstly, standard addition method was applied for analysis of these antibiotics in cow milk. For this purpose, five samples (10 ml each) from commercially available cow milk were spiked up to 0, 100, 200, 300 and 400 $ngml^{-1}$ level of the studied antibiotic. To each of the spiked samples, 5ml of 20% aqueous acetic acid was added in order to promote protein precipitation. The mixture was centrifuged at 4000 rpm for 10 min and the supernatant was collected by syringe then it was filtered through a 0.2 μm membrane filter. The filtrate was taken for the analysis by the on-line SPE flow system. Antibiotic compound in each sample was preconcentrated/separated and measured by the recommended method. Secondly, 10 ml human urine was spiked with 100 or 200 $ngml^{-1}$ of each antibiotic compound separately and the pH of the spiked sample was adjusted to the optimized value by ammonia buffer then filtered through 0.2 μm membrane filter. After filtration the sample was taken for analysis by the on-line SPE flow system where the signal height of the antibiotic in the sample was compared to a reference one in the calculation of percentage of recovery. Finally, 250 mg of the drugs under investigation (cefaclor acis, bacticlor, clorocef, amoxicillin acis or ampicillin) was weighed into 250 ml calibrated flask,. The solid was dissolved in the least amount of methanol and diluted to the volume with Millipore water. An aliquot containing 2.5 or 5 μg from each sample was injected to the preconcentration system under the optimized conditions. The amount of the antibiotic compound in the pharmaceutical sample was quantified after its elution from the analytical column then it was directly measured and the RSD % values were evaluated.

RESULTS AND DISCUSSIONS

5.1 Preconcentration/Separation and Determination of Heavy Metals

5.1.1 Why we Focus on Heavy Metals?

Human civilization and a concomitant increase in industrial activity has gradually redistributed many toxic metals from the earth's crust to the environment and increased the possibility of human exposure. Among the various toxic elements, heavy metals cadmium, lead, and mercury are especially prevalent in nature due to their high industrial use. These metals serve no biological function and their presence in tissues reflects contact of the organism with its environment. They are cumulative poison and are toxic even at low dose [164]. In addition there is an increase in the number of people who are suffering from chronic diseases such as kidney malfunction in the area in which we performed this study.

5.1.2 Hazards of Heavy Metals

Heavy metals are included within the category of environmental toxins: “Materials which can harm the natural environment even at low concentration, through their inherent toxicity and their tendency to accumulate in the food chain and/or have particularly low decomposition rates”. It is however a fact that many organisms need trace amounts of many metals to survive. Among those mercury, lead, arsenic, cadmium, aluminium, cobalt, nickel and copper are metals, which appear together in many real samples. So, it is very important to determine their concentrations [165]. Herein, some details about the hazards of copper, zinc, lead, cadmium and nickel are mentioned.

5.1.2.1 Copper

Copper is an essential element not only for life in mammals but also for plants and lower forms of organisms. It has varied and many biologic effects as an essential element as a toxic one. It is usually used as algicide and herbicide [166]. Copper is considered as an essential micronutrient. It is needed by plants at only very low levels and is toxic at higher levels. At these levels, copper can bind to the cell membrane and hinder the transport process through the cell

wall. Nearly 40 ng ml^{-1} is required for normal metabolism of many living organisms. On the other hand, copper is an important element in many industries [167].

5.1.2.2 Zinc

An essential trace element of great importance for humans, plants and animals, It plays an important role in several biochemical processes and their compounds have bactericidal activity. Zinc compounds have been employed in solutions as antiseptic and disinfectant agents. However, if it is in excess, this metal can also play an important role in the progression of several damages to human body, including disturbances in energy metabolism or increasing in oxidative stress. Waters from natural sources located in areas near to mining and smelters operation could be contaminated. Therefore, fauna, flora and soils that are in contact with these waters can also show high levels of zinc. Thus, zinc determination in natural waters, biological matrices, sediments, etc. is of great importance, considering that the concentration of this metal can serve as base to characterize the level of pollution of a certain area [132].

5.1.2.3 Lead

Lead is a toxic metal, which accumulates in the vital organs of man and animals. Its cumulative poisoning effects are serious hematological damage, anaemia, kidney malfunctioning, brain damage etc. In natural water its typical concentration lies between 2 and 10 ng ml^{-1}, whereas, the upper limit recommended by WHO is less than 10 ng ml^{-1}. Lead is a highly toxic cumulative poison in humans and animals. Chronic effects of lead on heme synthesis have been reported, with inhibition and reduction of various enzymes in blood and urine. Lead concentrations in urine reflect the amount of lead recently absorbed [168]. Environmental contamination of lead is widespread; the main anthropogenic source of this element is buming of leaded gasoline. As result the lead is frequently found in surface water and, recently, in polar snow. In humans, chronic lead poisoning is manifested by abnormalities such as encephalopathy, nervous irritability, kidney disease and altered heme synthesis and reproductive functions. Such poisoning is associated with low to intermediate levels of chronic exposure to lead, with primary sources being intake of food, water and air [169].

5.1.2.4 Cadmium

Cadmium may cause renal injuries and may interfere with the renal regulation of calcium and phosphate balance. Cadmium concentrations in urine reflect long term exposure and the quantity of cadmium stored in the body, particularly in the kidney and liver. When renal damage begins to occur, the excretion of cadmium can rise markedly. Cadmium is a natural constituent element from the earth's crust that is taken in by plants and then transferred to animals through the food chain. Its metabolism and toxicology are of great concern, since the element has accumulation capabilities in living organisms besides its high toxic potential. It's wide technological use fertilizers, mining, pigments, as well as its delivering from oil and coal burning and residues incineration; bring about an extensive anthropogenic contamination of soil, air and water. It was seen that exposure to abnormal levels of cadmium can result in its accumulation in the renal cortex, which causes a series of adverse subclinical reactions such as hypercalciurium, renal stones and renal tubular dysfunction besides probable development of carcinogenic activity in organisms [170].

5.1.2.5 Nickel

Nickel is a moderately toxic element as compared with other transition metals. However, it is known that inhalation of nickel and its compounds can lead to serious problems, including respiratory system cancer. Moreover, nickel can cause a skin disorder known as nickel-eczema. Such a disease, characterized as occupational disease is very usual in workers who handle great amounts of this element and has been the subject of many studies. This skin disorder can also appear in people who have great sensitivity to nickel, and can be caused by wearing of jewels made of nickel alloys, like rings, chains and bracelets. Studies proved that women are more liable to this disease. Medical diagnosis is currently established through nickel determination in blood and urine. Other studies show that disease incidence increased in patients who consume foods rich in nickel, such as oats, nuts, and beans and chocolate. So, an appropriate knowledge of the nickel content in foods could be of a great interest for the dietary control of nickel-eczema patients [171].

5.1.3 Characterization of the Sorbents

Characterization of the newly developed sorbents was carried out by both IR and elemental analysis. The IR bands of the isocyanate, hydroxyl, carbonyl, amino and azo groups in different types of PUFs are considered and presented in Table 5.1. Additional absorption bands for –N=N- and C=O and disappearance of the band correspondence to SCN group of the untreated foam at 2100 cm^{-1} was observed. The absorption band for NH_2 groups disappeared from the IR charts in the three kinds of functionalized PUF (HPANaph-PUF, HPAA-PUF and HPAPyr-PUF). This confirms that, the amino groups of AP-PUF were exhausted in bonding to the coupling compounds in the second step. Figure 5.1 shows the proposed structures of the novel PUF sorbents. Additionally, there are absorption bands for carbonyl groups in case of HPAAA-PUF and HPAPyr-PUF which are corresponding to amid C=O of the foam material and that of the ligand itself. However, the AP-PUF and HPANaph-PUF have single the carbonyl band corresponds to the foam material at 1661 and 1653 cm^{-1} respectively. The results of the elemental analysis of the functionalized PUF derivatives are given in Table 5.2. The results for carbon, hydrogen and nitrogen analysis show good agreement with the calculated values from the proposed formula. It is obvious that the analysis of the dried functionalized PUF agree with the values calculated by presuming the stoichiometry of their repeat units. The data reveals that each two repeated units of the untreated PUF coupled to one molecule of aminophenol. i.e. by ratio 2 PUF:1 aminophenol. The second coupling occurs by ratio1of AP-PUF: 1 of naphthol, acetylacetone or pyrazolone. Although, the elemental analysis data satisfy with the proposed empirical formula, it is too difficult to predict the repetitive unit of the ligands in the modified sorbent due to the complex structure of the PUF material which is unlike the silica gel or amberlite XAD-2.

The chemical stability was investigated by measuring the change of the sorption capacity towards studied metal ions after soaking of the modified foams for one hour in acids 1 – 6 mol l^{-1} HCl, 1 – 2 mol l^{-1} H_2SO_4, 1 – 2 mol l^{-1} HNO_3 and alkaline solutions of 1 – 4 mol l^{-1} NaOH, 1 – 4 mol l^{-1} NH_4OH, as well as organic solvents e.g. methanol, ethanol, isopropanol, n-butanol, acetone, chloroform and carbontetrachloride. However, the decomposition was negligibly small and no significant decrease in the sorption capacity was observed, which indicate that these chelating extractors are believed to be sufficiently stable.

Table 5.1: Important IR bands in the modified PUF sorbents

Sample designation	IR bands (wave number, cm^{-1})				
	υ_{NCS}	υ_{OH}	υ_{CO}	υ_{NH2}	$\upsilon_{N=N}$
Untreated PUF:	2100	3509-3311	1655	3111-3299	-
AP-PUF:	-	3565-3329	1661	2926-3297	1473
HPANaph –PUF:	-	3506-3300	1653	-	1473
HPAAA –PUF:	-	3447-3328	1653, 1716	-	1473, 1508
HPAPyr-PUF:	-	3436-3300	1653, 1705	-	1473, 1508

Table 5.2: Elemental analysis data of the modified foams

PUF Type	Formula	Calculated (%)			Experimental (%)		
		C	H	N	C	H	N
Untreated PUF	$C_{78}H_{134}N_5O_{23}$	62.07	8.8	4.6	62.09	8.9	4.6
AP-PUF	$(C_{78}H_{134}N_5O_{23})_2 + C_6H_3N_2O$	62.01	8.6	5.4	62.12	8.62	4.8
HPANaph-PUF	$C_{162}H_{271}N_{12}O_{47} + C_{10}H_5NO$	62.73	8.4	5.5	62.71	9.60	5.4
HPAAA-PUF	$C_{162}H_{271}N_{12}O_{47} + C_5H_6NO_2$	61.45	8.5	6.0	61.50	7.98	5.8
HPAPyr-PUF	$C_{162}H_{271}N_{12}O_{47} + C_{10}H_{10}N_3O$	62.1	8.5	6.3	61.30	7.79	6.4

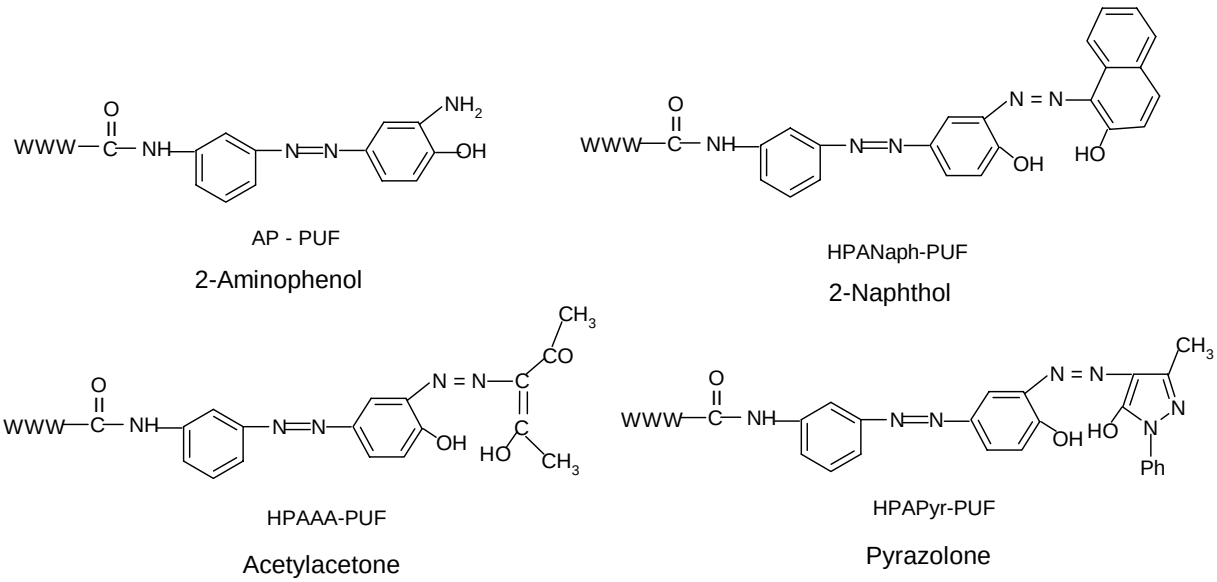


Fig. 5.1: Chemical structures of the modified PUF sorbents

5.1.4 Batch Procedure

5.1.4.1 Sorption of metal ions as a function of pH

The pH effect on the uptake metal ions was investigated by batch procedure. An aliquot of varying pH values containing each metal ion separately (Cu, Zn, Pb, Cd, or Ni) was stirred with 100 mg PUF for 1.0 h (Figs. 5.2 – 5.4). The percentage sorption of metal ion was calculated from the relation: *Sorption (%) = [C_o – C/ C_o] x100* Where C_o and C are the initial and remaining concentrations (µg l^{-1}) of the metal ion respectively.

Generally, it was found that the uptake increases by increasing pH till reaching a limiting value then leveling off within certain range of pH which is usually followed by a decrease. The optimum pH range for Cu, Zn, Pb, Cd and Ni are listed in Table 5.9. The decrease in percentage sorption higher than pH 8 may be attributed to the possible precipitation of metal hydroxides. By considering the higher affinity of the two times coupled foams (HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF) than AP-PUF since they have well-known chelating centers used as metal ion chelating sites. For example, the sorption of Zn (II) at pH 7.0 was 76 % with AP-PUF and 98 , 87

and 89 % with HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF respectively (Fig. 5.2). Similarly, at pH 6.0 the uptake of Cd (II) by AP-PUF is 47 % while it were 94, 90 and 100 % with HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF respectively, with an enhanced percentage uptake from 16 % to 26 % (Fig. 5.4). This due to the greater stability of metal complexes with the two times reacted foams. This proposal is confirmed through stable complexes formed between the metal ion and free electron pairs on the nitrogen atoms of azo group and the two oxygen atoms of the hydroxyl groups. Thus, the new modified foams are promising materials to be applied in metal ions removal from aqueous solutions. Obviously, it is concluded from the results that, AP-PUF has the lowest sorption capability for all studied metal ions. The HPANaph - PUF showed the largest uptake with Zn (II) while HPAPyr-PUF has greater extraction affinity for Cd than the rest of these materials. At low pH values (pH<5), the chelating groups (-N=N- and OH) in the sorbent become protonated and might behave as weak anion exchanger which reduce the tendency of the sorbent towards the positively charged metal ions and reach zero uptake at about pH 1.

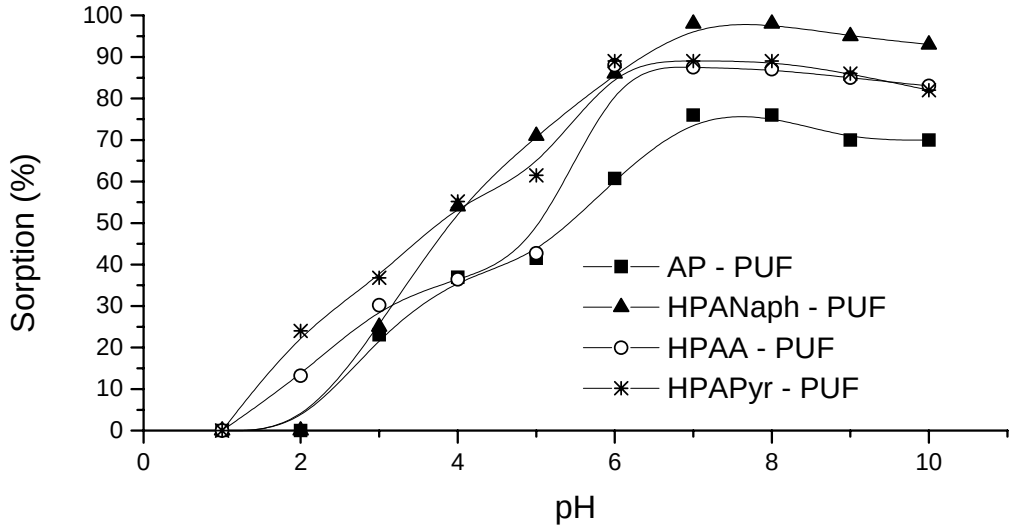


Fig. 5.2: Effect of pH on the extraction of Zn (II) with different modified PUF sorbents

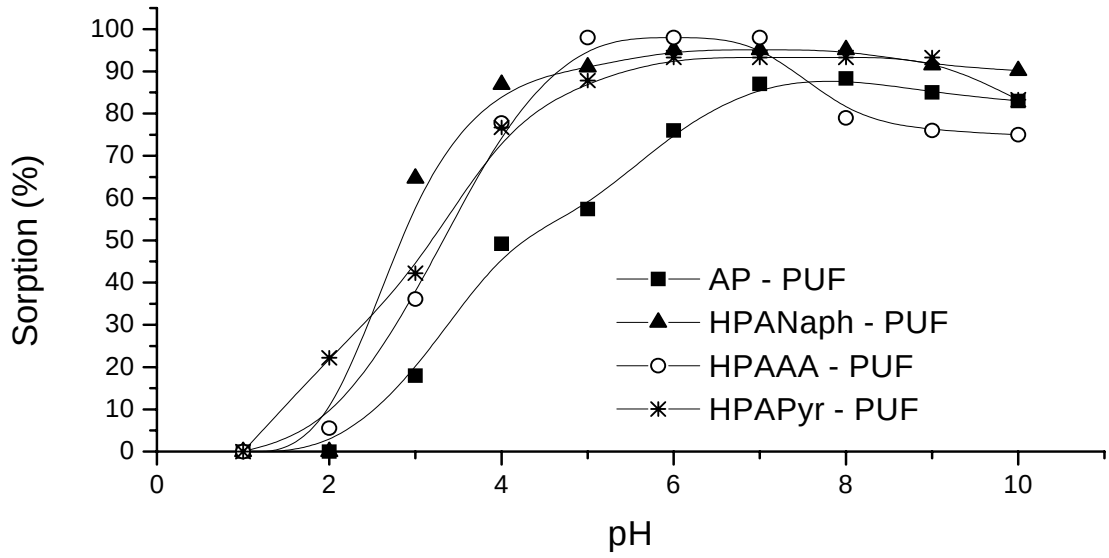


Fig. 5.3: Effect of pH on the extraction of Pb (II) with different modified PUF sorbents

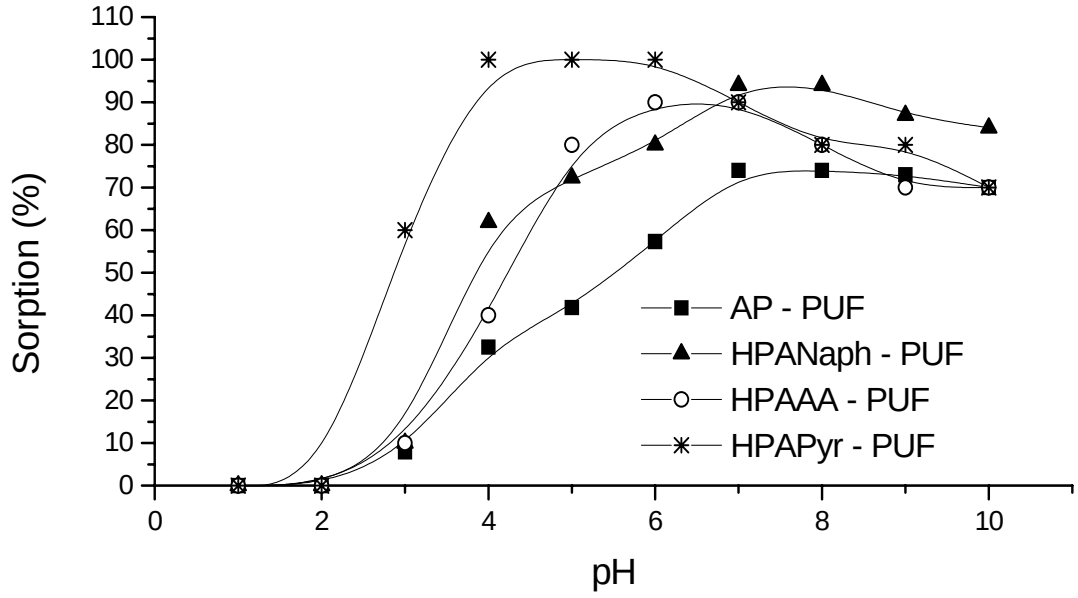


Fig. 5.4: Effect of pH on the extraction of Cd (II) with different modified PUF sorbents

5.1.4.2 Sorption Kinetics

The rate of loading of Cu, Zn, Pb, Cd and Ni on the functionalized PUFs was investigated. The sorption kinetics of the elements is shown in Figs 5.5 and 5.6 in case of Zn (II) and Ni (II) respectively. It was observed that, the shaking time necessary to attain equilibrium state are for Cu (II): 25 min (AP-PUF), 10 min (HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF); for Zn (II): 30 min (AP-PUF), 15 min (HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF); for Pb (II): 25 min (AP-PUF), 15 min (HPANaph-PUF), 20 min (HPAAA-PUF and HPAPyr-PUF); for Cd (II): 25 min (AP-PUF), 15 min (HPANaph-PUF), 10 min (HPAAA-PUF and HPAPyr-PUF); for Ni (II): 30 min (AP-PUF), 20 min (HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF). Stirring of about 5-10 min was required for 95-100 % uptake of Cd, and 10 min was required for 93% sorption of Pb by HPAPyr-PUF, 15-20 min was required for 93-99 % uptake of Ni and Cu by HPANaph-PUF. On the other hand, 30 min is required for 75, 96, 87 and 81 % sorption of Zn with AP-PUF, HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF respectively. This low uptake of Zn ions on the modified foams reflects the low stability of Zn – foam complexes because the nearly filled d-orbital of Zn ion which reflect its lower affinity to the three PUFs. The faster rate of extraction of Cu, Pb, Cd and Ni ions with these sorbents may reflect a better accessibility of the studied metal ions to the chelating sites (two OH and -N=N- groups), and therefore strong bond formation with the immobilized ligands. Generally, the AP-PUF requires longer shaking time with all metal ions than the HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF to attain the equilibrium state. This investigates the accessibility of the latter three sorbents in column technique where fast chelation is a pronounced factor.

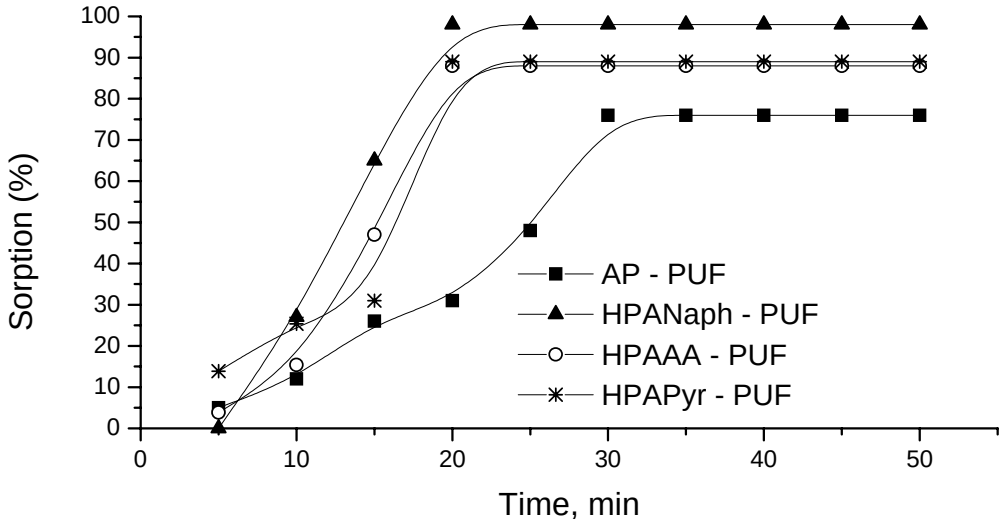


Fig. 5.5: Rate of sorption of Zn (II) with modified PUF sorbents

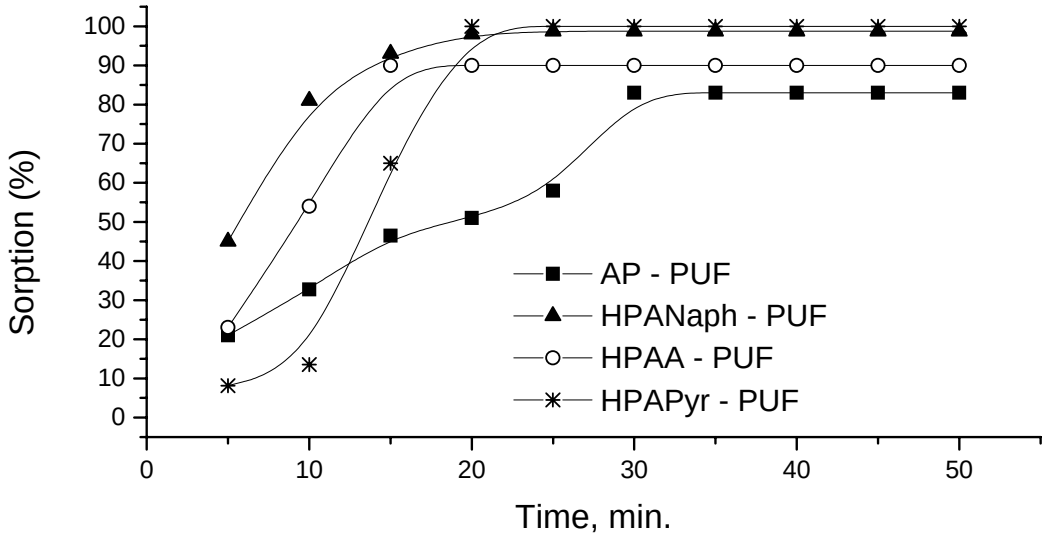


Fig. 5.6 : Rate of sorption of Ni (II) with modified PUF sorbents

The loading half time $t_{1/2}$, defined as the time required for reaching 50% of the total uptake, was estimated from the data in Figs. 5.5 and 5.6. Lagragren plot is a very useful mathematical method to calculate the half-life time ($t_{1/2}$) for adsorption process that proceed via first-order type. Thus, if q_e and q_t are the amount of metal ion (µg g^{-1}) sorbed by 100 mg PUF after reaching the equlibrium and at any time t (min) respectively. The equation reported [172] is: $\log (q_e - q_t) = \log q_e - Kt/2.303$, where K is the rate constant (min^{-1}). Since linear plott obtained, we could calculate, from the slope the values of the first order overall rate constant and the $t_{1/2}$ which can be calculated from the relation $t_{1/2} = 0.693/K$. Figures 5.7 and 5.8 depict the plotting of Lagragren relation in case of Cu (II) and Ni (II) and the values for K and $t_{1/2}$ which are recorded in Table 5.3. To summerize, the $t_{1/2}$ values for HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF are very small compared to those with AP –PUF (for Ni (II) with HPAPyr –PUF is one-fourth less than that of AP –PUF) and it is varying between 1.3 to 14.6 min.

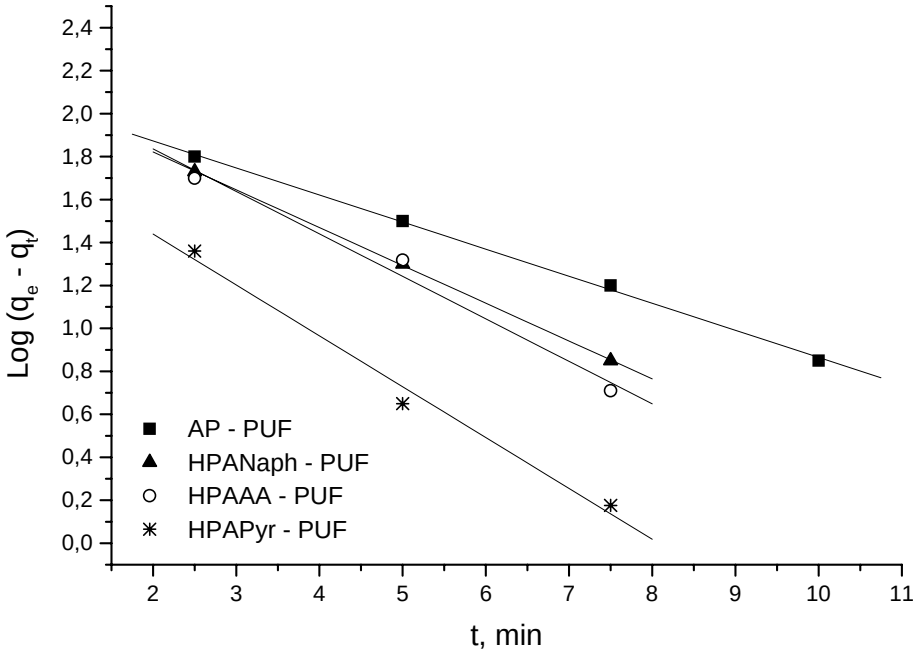


Fig. 5.7: Lagragren plottings for Cu(II) with modified PUF sorbents

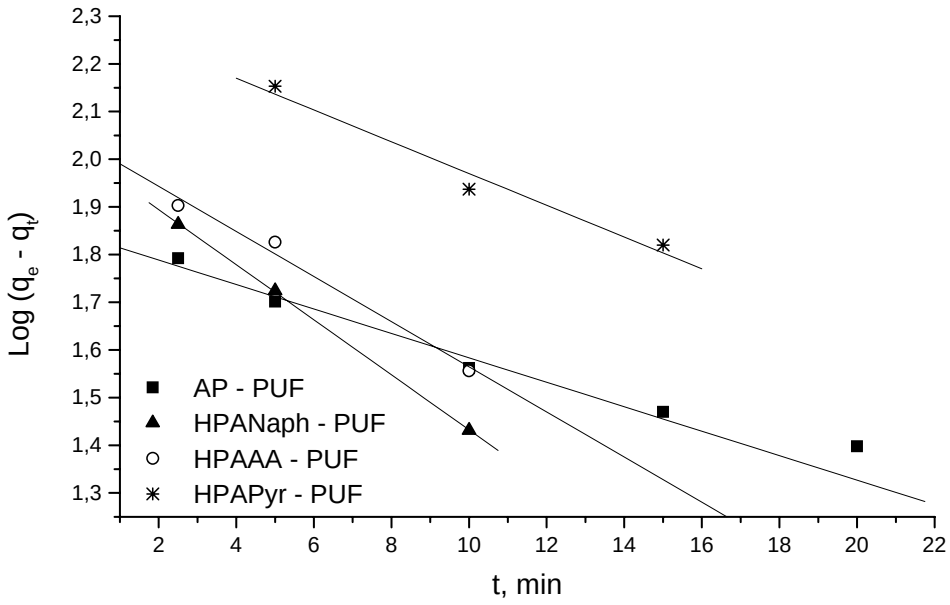


Fig. 5.8: Lagragren plotting for Ni (II) with modified PUF sorbents

Table 5.3: Kinetic data from Lagregren plotting with modified PUF sorbents at 25 °C.

PUF type	Parameter	Cu (II)	Zn (II)	Pb (II)	Cd (II)	Ni (II)
AP – PUF	K, min^{-1}	0.063	0.032	0.055	0.049	0.043
	$t_{1/5,}$ min	11.0	21.6	12.5	14.1	16.1
HPANaph-PUF	K, min^{-1}	0.408	0.099	0.506	0.334	0.133
	$t_{1/5,}$ min	1.7	7.0	1.4	2.1	5.2
HPAAA-PUF	K, min^{-1}	0.455	0.048	0.348	0.109	0.094
	$t_{1/5,}$ min	1.5	14.6	2.0	6.3	7.4
HPAPyr-PUF	K, min^{-1}	0.545	0.053	0.208	0.144	0.056
	$t_{1/5,}$ min	1.3	13.0	3.3	4.8	12.4

5.1.4.3 Capacity of the sorbents

An aliquot of 20 ml solutions containing excess concentration of metal ion were adjusted to the optimum pH and shaken for 1.0h with 100 mg modified PUF sorbents at room temperature. The amount of extracted amount was eluted with 0.1 mol l^{-1} nitric acid and the metal ion was determined by the recommended method. The total sorption capacity Q ($\mu g\ g^{-1}$) per gram of PUF was calculated from the equation: $Q = [(Co - C)xV] / m$, where V the sample volume in liter and m is the weight of PUF in grams. The calculated capacities for three replicate measurements for each metal ion at saturation of the PUF sorbents are listed in Table 5.4. The results show that, for all metal ions the two times functionalized PUF sorbents have higher capacities than AP - PUF. Moreover, the HPANaph-PUF has the greatest capacity for the majority of metal ions. This may be attributed to the fact that, coupling of diazonium ions to phenolic compound (2-Naphthol) is much easier than to active methylene group (acetylacetone or Pyrazolone) which in turn will affect the ultimate number of chelating sites. Naphthols couple considerably more readily than reactive methylene compounds which behave similarly to hydroxybenzene derivatives. Also, the existence of negative substituents, such as carbonyl group, retard the reaction when present in the acetyl acetone or pyrazolon coupling component [173].

Table 5.4: Total capacity of Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) with PUF sorbents

	Capacity (Mean ±S.D, n =4) mg g^{-1}				
PUF sorbent	Cu (II)	Zn (II)	Pb (II)	Cd (II)	Ni (II)
AP- PUF	0.75±0.02	0.22±0.06	0.72±0.08	0.30± 0.11	1.08±0.18
HPANaph-PUF	1.18± 0.03	0.56 ±0.06	1.26±0.16	0.45±0.09	1.24±0.07
HPAAA - PUF	1.05±0.04	0.56 ±0.10	1.25±0.03	0.84±0.05	1.47±0.21
HPAPyr - PUF	1.12±0.36	0.68 ±0.04	1.20±0.06	0.80 ±0.14	1.59±0.18

5.1.4.4 Precision of the method

In order to determine the precision of the applied procedure and its validity for applications in the analysis of real samples, the reproducibility test was examined. Similar ten samples of 20 ml volume and concentration of 5 µg ml^{-1} each element were stirred with 0.10 g foam sorbent for 1.0h. The uptake capacities were determined by the appropriate method of determination. From the data obtained in Figs (5.9 & 5.10), it was found that the relative error, expressed as RSD% doesn't exceed 1.5 % which confirm the possibility of using this method for determination of these metal ions in natural samples. Moreover, the results obtained from the sorbent HPANaph-PUF have less relative error compared to the AP – PUF. The precision of Methods based on the HPAAA – PUF and HPAPyr – PUF are depicted in Table 5.9.

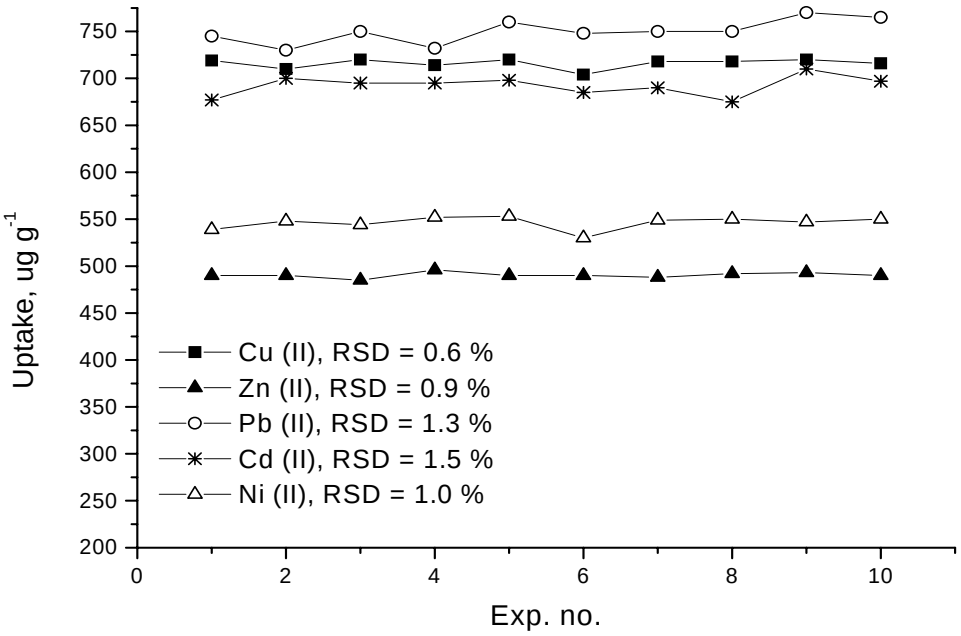


Fig 5.9: Precision of the sorption capacity of AP – PUF sorbent with Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II)

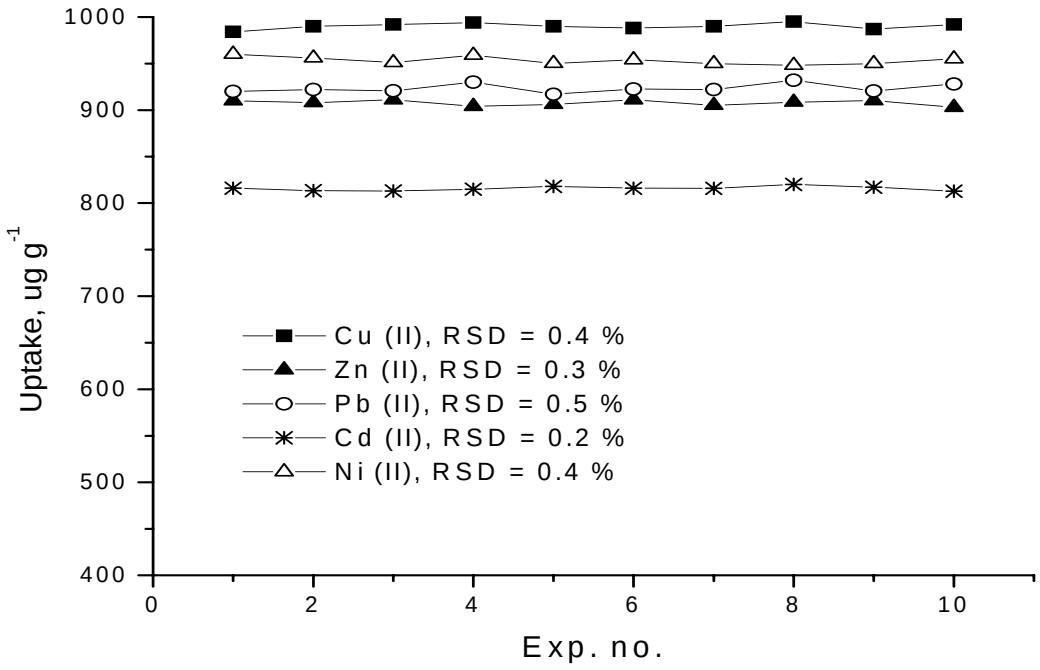


Fig 5.10: Precision of the sorption capacity of HPANaph-PUF with Cu (II),Zn (II), Pb (II), Cd (II) and Ni (II).

5.1.4.5 Durability of the Sorbents

The reusability of the PUF sorbents was tested by loading Cu, Cd, Zn, Cd or Ni several times from 20 ml sample solutions having a concentration of 10µg ml^{-1}. After this, desorption of the sorbed metal ions have been done by shaking the sorbent with 20 ml 0.1mol l^{-1} HNO_3 solution. The recovered metal ion was determined by the recommended procedure and the experiment was repeated 12 cycles on the same sorbent. It was found that, the exchange capacity of the material after 12 cycles of sorption and desorption does not vary more than 2.2%, 2.3%, 2.5% and 3.1% with AP-PUF- PUF, HPANaph- PUF, HPAAA-PUF and HPAPyr- PUF respectively (as depicted in Figs 5.11 & 5.12). Therefore, repeated use of the BPUF is feasible. Also, the uptake capacity of the PUF sorbents that were stored for more than 17 months in closed dark bottles under ambient conditions has been found to be practically unchanged.

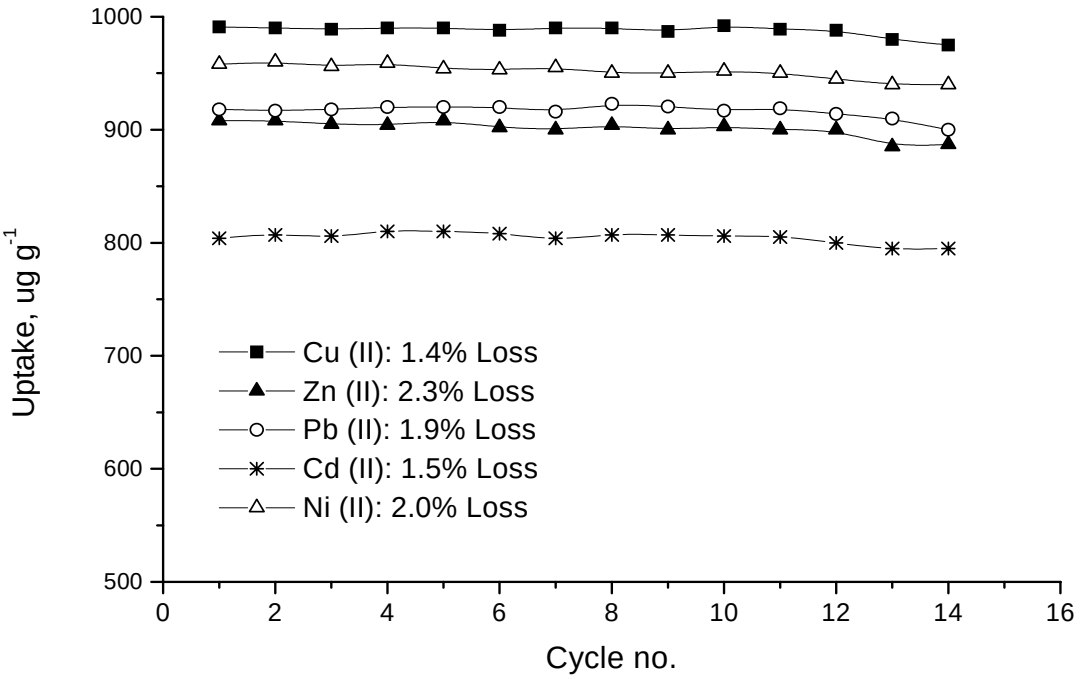


Fig 5.11: Reusability of HPANaph-PUF with Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II)

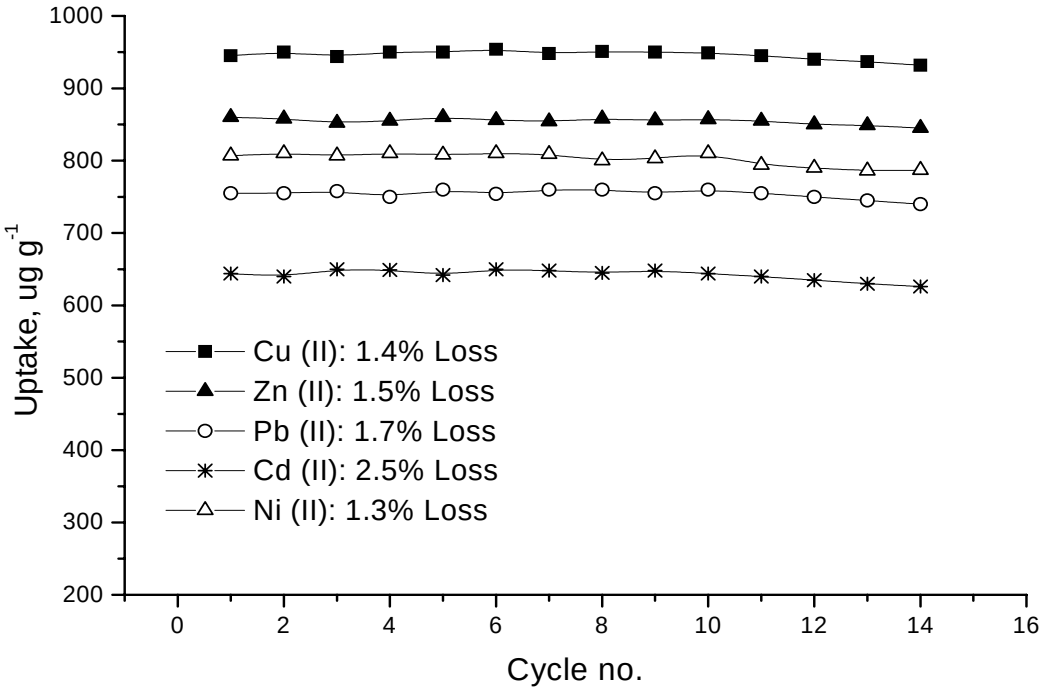


Fig 5.12: Reusability of HPAAA - PUF with Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II)

5.1.4.6 Interference Study

The interference effect is one of the major problems that influence atomic spectroscopic technique in the determination of the metal ions at trace levels. The matrix effect in complex samples such as biological materials or highly saline samples such as sea water is a pronounced factor that strongly influences the analyte determination. The influence of foreign ions that might interfere with the sorption of metal ions under investigation by PUFs was studied in order to identify the potential interference of these ions. The ions Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Fe^{+++}, Mn^{++}, Cl^{-}, Br^{-}, I^{-}, SO_4^{--}, NO_3^{-}, PO_4^{---}, citrate and glycinate ions (added in mg l^{-1} according to [174]) were selected as diverse substances since they may coexist, compete or mask the analyte from the sorbent. These ions (Ca and Mg) might be present in real water samples at higher concentration and because of some ions capacity to form chelates with modified foams under the same conditions. For this purpose, solutions of 20 ml containing 1µg ml^{-1}adjusted at pH 7.0 and varying amounts of interfering ion was added and stirred for 1.0 h with 100 mg sorbent and the percentage sorption. Level of tolerated concentration, defined as maximum concentration of the interfering substance found to cause change in signal ≥ 5% compared to the signal of metal ion alone (*underlined*). The results reveal that most of the ions normally present in natural water don't interfere under the experimental conditions. Also, it is obvious that the recovery of metal ions by using AP –PUF or HPAAA –PUF are more affected by the added foreign ions than in case of HPANaph–PUF or HPAPyr–PUF (Tables 5.5-5.8). This effect may occurs because the new chelating sites is more selective for the studied metal ions and interfering chelates in case of Ca and Mg, Mn and Fe or the stability of the formed complexes with sorbents is high enough so that it could not be affected by interfering ligands such as glycinate or citrate. Moreover, it is clear from the data that, the proposed sorbents could be applied for the preconcentration and separation of these elements from highly saline water samples. Obviously, this sorbents feature has enabled successful separation of these trace metal ions from different complex matrices such as seawater natural water and biological samples with quantitative recovery. Finally, this data indicate more selectivity of these materials since glycine or citrate can be used as masking agents and no meaningful change in the uptake in case of the four modified foams are more selective for the studied metal ions.

Table 5.5: Interference effect of Cu, Zn, Pb, Cd and Ni with AP –PUF

Ion	*Added as*	*Concentration (mg l^{-1})*	*Recovery (%)*				
			Cu (II)	*Zn (II)*	*Pb (II)*	*Cd (II)*	*Ni (II)*
-	-	-	89.0	76.0	88.0	74.0	83.0
Na^{+}	NaCl	15000	80.3	70.0	75.9	70.3	80.7
K^{+}	KCl	1000	80.3	69.8	79.8	72.0	81.0
Ca^{++}	$Ca(NO_3)_2$	50	86.5	75.7	88.7	73.3	83.3
Mg^{++}	$Mg(NO_3)_2$	50	84.4	75.9	88.0	72.8	82.7
Fe^{+++}	$FeCl_3$	0.30	84.8	76.2	86.9	73.1	83.7
Mn^{++}	$MnCl_2$	0.10	85.0	76.0	87.3	74.0	82.4
Cl^{-}	NH_4Cl	1500	81.0	71.6	76.3	71.3	81.0
Br^{-}	NaBr	100	79.7	74.5	66.7	71.0	80.0
I^{-}	NaI	100	82.2	75.3	81.2	71.0	78.0
SO_4^{--}	Na_2SO_4	500	77.8	72.4	86.0	73.3	82.8
NO_3^{-}	$NaNO_3$	1000	85.8	71.6	86.4	74.0	82.1
PO_4^{---}	Na_3PO_4	150	74.6	72.4	84.0	68.4	81.3
Citrate	Na-Citrate	100	80.5	74.9	83.3	70.2	82.5
Glycinate	Glycine	100	81.7	74.0	84.0	69.0	80.6

Table 5.6: Interference effect of Cu, Zn, Pb, Cd and Ni with HPANaph – PUF

Ion	*Added as*	*Concentration (mgl^{-1})*	*Recovery (%)*				
			Cu (II)	*Zn (II)*	*Pb (II)*	*Cd (II)*	*Ni (II)*
-	-	-	100.0	98.0	95.1	94.0	98.0
Na^{+}	NaCl	15000	96.8	94.5	90.0	97.7	98.0
K^{+}	KCl	1000	97.0	97.6	94.8	95.0	96.0
Ca^{++}	$Ca(NO_3)_2$	50	97.5	96.7	98.8	94.4	97.4
Mg^{++}	$Mg(NO_3)_2$	50	95.8	96.2	96.7	98.1	95.0
Fe^{+++}	$FeCl_3$	0.30	96.0	97.3	92.0	98.1	96.5
Mn^{++}	$MnCl_2$	0.10	95.8	95.0	93.9	99.2	94.1
Cl^{-}	NH_4Cl	1500	98.0	100.0	94.8	96.1	100.0
Br^{-}	NaBr	100	95.8	98.8	92.0	87.0	98.7
I^{-}	NaI	100	95.8	97.0	91.8	87.0	98.2
SO_4^{--}	Na_2SO_4	500	97.2	87.6	95.0	90.0	95.0
NO_3^{-}	$NaNO_3$	1000	100.0	97.1	95.0	97.1	98.2
PO_4^{---}	Na_3PO_4	150	95.0	96.4	94.7	98.0	96.0
Citrate	Na-Citrate	100	96.7	94.0	94.4	97.0	99.0
Glycinate	Glycine	100	92.5	93.8	91.6	94.1	93.5

Table 5.7: Interference effect of Cu, Zn, Pb, Cd and Ni with HPAAA –PUF

Ion	*Added as*	*Concentration (mgl^{-1})*	*Recovery (%)*				
			Cu (II)	*Zn (II)*	*Pb (II)*	*Cd (II)*	*Ni (II)*
-	-	-	95.3	87.0	98.0	90.0	100.0
Na^{+}	NaCl	15000	95.4	80.7	88.9	95.0	98.5
K^{+}	KCl	1000	92.0	86.0	94.0	92.6	95.5
Ca^{++}	$Ca(NO_3)_2$	50	97.2	81.1	95.7	93.3	96.2
Mg^{++}	$Mg(NO_3)_2$	50	95.3	84.6	100.0	96.3	95.0
Fe^{+++}	$FeCl_3$	0.30	95.0	88.2	91.0	98.4	97.6
Mn^{++}	$MnCl_2$	0.10	93.2	95.4	97.0	94.6	95.0
Cl^{-}	NH_4Cl	1500	95.0	82.3	86.4	97.0	98.0
Br^{-}	NaBr	100	83.3	80.7	88.9	90.0	91.2
I^{-}	NaI	100	83.3	81.0	90.0	86.1	87.3
SO_4^{--}	Na_2SO_4	500	91.7	80.7	86.2	90.0	93.7
NO_3^{-}	$NaNO_3$	1000	93.0	81.6	97.2	90.0	94.0
PO_4^{---}	Na_3PO_4	150	92.7	83.0	94.6	92.0	91.3
Citrate	Na-Citrate	100	83.0	92.3	84.0	100.0	87.3
Glycinate	Glycine	100	90.5	92.9	95.0	91.6	95.3

Table 5.8: Interference effect of Cu, Zn, Pb, Cd and Ni with HPAPyr –PUF

Ion	*Added as*	*Concentration (mgl^{-1})*	*Recovery (%)*				
			Cu (II)	*Zn (II)*	*Pb (II)*	*Cd (II)*	*Ni (II)*
-	-	-	98.0	89.0	93.3	100.0	96.0
Na^{+}	NaCl	15000	96.1	84.5	94.7	90.5	98.1
K^{+}	KCl	1000	93.4	90.0	99.5	98.0	96.1
Ca^{++}	$Ca(NO_3)_2$	50	98.3	88.7	97.8	96.3	100.0
Mg^{++}	$Mg(NO_3)_2$	50	97.0	90.3	100.0	100.0	98.2
Fe^{+++}	$FeCl_3$	0.30	95.0	88.1	96.2	98.0	96.4
Mn^{++}	$MnCl_2$	0.10	96.4	91.0	95.7	99.3	100.0
Cl^{-}	NH_4Cl	1500	95.0	82.5	93.0	91.7	96.4
Br^{-}	NaBr	100	91.6	83.6	92.7	96.4	98.1
I^{-}	NaI	100	92.0	83.0	93.0	97.0	96.3
SO_4^{--}	Na_2SO_4	500	93.0	84.5	95.0	98.0	92.8
NO_3^{-}	$NaNO_3$	1000	96.0	84.6	97.4	100.0	99.0
PO_4^{---}	Na_3PO_4	150	91.0	80.4	93.0	94.8	95.1
Citrate	Na-Citrate	100	92.0	89.1	98.0	100.0	98.5
Glycinate	Glycine	100	93.7	90.8	86.1	93.3	95.9

Table 5.9: Optimum conditions for sorption of Cu, Zn, Pb, Cd and Ni by batch method

Sorbent	*Parameter*	*Metal ion*				
		Cu (II)	*Zn (II)*	*Pb (II)*	*Cd (II)*	*Ni (II)*
AP-PUF:	pH range	7-8	7-8	7-8	7-8	7-8
	$T_{0.5}$, min	12.0	15.0	7.0	12.0	10.0
	Capacity (mg g^{-1})	0.75	0.22	0.72	0.30	1.08
	Precision as RSD (%)[a]	0.6	0.9	1.3	1.5	1.0
HPANaph–PUF	pH range	6-8	7-8.5	6-8	7-8	7-8.5
	$T_{0.5}$, min	5.0	10.0	5.0	10.0	5.0
	Capacity (mg g^{-1})	1.18	0.56	1.26	0.45	1.24
	Precision as RSD (%)[a]	0.4	0.3	0.5	0.2	0.4
HPAAA-PUF:	pH range	6-7	6-8	5-7	6-7	6-9
	$T_{0.5}$, min	6.0	10.0	3.0	2.5	25.0
	Capacity (mg g^{-1})	1.05	0.56	1.25	0.84	1.47
	RSD (%)[a]	0.9	0.7	0.4	0.8	0.5
HPAPyr-PUF:	pH range	5-7	6-7.5	6-8	4-6	7-8.5
	$T_{0.5}$, min	2.5	25.0	3.5	2.0	30.0
	Capacity (mg g^{-1})	1.12	0.68	1.20	0.80	1.59
	Precision as RSD (%)[a]	0.5	0.8	0.6	0.7	0.3

(a) RSD = Relative standard deviation for ten replicates

5.1.5 Column Procedure

5.1.5.1 Breakthrough capacity

In dynamic mode, one of the most important parameters that determine the operational characteristics of an adsorbent is the column capacity which is proportional to the effective number of active sites per unit mass of the sorbent. Theoretically, the value of capacity depends upon the nature of the matrix and the properties of the incorporated ligand. Usually in the column mode, the operational capacity is lower than the expected value, and it relies on several parameters such as the column bed height, sample flow rate, temperature of the solution, particle size and concentration of the feeding solution. Besides, the 'breakthrough' of solution from the column defines a working capacity, which is lower than the total capacity. The working capacity corresponds to the maximum amount of analyte that is retained with minimum leakage of the element in the influent solution. Moreover, the volume of solution percolated from the breakthrough point to the point of leveling of the loading curve for a given solution flow rate also depends upon the kinetics of exchange [175]. The breakthrough capacities are more significant and useful than batch capacities in foam chromatographic applications. In the present investigations, the breakthrough studies were carried out by 1.0 g of sorbent each in a separate column, and then the column was washed with DDW and treated with separate solutions for each metal ion at concentration of 10 µg ml^{-1} and 3.0 ml min^{-1} flow rate after adjustment to pH 7.5. From the Figs (5.13 - 5.15), the saturation of all columns was achieved after passing of 35 – 100 ml of the feeding solution. The breakthrough capacity per one gram (µmol g^{-1}) of PUF sorbent was calculated according to the equation, *dynamic capacity* = $(V_{50\%} \,.\, C_o)/\, m$ [176], where $V_{50\%}$ is the effluent volume (ml) at 50% breakthrough, C_o is the concentration of the feed solution (µmol ml^{-1}), and *m* is the mass of adsorbent (PUF) in grams. Accordingly, the dynamic capacities (µmol g^{-1}) for Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) are estimated to be 4.3, 3.2, 0.9, 1.8 and 3.1 with AP –PUF; 11.9, 4.2, 2.8, 4.7 and 7.1 with HPANaph–PUF; 6.3, 6.6, 2.2, 2.9 and 4.3 with HPAAA–PUF; and 7.9, 8.5, 1.1, 3.0 and 5.9 with HPAPyr-PUF respectively. Finally, it is clear that the steep curves at breakthrough point for all metal ions suggest the ability of all modified foams for separation and preconcentration of these metal ions.

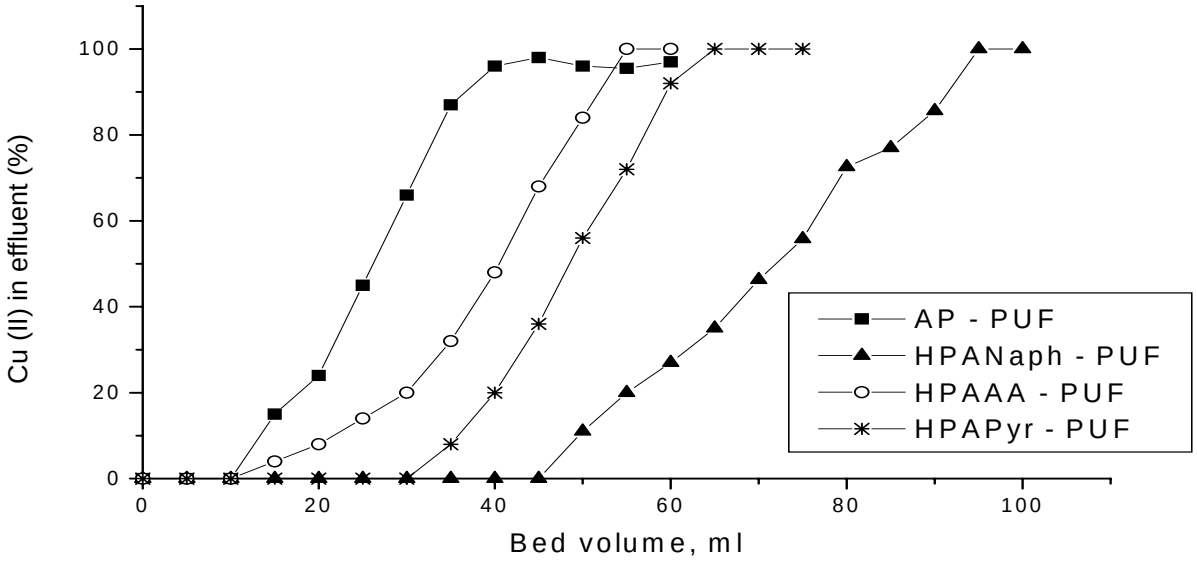


Fig. 5.13: The amount of Cu (II) released in the effluent versus the bed volume in the determination of the breakthrough capacity with modified PUF sorbents at 3 ml min^{-1} and 10 µg ml^{-1} feeding solution

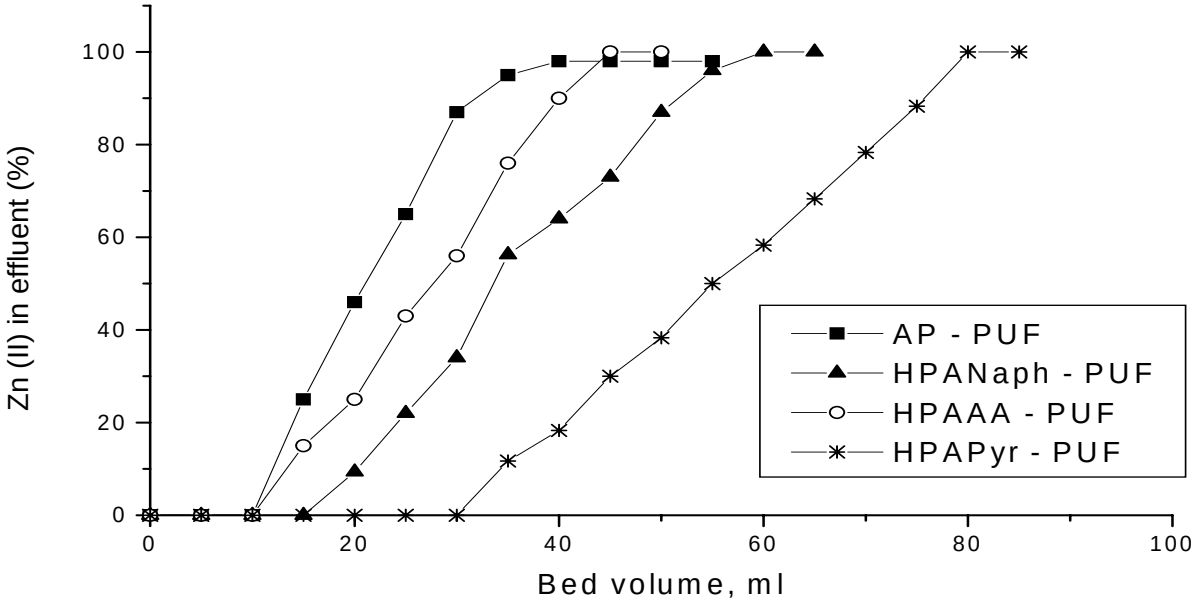


Fig 5.14: The amount of Zn (II) released in the effluent versus the bed volume in the determination of the breakthrough capacity with modified PUF sorbents at 3 ml min^{-1} and 10 µg ml^{-1} feeding solution

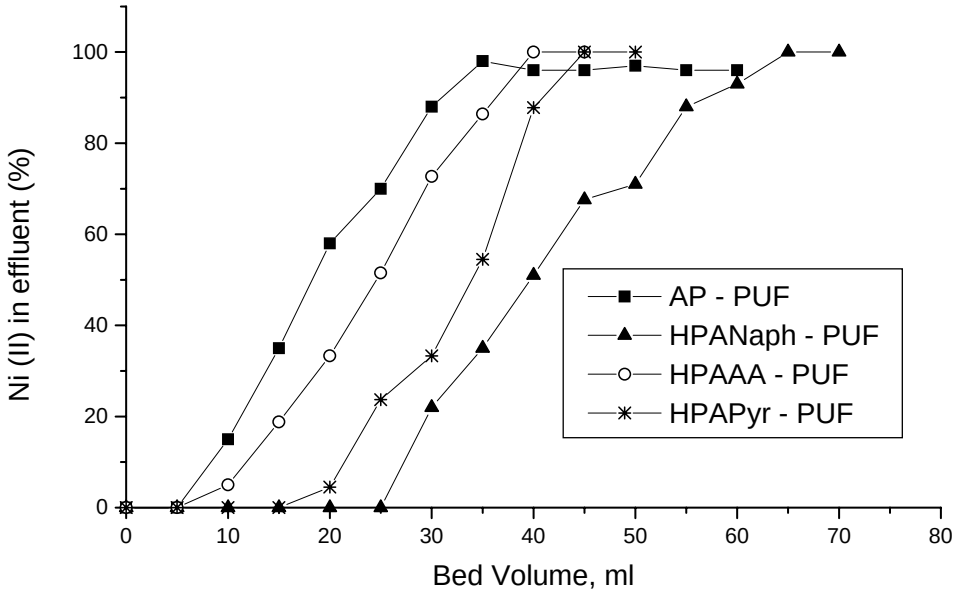


Fig 5.15: The amount of Ni (II) released in the effluent versus the bed volume in the determination of the breakthrough capacity with modified PUF sorbents at 3 ml min^{-1} and 10 µg ml^{-1} feeding solution

Worthmention, capacity in case of two times coupled PUFs exceeds that obtained for AP –PUF sorbent. Also, the HPANaph –PUF shows higher capacity than all the modified sorbents under investigation. Besides, estimation of the breakthrough capacity is very important in order to control the concentration of the sample to be analyzed and achievement of quantitative retention of the metal ion. Therefore, the point in the curve at which the analyte emerges was selected and utilized to calculate the breakthrough capacity. Table 5.10 depicts the estimated breakthrough and dynamic capacities. From these data we have concluded that the capacity is increasing in the order form AP –PUF. HPAAA –PUF, HPAPyr –PUF to HPANaph – PUF. This difference in capacity may be attributed to the variation in chemical stability between the metal ions and the ligand compound immobilized on the PUF and the difference in ionic sizes of the metal ions. Finally, the capacity of all sorbents under dynamic conditions is less than that one under static mode.

Table 5.10: Breakthrough and dynamic capacities of 10 µg ml^{-1} of Cu, Zn, Pb, Cd or Ni with1.0 g column of modified PUF at 3.0 ml min^{-1} flow rate.

Sorbent	*Breakthrough Capacity (µmol g^{-1})*					*Dynamic Capacity (µmol g^{-1})*				
	Cu(II)	Zn(II)	Pb(II)	Cd(II)	Ni(II)	Cu(II)	Zn(II)	Pb(II)	Cd(II)	Ni(II)
AP – PUF	1.9	1.5	0.5	0.9	0.9	4.3	3.2	0.9	1.8	3.1
HPANaph – PUF	7.1	1.5	1.0	1.8	4.3	11.9	4.2	2.8	4.7	7.1
HPAAA – PUF	1.6	2.3	0.7	1.3	0.8	6.3	6.6	2.2	2.9	4.3
HPAPyr – PUF	4.8	4.6	0.45	1.3	2.6	7.9	8.5	1.1	3.0	5.9

5.1.5.2 Effect of sample flow rate

The influence of the sample flow rate on the adsorption of metal ions onto PUF columns was investigated. Flow rates in the range 1 – 15 ml min^{-1} were examined for each metal ion separately in standard solution (1µg ml^{-1}) at optimized pH. Quantitative recoveries and desorptions of metal chelates on SPE are affected by two important factors that are the flow rate of sample and nature and concentration of the eluent [174]. The retention profiles of the elements to the modified sorbents at different sample flow rates in case of AP-PUF, HPANaph-PUF and HPAPyr-PUF are depicted in Figs 5.16-5.18. The retention profile of the elements with the HPAPyr – PUF sorbent is similar to the data in these figures. The results indicated that, the optimum flow rate for loading metal ions is 1- 5 ml min^{-1} with AP –PUF, 1 – 7 ml min^{-1} with HPANaph-PUF and HPAAA –PUF and 1 – 9 ml min^{-1} with HPAPyr –PUF. Slower flow rates than 3 ml min^{-1} was not recommended to avoid the longer analysis time. However, at flow rates greater than 5, 7, 7 and 9 ml min^{-1} with AP –PUF, HPANaph - PUF, HPAAA –PUF and HPAPyr –PUF respectively, a decrease was observed in the efficiency of retention onto the column, probably because the elements couldn't be significantly equilibrated with the chelating centers of the modified foam since it needs longer contact time to the sorbent. Furthermore, some metal ions might be retained at faster flow rates than another to the same sorbent. For example, Cu (II) can be quantitatively loaded onto AP –PUF up to flow rate of 11 ml min^{-1} while Cd (II) up to 5 ml min^{-1}. Also, Zn (II) could be quantitatively collected by the HPANaph –PUF and HPAAA – PUF columns up to 11 ml min^{-1} while Cd (II) up to 7.5 ml min^{-1} and Pb (II) could be percolated the HPAPyr –PUF column at 13.5 ml $min^{-1.}$ Hence, the 3 ml min^{-1} flow rate was chosen for further column experiments in order to ensure better retention along with relevant duration time. The results of influence of the flow rate is directly correlated to the kinetic data where the sorbents which withstand quantitative retention efficiency at higher flow rate such as HPANaph-PUF and HPAAA-PUF (≤7 ml/min) and HPAPyr (≤ 9 ml/min) have showed faster kinetics of sorption (greter rate constant) than AP-PUF (≤5 ml/min) as previously indicated in Table 5.3

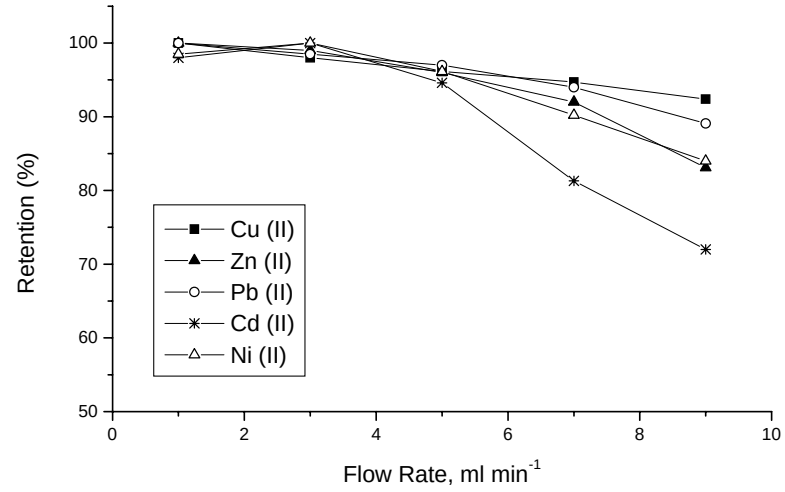


Fig 5.16: Effect of sample flow rate on the retention of Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) (50 ml, 1ug ml^{-1}) with 1.0 g AP-PUF column

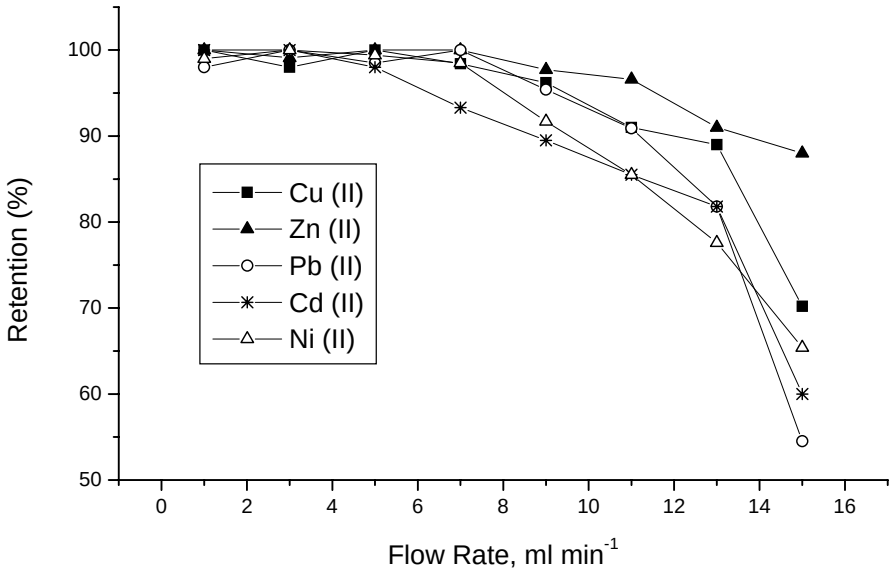


Fig 5.17: Effect of sample flow rate on the retention of Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) (50 ml, 1ug ml^{-1}) with 1.0 g HPANaph-PUF column

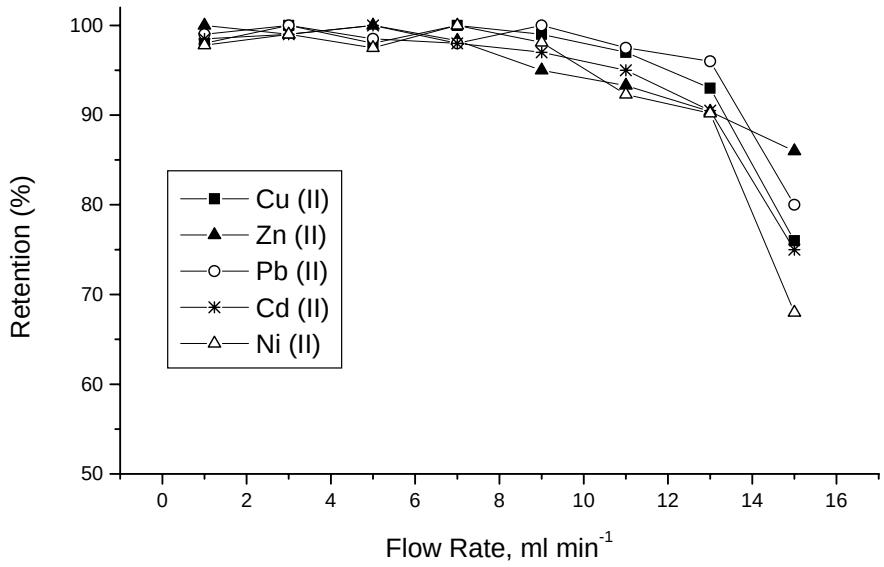


Fig 5.18: Effect of sample flow rate on the retention of Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) (50 ml, 1ug ml^{-1}) with 1.0 g HPAPyr-PUF column

5.1.5.3 Preconcentration and recovery

The column technique is a common procedure for preconcentration and separation of metal ions from large sample volumes. In this context, PUFs columns were applied to preconcentrate the elements from synthetic solutions. The limit of preconcentration was estimated using the recommended column procedure. This could be performed by increasing the volume of solution and the total amount of each metal ion added to the sample is kept constant at 25 µg. The results revealed that, quantitative recovery was achieved up to sample volume of 1000 ml with AP – PUF and 1500 ml with HPANaph-PUF and HPAPyr-PUF (except for Cd up to 1000 ml with HPAPyr-PUF) and up to 1000 ml with HPAAA – PUF. At higher volumes, the percentage recovery decreases abrubtly. Additionally, the volume of stripping solution (0.1 mol l^{-1} HNO_3) necessary to get 95 % recovery was examined to avoid dilution of the eluate and to obtain higher concentration factors (CF). It was found that, sorbed metal ions could be eluted by using 8.0, 5.0, 5.0 and 6.0 ml from AP-PUF, HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF columns respectively. Moreover, the eluent volume for desorption was estimated from the desorption

breakthrough. Figure 5.19 and 5.20 illustrates the elution profile incase of HPANaph-PUF and HPAAA – PUF sorbents. Similar results behavior was obtained in case of AP- PUF and HPAPyr – PUF sorbents. The eluent volumes at 95 % recovery was selected for further preconcentration steps. Accordingly, 8.0, 5.0, 5.0 and 6.0 ml from the eluent were used for desorption from AP-PUF, HPANaph-PUF, HPAAA-PUF and HPAPyr-PUF respectively. The CF was calculated from the ratio of initial (sample) to the final (eluent) volume and the results are recorded in Table 5.11. From these data it is clear that, the HPANaph-PUF has the highest concentration factor (up to 500), followed by HPAPyr-PUF (up to 375) (except for Cd with it is 167). The HPAAA-PUF has the lowest concentration factor (up to 285). This reflects the stronger binding of metal ions to HPANaph - PUF chelating sorbent. The CFs of all types of two times coupled foams are superior to that of the AP - PUF (125) which reveals weak chelation capacity than all twice coupled foams.

These results show that the tested metal ions can be concentrated effectively from large volumes of dilute aqueous solutions, and hence can be applied for further determinations at ppb level by using the four modified foams. Also, this confirms high selectivity of this analytical methodology for the collection of studied metal ions from large volumes. The quantitative recoveries of the present elements at lower concentration and their faster rate of sorption and desorption added to the good reusability and stability of the sorbents are distinct advantage. Moreover, the less eluent concentration required for desorption is (0.1 mol l^{-1} nitric acid solution with all types of these foams) at higher concentration factor obtained is a sorbent superior property.

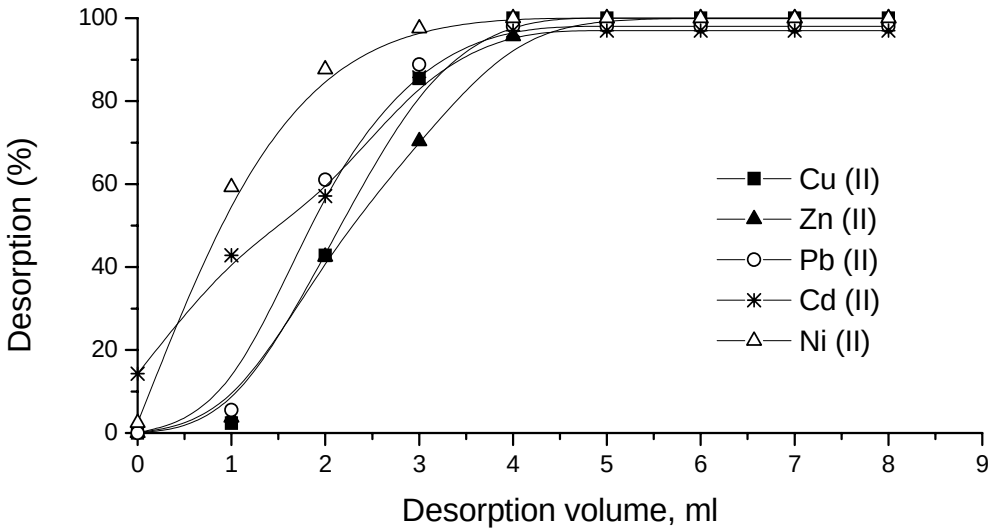


Fig. 5.19: Desorption of Cu, Zn, Pb, Cd and Ni from HPANaph- PUF column with 0.1 M HNO_3 at 3 ml min^{-1}

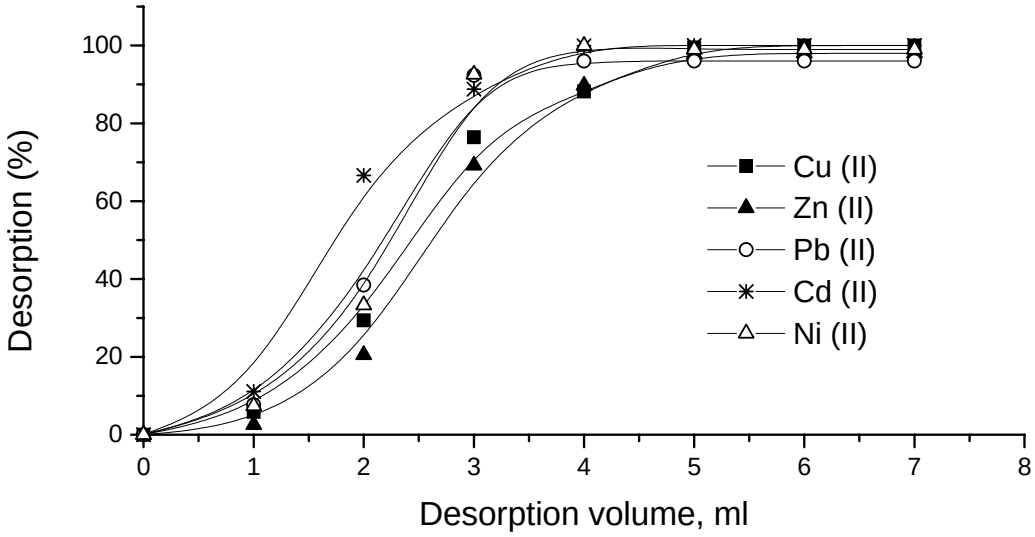


Fig. 5.20: Desorption of Cu, Zn, Pb, Cd and Ni from HPAAA- PUF column with 0.1 M HNO_3 at 3 ml min^{-1}

5.1.5.4 Analytical performance

The linear range of the proposed PUF sorbents was investigated for the metal ions in the concentration range 1 – 200 µg l^{-1} using the analytical column under the optimized conditions. The analytical curves shown in Figs 5.21 and 5.22 indicate the linear range that could be used for the method application in the real analysis. The LOD calculated from the slope of the analytical curve based on the three and ten times standard deviation of the blank measurements was achieved. The equation LOD = 3 S_b / k_m was applied to find out the LOD where: S_b is the standard deviation of the measurement of the blank solution and k_m is the slope of the analytical curve. Table 5.12 presents the analytical parameters of the proposed sorbents.

The values of LODs for all metal ions with all PUFs are relevant and lower than the concentration of the investigated elements in most of the natural samples. This enables the use of these materials in quantitative collection of these metal ions at trace concentration prior to their determination with higher accuracy. This is an advantage since the higher sensitivity is a crucial demand in any proposed analytical method.

Table 5.11: Recovery and preconcentration factor of 25 µg Zn (II), Pb (II), Cd (II), and Ni (II) with PUF sorbents.

Sorbent	Metal ion	Initial volume (ml)	Desorption volume (ml)	Recovery (%)	CF
AP-PUF					
	Cu (II)	1000	8.0	94	125
	Zn (II)	1000	8.0	96	125
	Pb (II)	1000	8.0	98	125
	Cd (II)	1000	8.0	99	125
	Ni (II)	1000	8.0	95	125
HPANaph-PUF					
	Cu (II)	1500	3.5	96	428
	Zn (II)	1500	4.0	97	375
	Pb (II)	1500	3.5	98	428
	Cd (II)	1500	4.0	99	375
	Ni (II)	1500	3.0	97	500
HPAAA -PUF					
	Cu (II)	1000	5.0	96	200
	Zn (II)	1000	4.5	98	222
	Pb (II)	1000	3.5	99	285
	Cd (II)	1000	3.5	97	285
	Ni (II)	1000	3.5	98	285
HPAPyr -PUF					
	Cu (II)	1500	5.0	95	300
	Zn (II)	1500	6.0	96	250
	Pb (II)	1000	5.0	96	200
	Cd (II)	1500	6.0	97	250
	Ni (II)	1500	4.0	98	375

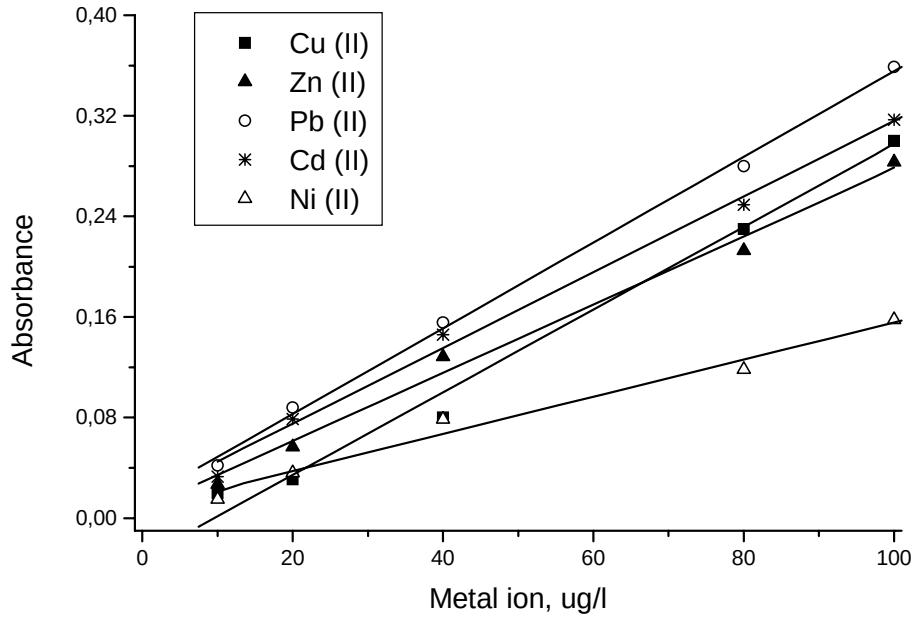


Fig. 5.21: Linear range of Cu, Zn, Pb, Cd and Ni with AP - PUF sorbent: 3 ml min^{-1} sample flow at pH 8 and 1 g foam column

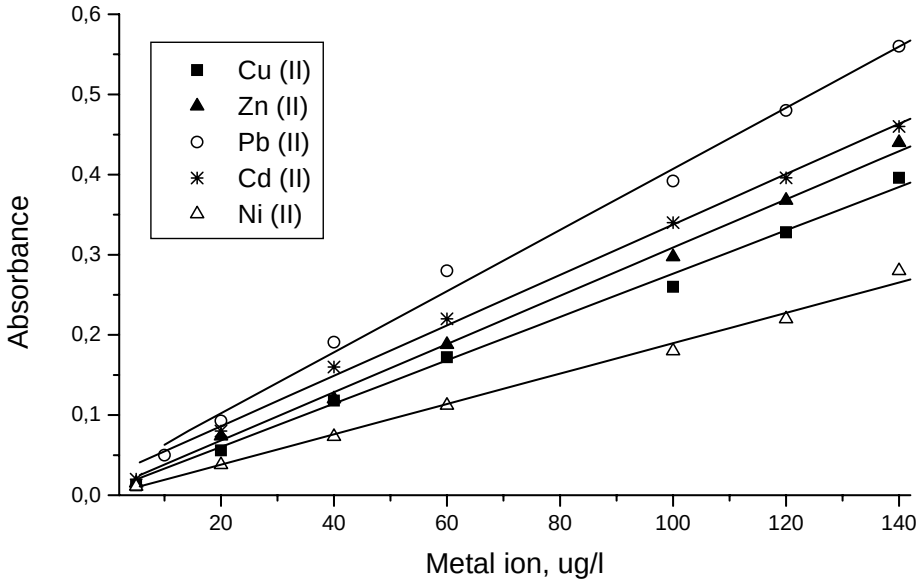


Fig. 5.22: Linear range of Cu, Zn, Pb, Cd and Ni with HPANaph - PUF sorbent:3 ml min^{-1} sample flow at pH 8 and 1.0 g foam column

Table 5.12: Analytical parameters of the 1.0 g modified PUF column for Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II).

Sorbent	Metal ion	Linear Equation	S_b (N=5, µg l^{-1})	LOD(µgl^{-1})	Linear range (µgl^{-1})
AP – PUF	Cu (II)	A = (0.002227) C – 0.00608, R = 0.99569	1.1E-3	1.5	5-60
	Zn (II)	A = (0.00271) C + 0.00718, R = 0.99641	1.3E-3	1.4	5-80
	Pb (II)	A = (0.003408) C + 0.00146, R = 0.99903	1.6 E-3	1.4	5-80
	Cd (II)	A = (0.003109) C + 0.00151, R = 0.99799	2.1 E-3	2.1	10-80
	Ni (II)	A = (0.001476) C + 0.00801, R = 0.99231	1.3 E-3	2.6	10-110
HPANaph –PUF	Cu (II)	A = (0.002702) C + 0.00614, R = 0.99806	1.1 E-3	1.2	5-140
	Zn (II)	A = (0.003009) C + 0.00812, R = 0.99875	0.6 E-3	0.6	5-140
	Pb (II)	A = (0.003811) C + 0.002591 R = 0.99746	4.0 E-3	3.1	10-140
	Cd (II)	A = (0.003145) C + 0.002317, R = 0.99902	1.2 E-3	1.0	5-140
	Ni (II)	A = (0.001893) C + 0.00026, R = 0.99643	0.9 E-3	1.4	5-140
HPAAA –PUF	Cu (II)	A = (0.003809) C + 0.00176, R = 0.99732	3.2 E-3	2.5	10-100
	Zn (II)	A = (0.003107) C + 0.00107, R = 0.99487	1.4 E-3	1.4	5-120
	Pb (II)	A = (0.003865) C - 0.00265, R = 0.99857	2.7 E-3	2.1	10-120
	Cd (II)	A = (0.003472) C - 0.00202, R = 0.99544	4.2 E-3	3.6	15-80
	Ni (II)	A = (0.002421) C + 0.00419, R = 0.99566	2.2 E-3	2.7	10-100
HPAPyr –PUF	Cu (II)	A = (0.002486) C + 0.00314, R = 0.98854	1.6 E-3	1.9	10-150
	Zn (II)	A = (0.003252) C + 0.0094, R = 0.99703	0.7 E-3	0.6	5-100
	Pb (II)	A = (0.004145) C - 0.00521, R = 0.99699	1.8 E-3	1.3	5-150
	Cd (II)	A = (0.003429) C + 0.00121, R = 0.99734	0.5 E-3	0.4	5-120
	Ni (II)	A = (0.001993) C + 0.0082, R = 0.99226	2.4 E-3	3.6	15-150

5.1.6 Analytical Applications

5.1.6.1 Analysis of certified sample

Certified reference material NIST – SRM 1577b (Bovine liver) was analysed by the proposed SPE method and the modified PUF sorbents. The obtained data are listed in Table 5.13. It is obvious from the data of recovery percentage and the RSD% for three measurements that, all of these sorbents are extensively efficient for analytical determination of these elements this biological sample which has complex matrix. The obtained recovery and RSD % values indicate that the recovery percentages vary from 89.0 to 104% and the RSD are in the range 1.3 to 8.2%. It is clear that the recovery is quantitative (≥ 95 %) with most measurements and RSD % is satisfactory (less than 10 %).

Table 5.13: Analysis of certified reference material NIST – SRM 1577b (Bovine liver) using PUF sorbents

Sorbent	Element	Certified (µg/g)	Found (µg/g)	Recovery (%)	RSD (%)
AP – PUF:					
	Cu	160.0	153.9	96	2.3
	Zn	127.0	115.5	91	6.8
	Pb	0.13	0.12	95	1.9
	Cd	0.50	0.44	89	5.0
HPANaph – PUF:					
	Cu	160.0	163.4	102	1.4
	Zn	127.0	124.1	98	3.0
	Pb	0.13	0.127	98	2.1
	Cd	0.50	0.52	104	3.7
HPAAA – PUF:					
	Cu	160.0	161.5	101	7.5
	Zn	127.0	118.8	93	2.8
	Pb	0.13	0.131	102	6.0
	Cd	0.50	0.48	96	6.4
HPAPyr – PUF:					
	Cu	160.0	155.1	97	1.3
	Zn	127.0	120.9	95	4.5
	Pb	0.13	0.124	96	8.2
	Cd	0.50	0.51	102	5.6

5.1.6.2 Analysis of tap water

In order to evaluate the applicability of the PUF sorbents in real analytical situations, it was utilized for analysis of tap water sample. The preconcentration step for the natural sample, to achieve sample concentration and matrix removal, was carried out prior to the determination by flame AAS. Accordingly, the elements, Cu (II), Zn (II), Pb (II), Cd (II) and Ni (II) in tap water (obtained from our research lab. in the Faculty of Science at Fayoum City, Egypt) were enriched and measured. The RSD % data reported in Table 5.14 were obtained for three replicate measurements. The relevant values for the RSD % (less than 10 %) indicate susceptible precision of the developed method. The overall consideration of the recovery data indicates that it is quantitative for all metal ions with all PUFs. The RSD % varies from 1.3 to 6.6 % which are satisfactory. The sensitivity of the method is also reasonably high. Finally, satisfactory results were obtained for the elements examined which confirm the selectivity and validity of the proposed method for the preconcentration of the investigated metal ions from drinking water. These results could be recognized as true values since the method is validated via analysis of the NIST – SRM 1577b certified material and the possible the interference effect in drinking water in section *4.1.4.6.*

Table 5.14: Analysis of tap water sample by PUF sorbents

Sorbent	Metal Ion	Found ($\mu g\ l^{-1}$)	RSD* (%)
AP- PUF	Cu	48.0	4.9
	Zn	130.1	7.0
	Pb	3.7	5.0
	Cd	5.3	3.3
	Ni	13.9	6.1
HPANaph-PUF	Cu	46.4	4.2
	Zn	123.8	1.4
	Pb	4.1	4.4
	Cd	5.2	3.4
	Ni	14.7	2.9
HPAAA-PUF	Cu	44.3	1.3
	Zn	139.2	1.8
	Pb	4.4	1.5
	Cd	4.8	5.1
	Ni	14.5	2.1
HPAPyr-PUF	Cu	47.2	4.8
	Zn	131.3	6.5
	Pb	4.0	5.5
	Cd	4.7	6.6
	Ni	15.9	3.7

** Measured by flame AAS: With three fold measurements (N = 3) by flame AAS.*

5.1.7 Comparison between the proposed sorbents

An overall consideration of the four new PUF materials (Table 5.15), we could conclude the following: The optimum pH range for maximum extraction is in the faint acidic to faint alkaline (i.e. neutral medium) which is an advantage where these sorbents are ready for application in natural water samples without addition of any acid or base The loading half – life time is short periode and is comparable in all sorbents (except Ni with HPAA -PUFand Ni and Zn with HPAPyr - PUF which are 25 and 30 min respectively). The HPANaph - PUF, HPAA - PUF and HPAPyr - PUF showed smaller $t_{1/2}$ values than AP –PUF with the latter elements. The saturation capacities for the two times coupled foams (HPANaph - PUF, HPAAA - PUF and HPAPyr - PUF) are larger than one time coupled foam (AP – PUF) with all studied elements. The maximum flow rate at quantitative retention is greater for most elements with HPANaph - PUF, HPAA - PUF and HPAPyr - PUF than AP – PUF. The flow rate reaches 15 ml min^{-1} with the former but it is 7 ml min^{-1} with the latter. The CF is a discriminating factor where it increases in the order AP - PUF, HPAAA - PUF and HPAPyr – PUF and HPANaph - PUF respectively. The maximum value is 500 with HPANaph –PUF and the minimum value is 125 with AP –PUF. From this overall comparison, we deduced that, the HPNaph –PUF is the best one of the four synthesized PUFs since it has the maximum CF, least interference effect by foreign ions and highest capacity with most elements.

Based on the above conclusion, we tried to confirm the validity of the HPANaph-PUF sorbent by extending its application to analysis of other two samples namely the synthetic seawater and apple leaves. In Tables 5.16 and 5.17 below, the results from the analysis of these two samples and the calculated RSD % from three times measurements showed satisfactory results. The sorbent showed good recovery percentage (96– 105 %) and RSD values (1.4 – 7.1%).

Table 5.15: Comparison between the developed PUF sorbents

Sorbent/Metal ion	pH range	Loading half time,t0.5	Total capacity ($\mu mol\ g^{-1}$)	Dynamic Capacity ($\mu mol\ g^{-1}$)	Sample flow rate, ml min^{-1}	CF
AP-PUF						
Cu	7-8	12.0	11.90	4.30	1-5	125
Zn	7-8	15.0	3.38	3.23	1-5	125
Pb	7-8	7.0	3.47	0.87	1-6	125
Cd	7-8	12.0	2.67	1.74	1-5	125
Ni	7-8	10.0	18.6	3.10	1-5	125
HPANaph-PUF						
Cu	6-8	5.0	18.7	11.9	1-9	428
Zn	7-8.5	10.0	8.6	4.15	1-11	375
Pb	6-8	5.0	6.1	2.80	1-9	428
Cd	7-8	10.0	4.02	4.73	1-7	375
Ni	7-8.5	5.0	21.3	7.07	1-8	500
HPAAA-PUF						
Cu	6-7	6.0	16.7	6.30	1-7	200
Zn	6-8	10.0	8.6	6.61	1-15	222
Pb	5-7	3.0	6.03	2.17	1-7	285
Cd	6-7	2.5	7.5	2.86	1-7	285
Ni	6-9	25.0	25.3	4.31	1-7	285
HPAPyr –PUF						
Cu	5-7	2.5	17.8	7.90	1-11	300
Zn	6-7.5	25.0	10.5	8.46	1-9	250
Pb	6-8	3.5	5.8	1.09	1-13	200
Cd	4-6	2.0	7.14	2.95	1-11	250
Ni	7-8.5	30.0	27.4	5.86	1-9	375

Table 5.16: Analysis of spiked synthetic seawater sample by HPANaph-PUF sorbent

Metal Ion	Added to seawater ($\mu g\ l^{-1}$)	Found ($\mu g\ l^{-1}$)	Recovery (%)	RSD* (%)
Cu	21.0	20.9	99	2.9
Zn	19.8	20.3	103	1.5
Pb	20.0	19.2	96	4.9
Cd	18.0	18.4	102	6.6
Ni	28.4	29.7	105	5.2

Table 5.17: Analysis of apple leaves by HPANaph-PUF sorbent.

Metal Ion	Found ($\mu g\ g^{-1}$ dry leaves)	RSD% (n=3)
Cu	21.4	1.4
Zn	8.4	2.8
Pb	51.7	2.1
Cd	7.0	7.1
Ni	81.0	5.7

5.1.8 Comparison with other sorbents

A comparison of the proposed procedure with other preconcentration system using several functionalized PUF sorbents is given in Table 5.18. The sorption capacity for all elements with the developed sorbents is lower that those values obtained with o-aminophenol functionalized amberlite XAD-2. However, we have achived higher CF. The silica based sorbent 1,8-DHA-SG enables much higher sorption capacity however the CF still comparable or less that HPANaph-PUF, HPAAA – PUF and HPAPyr- PUF sorbents. Also, the investigated sorbents have shown superiority in CF to some sorbents having comparable sorption capacity. For example, hydroxytoluene and hydroxyacetophenone functionalized PUF revealed CF lower than all developed sorbents.

Table 5.18: Comparison of sorption capacity and CF among different sorbents.

Sorbent	Capacity (μ mol g^{-1})					CF					Ref.
	Cu	Zn	Pb	Cd	Ni	Cu	Zn	Pb	Cd	Ni	
AP – PUF	11.9	3.4	3.5	2.7	18.6	125	125	125	125	125	This work
HPANaph – PUF	18.7	8.6	6.1	4.0	21.3	428	375	428	375	500	,,
HPAAA – PUF	16.7	8.6	6.0	7.5	25.3	200	222	285	285	285	,,
HPAPyr – PUF	17.8	10.5	5.8	7.1	27.4	300	250	212	250	375	,,
1-Naphthol – PUF	200.0	-	-	-	220.0	-	-	-	-	-	[148]
Oxine – PUF	-	270.0	-	160	-	-	50-	-	50	-	[177]
Hydroxytoluene-PUF	-	3.9	1.6	2.9	-	-	100	100	100	-	[150]
Hydroxyacetophenon	-	3.8	1.4	2.5	-	-	100	100	100	-	[150]
Pyrocatechol – PUF	29.6	-	-	-	24.7	42	-	-	-	54	[151]
1,8-DHA-SG	-	180.0	76.0	70.2	-	-	200	200	200	-	[178]
o-aminophenol-XAD-2	53.5	45.2	16.0	30.5	55.9	50	40	40	50	65	[179]

5.2 Preconcentration/Separation and Determination of β – Lactam Antibiotics

5.2.1 Why we Focus on BLAs?

Recent studies have shown that a multitude of drugs are present in aquatic systems. The interest in the analysis of antibiotic residues in the environment arises from the fact that they are suspected of being responsible for the appearance of bacterial strains that are resistant to antibiotics which are important drugs for the treatment of many serious infections [180].

BLAs is the oldest, most famous, most widely used group of antibiotics till now involves more than fourteen kinds of BLAs antibiotics. Since manufacture and consumption of pharmaceuticals continuously increases, antibiotics are used not only in medicine but also in the food industry for food preservation, processing, and transportation, right down to the illegal introduction of antibiotics, for example, into dairy products in order to decrease the total number of microbes. The main channels for contaminating food with antibiotics are the use of antibiotics as veterinary drugs and as fodder additives. In this context, stringent drug quality control is required, and the concentrations of drugs in human and animal biological fluids, food stuffs, pharmaceutical industry wastewater, etc., should be determined [181].

5.2.1.1 BLAs in the Environment

The administrated amount of the BLAs is not totally absorbed in the body and most of it remains unaltered. Unless removing or completely deactivated in the sewage treatment plant (STP), the antibiotic will be released to the surface water and take part in the topsoil in fields [105]. This coming of pollutants is uncontrolled since there is no STP in most hospitals and husbandry farms. The estimated concentrations of BLAs in wastewater in USA, including metabolism, show values of 16 µg/l amoxicillin, 12 µg/l for cephalexin and 2.8 µg/l for penicillin G. This estimation has only considered the antibiotics used in human medicine [182]. A study for the consumption of antibiotics in Germany in hospitals, households and their corresponding emissions into the effluents and municipal sewage indicated that β – lactam antibiotics represents 65 % (penicillins with 49 % and cephalosporins with 16 %) of the total amount of antibiotics used. In the emitted antibiotics in the sewage water system, BLAs compromise 72 % (penicillins with 53 % and cephalosporins with 19 %) of the total released amount [183].

5.2.1.2 Hazards of BLAs

Antibiotics are widely used at therapeutic levels, in humans as well as animals, for the treatment of bacterial infections. However, the use of subtherapeutic levels of antibiotics in food producing animals to increase bulk may lead to the emergence of antibiotic – resistant bacterial strains. In addition, antibiotics residues in milk or edible tissues are potential risk for individuals who are hypersensitive to antibiotics. In consequence, the dual use of antibiotics has become an increasingly important public health concern. Antibiotics approved for use in food – producing animals have established tolerance for detection of their residues in milk, kidney, muscle and liver [184]. Because of the allergic reaction due to the β - lactam antibiotic residues in milk, the molecules belonging to the group of β – lactams have the lowest tolerance in the EU among all the antimicrobials. The EU regulations 2377/90 set these maximum residue limit (MRL) for some BLAs in milk: penicillin G (4µg/l), ampicillin (4 µg/l), oxacillin (30 µg/l), cephalexin (100 µg/l), amoxicillin (4 µg/l) and cephapirin (60 µg/l) [185].

5.2.1.3 Determination of β-Lactam Antibiotics

High pressure liquid chromatography coupled with UV detector (HPLC – UV) is the technique usually adopted as a confirmatory method for antibiotic residues. This technique has some limitations: mainly it has low sensitivity and selectivity; therefore many purification steps are needed. Sometimes, in order to detect the analytes through a fluorescence detector, also a derivatisation step must be used to achieve higher sensitivity [186]. Moreover, the HPLC – UV confirmation, even using a diode array detector, is not completely reliable. In any case the method is quite time consuming and unsuitable to process a great number of samples. This could be one of the reasons of the lack of data in national and international literature about the residual molecules present in milk. Liquid chromatography coupled with mass spectrometry (HPLC – MS) could represent a highly sensitive and selective tool to detect antibiotic residue in milk without the need of many purification steps. There are already some methods to detect residues of β - lactam antibiotics in milk using HPLC – MS, but in many cases they are unsatisfactory because they are unable to reach the sensitivity required by the tolerance set by the EU Regulations 2377/90. Only recently some technical improvement of the HPLC – MS technique, in particular the interface, allowed some sensitive methods to be set up [187]. Table 5.19 summarizes some methods for the determination of the BLAs.

Table 5.19: Methods of determinations of β - lactam antibiotics (amoxicillin, ampicillin, cefaclor and cefotaxime):

BLAs	Sample	Analytical technique	LOD	Ref.
Amoxicillin	Pharmaceutical formulations	Fl	10mg/ml	[188]
Amoxicillin	-	Visible	3 mg/ml	[189]
Cefaclor	-	Visible	3 mg/ml	[189]
Ampicillin	-	Visible	3 mg/ml	[189]
14 βLA	Bovine muscles	HPLC-UV	0.04 mg/l	[190]
Amoxicillin	Drug	HPLC-UV	0.04 mg/ml	[191]
Cefaclor	Pharmaceutical sample	AAS/colorimetric	10 µg/ml	[192]
Cefotaxime	Pharmaceutical sample	AAS/colorimetric	5 µg/ml	[192]
Amoxicillin	Drug samples	Visible	5.0µg/ml	[193]
Cefotaxime	Human plasma	HPLC-UV	1 µg/ml	[194]
Amoxicillin	Pharmaceutical samples	Visible	1 µg/ml	[195]
Amoxicillin	Human plasma	HPLC-UV	1 µg/ml	[196]
Ampicillin	Pharmaceutical dosages	Visible	0.2 µg/ml	[197]
Amoxicillin	Bovine milk	HPLC-MS	100 µg/l	[198]
Ampicillin	Urban wastewater	HPLC-MS	10 ng/l	[199]
Cefaclor	Human serum	UV (Fl)	3.6 ng	[200]

5.2.1.4 SPE Procedures for Preconcentration of BLAs

The determination of BLAs by liquid chromatography suffers from many disadvantages such as the presence of complex matrices that might clog the HPLC column in addition to the need for measurement at lower detection limits using simple detection techniques. The combination of SPE methodology to simple measuring instruments like spectrophotometry has provided many researchers to gain more accurate and satisfactory results. Moreover, the uses of SPE systems are combined to more sophisticated analytical techniques such as LC with UV or mass spectrometric detector. Although the use of MS, and especially MS/MS, allows increasing the sensitivity, a pre-concentration step is still necessary to reach suitable LOD. Until now, the most common sample treatment applied to the determination of antibiotics in water has been off-line SPE in combination with LC–MS [201].

Direct sample injection may solve the problems of extensive sample handling, contamination and sample loss, complex and time-consuming steps due to treatments of the sample before injection into the chromatograph. With a column-switching technique, the manual sample preparation steps are drastically reduced, or even eliminated. In addition to being fast, efficient and easily automated, SPE is a clean analytical procedure. This technique is particularly attractive since it allows the simultaneous removal of matrix components and preconcentration of the analytes [202].

Another feasible alternative is to perform the SPE pre-concentration on-line mode. This has several advantages, as the reduction of sample manipulation, sample size and organic solvent consumption, as well as an easier automation that leads to an important reduction in the analysis time [203]. In chromatographic analysis of biological samples, sample preparation generally is the bottleneck: it often involves several manual steps and can easily become a time-consuming process. Many chromatographic methods used require extensive manual sample preparation. Since pharmacokinetic studies normally generate a large number of samples, the application of an automated sample-preparation method will be advantageous, because of an increased sample throughput. One of the most important goals of sample preparation is the removal of macromolecules, which makes the application of on-line analysis an interesting approach. This technique allows the automated removal of proteins from a variety of complex samples in a simple and flexible way [204].

5.2.2 PCTDD – PUF as Sorbent for BLAs

PUF is selected in this work because of its hydrodynamic and chemical properties. The material is highly porous, therefore, it permits working under higher flow rates without suffering from the backpressure problems within the analytical columns. Higher flow rate is strongly needed when using preconcentration systems because it provides higher concentration factors. In addition, there are several functional groups in this material that can be chemically anchored to several reagents. For example, it contains free amino, imino and hydroxyl groups. The only limitation for anchoring any ligand to PUF surface is that it must react to the PUF under mild conditions since PUF may decompose under severed conditions. Therefore, the intended ligand should be very reactive so that it will react to the available functional groups without decomposition of the PUF.

Accordingly, the polyelectrolyte copolymer PCTDD is recommended in this synthesis due to the presence of very reactive chlorine atoms which can undergo elimination reaction to the amino, imino and hydroxyl groups in PUF under mild conditions. The PCTDD was prepared by elimination reaction between N-Chloranil and tert-amine compound (N,N,N',N'– tetramethylethylenediamine) in dry toluene where the chloride ion eliminated and the lone pair of electrons on the nitrogen atom make the covalent bond with the carbon atom of the chloranil ring resulting in the formation of permanent positive charge on the nitrogen atom. Anchoring of PCTDD to the PUF was performed by stirring the copolymer with PUF plugs in toluene at 90 ^{o}C for 72 h. The cationic centers on the PCTDD have the capability to adsorb negatively charged carboxylate anions of the β - lactam antibiotics via the formation of ion – associates as depicted in Fig 5.23.

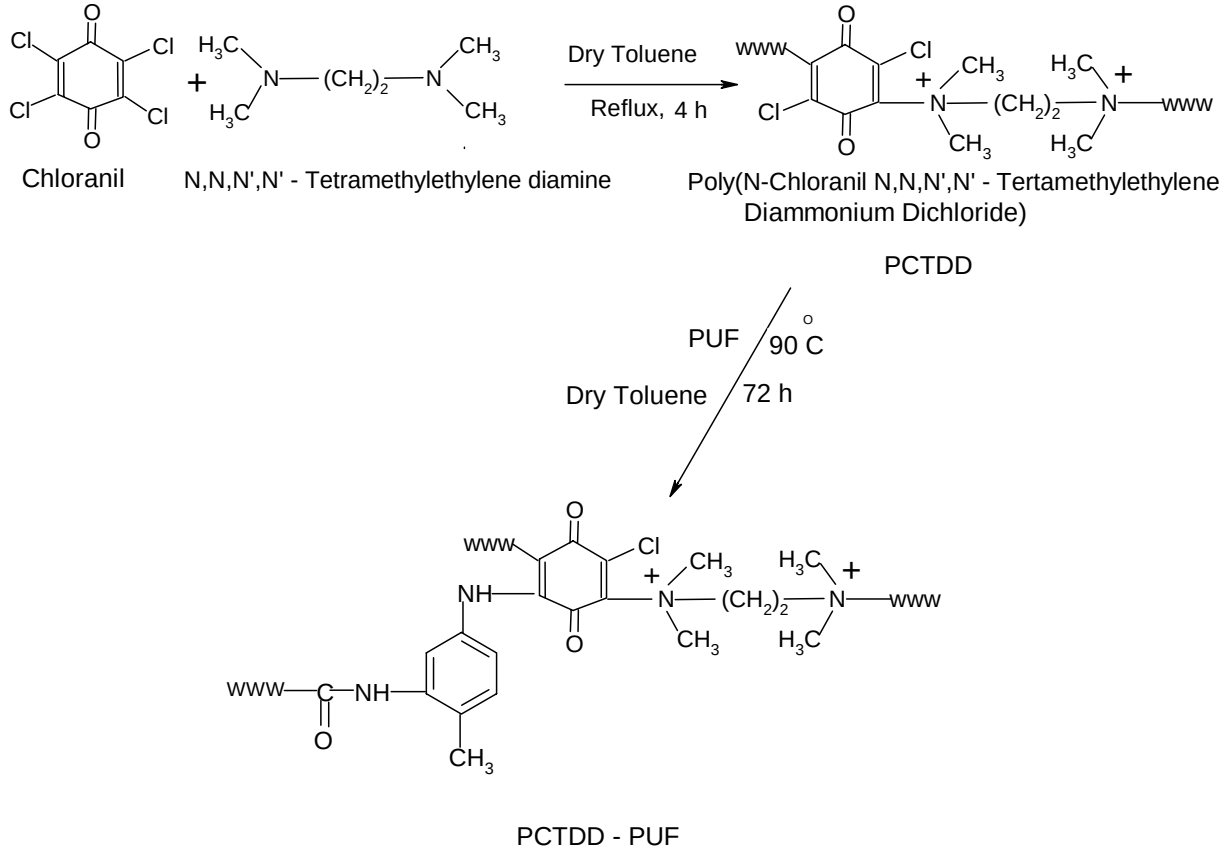


Fig. 5.23: Schematic diagram for the synthesis of PCTDD – PUF sorbent

5.2.3 Setup of the On-Line SPE System

A fully automated SPE flow system was made up of a peristaltic pump furnished with tygon tubes to deliver all solutions, the pump is provided with four-way valve and a minicolumn packed with polyurethane foam material functionalized with cationic polyelectrolyte copolymer (PCTDD – PUF). The foam column is coupled to a flow cell of UV-Visible spectrophotometer. The flow system (Fig. 5.24) was operated in a time-based mode and Millipore water served as the carrier stream. The minicolumn was fabricated by using polyethylene tube, 30 mm length and 3.0 mm i.d. Solid sorbent plugs (100 mg of PCTDD - PUF) was packed in the minicolumn with vacuum pump and compressed in order to avoid channel formation. The minicolumn was subsequently washed thoroughly by 0.5 M hydrochloric acid followed by Millipore water until the effluent is acid free.

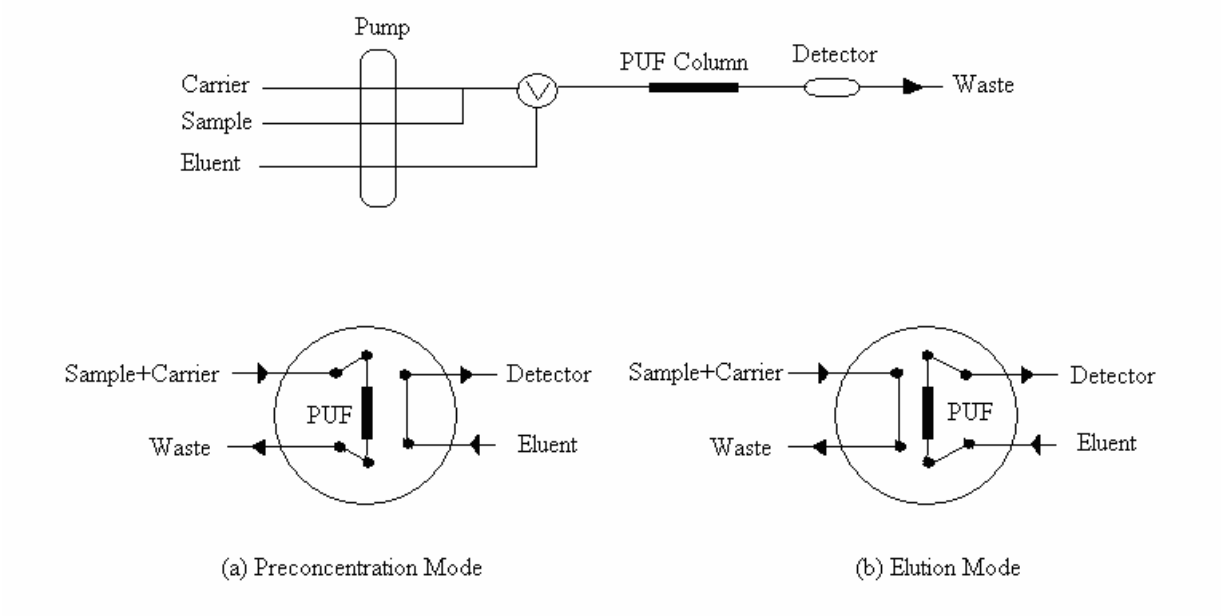


Fig. 5.24: Diagram for the setup of the on-line SPE preconcentration sytem.

5.2.4 UV Spectra of the Antibiotics

The UV spectra of the BLAs (cefaclor, amoxicillin, ampicillin and cefotaxime) are shown in Fig. 5.25. The four antibiotic compounds have reached the maximum absorption at wavelength close to 200 nm. But due to the hydrochloric acid also absorb at this value, the measurement wavelength was selected higher than 200 nm to avoid the noise from the acid.

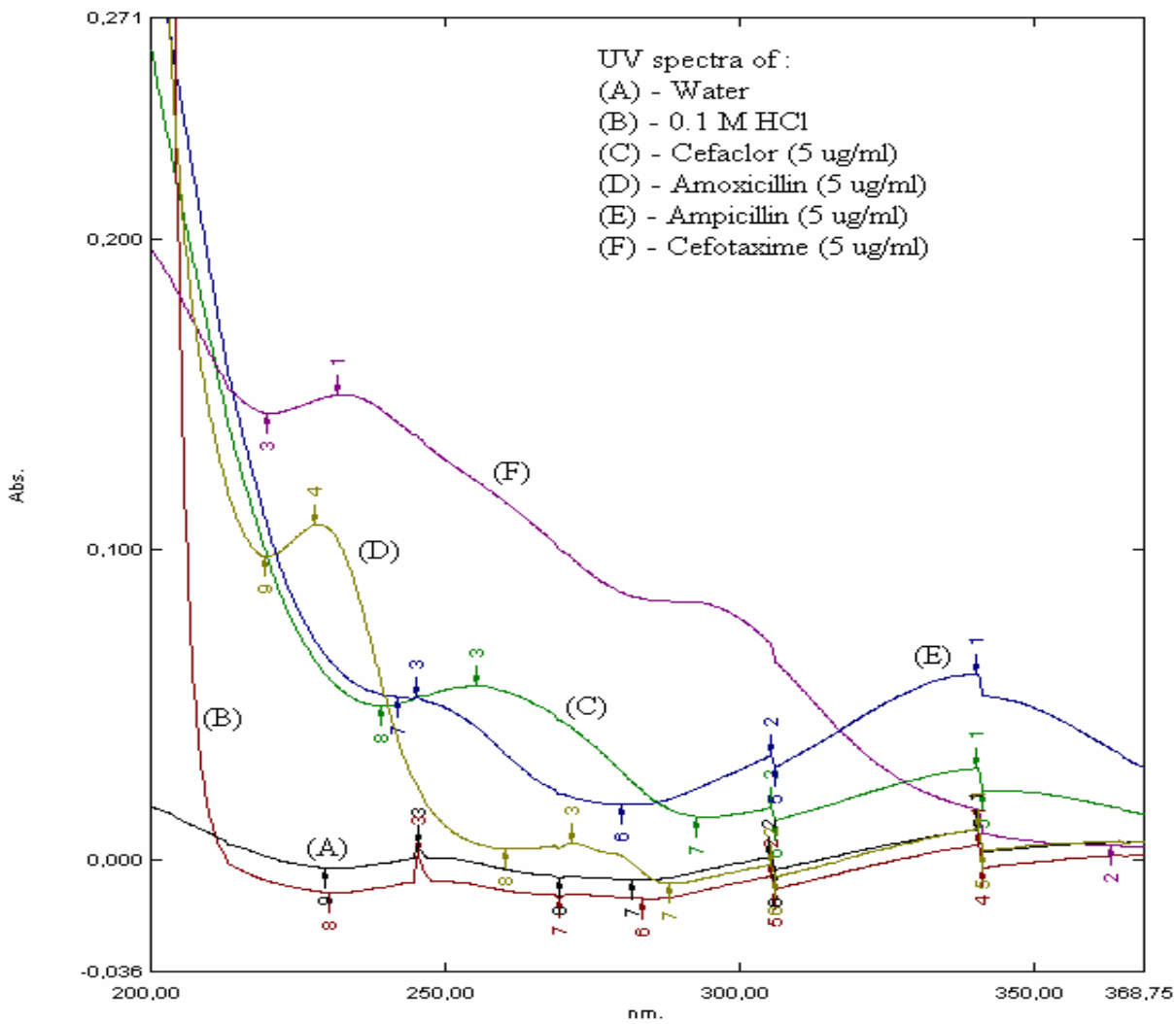


Fig. 5.25: Ultraviolet absorption spectra of the BLAs

5.2.5 Factors Affecting the Analytical Signal

In order to set the on – line SPE system at the optimum preconcentration conditions for the antibiotics under consideration, several parameters, which may affect the height of the analytical signal, have to be firstly optimized. These parameters include selection of the wavelength for measurement, signal to noise ratio, flow rate of the carrier solution, volume of the injected sample, and the concentration of the eluent (hydrochloric acid). All experiments were carried out without the connection of the PCTDD – PUF column into the system.

5.2.5.1 Signal to Noise Ratio

The effect of the blank signal on the analyte signal was studied in order to choose the wavelength for further measurements under the minimum signal interference conditions. Figures (5.26 & 5.27) indicate that by shifting the detection wavelength from 200 nm to higher values the blank signal extensively decrease and reach its minimum at 210 nm. Accordingly, the 210 nm was selected for the further measurements.

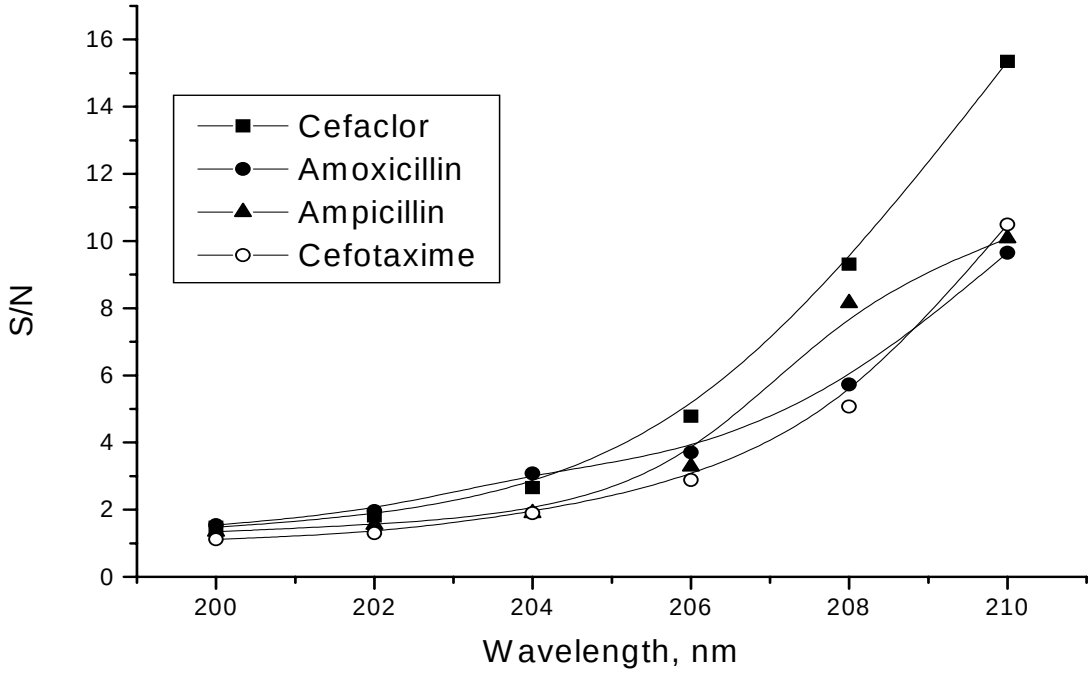


Fig 5.26: Influence of the wavelength on the signal to noise ratio of BLAs (cefaclor, amoxicillin, ampicillin and cefotaxime):3 ug/injection,1.5 ml/min, 200 uL loop and the blank 0.1 M HCl

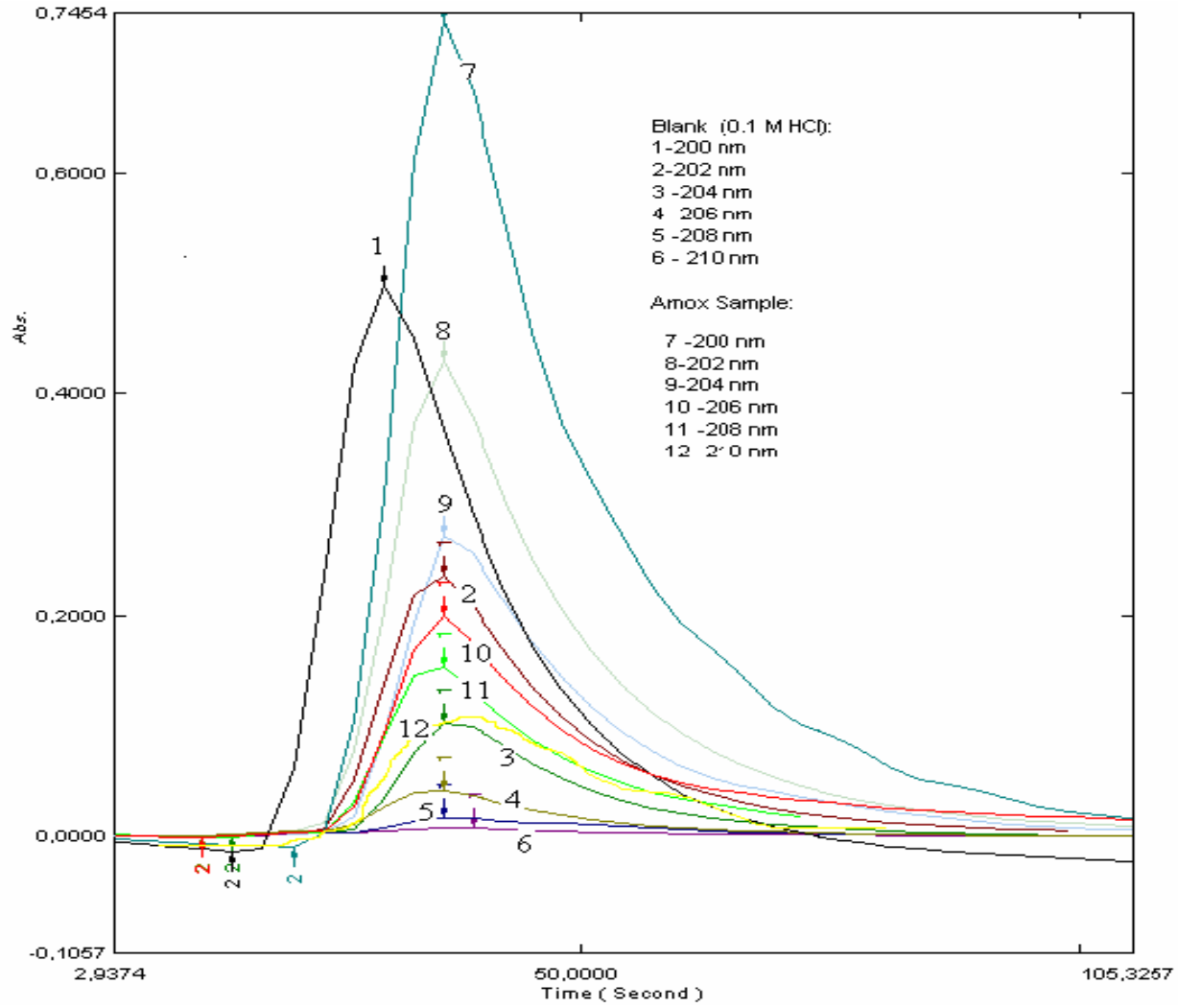


Fig.5.27: Influence of the measurement wavelength on the signal to noise ratio for Cefaclor sample (3 µg/injection), 200 µl loop, 0.1 M HCl

5.2.5.2 Effect of the Carrier Flow Rate

In the on – line FIA systems, the carrier flow rate is one of the most important parameters, because it controls the amount of analyte that passes through the minicolumn per unit time and accordingly the signal height. Therefore, the influence of this parameter must be carefully investigated in order to set an optimum carrier flow rate that allows a maximum mass transfer without loss of analytical significance. The carrier solution is that used to bring the analyte or

eluent into the analytical column where the sorption or desorption of the antibiotics takes place. Normally, the carrier solution should not be involved in the chemistry of the chromatographic process. In this work, Millipore water was selected as the recommended carrier since it shows no absorbance signal at the wavelength selected for measurement of the antibiotics and it has no ability to disrupt the bonded antibiotics with the PCTDD – PUF sorbent. Usually in the on-line SPE procedures the peak height of the signal is taken as function of the analyte concentration [27, 155, 205, and 206]. Accordingly, in all subsequent experiments we measured the peak height as function of the BLAs concentration. The obtained results when varying the carrier flow rate show that the maximum signal height for the analytes could be achieved when the carrier flow rate is around 3.0 ml min^{-1}. Higher flow rates than 3.0 ml min^{-1} lead to significant decrease in the signal which might be due to the dilution effect by the carrier solution. Figures (5.28 & 5.29) illustrate the recovery data obtained by changing the carrier speed from 1.0 up to 5.0 ml min^{-1}.

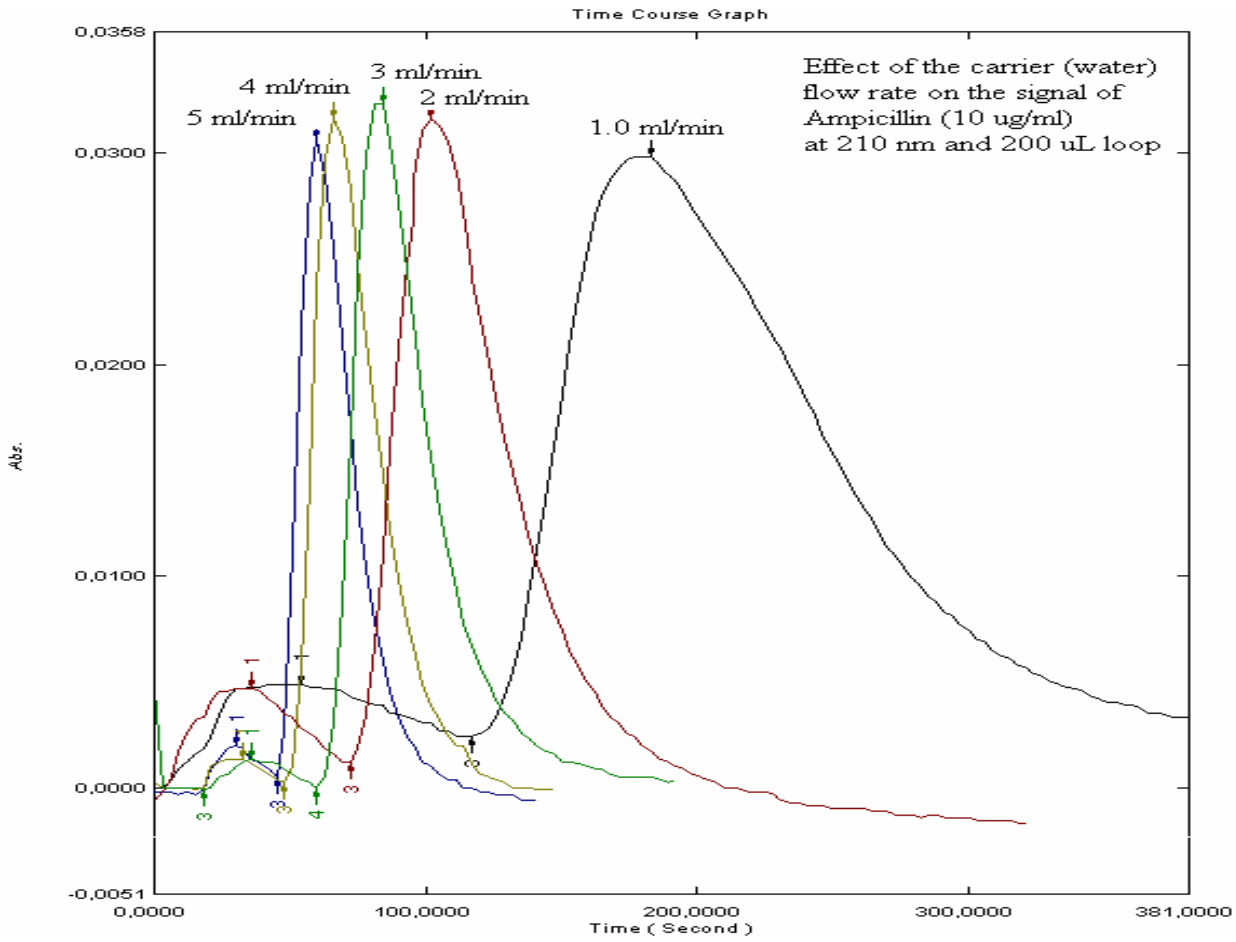


Fig. 5.28: Effect of the carrier flow rate on the signal of ampicillin (2µg injected)

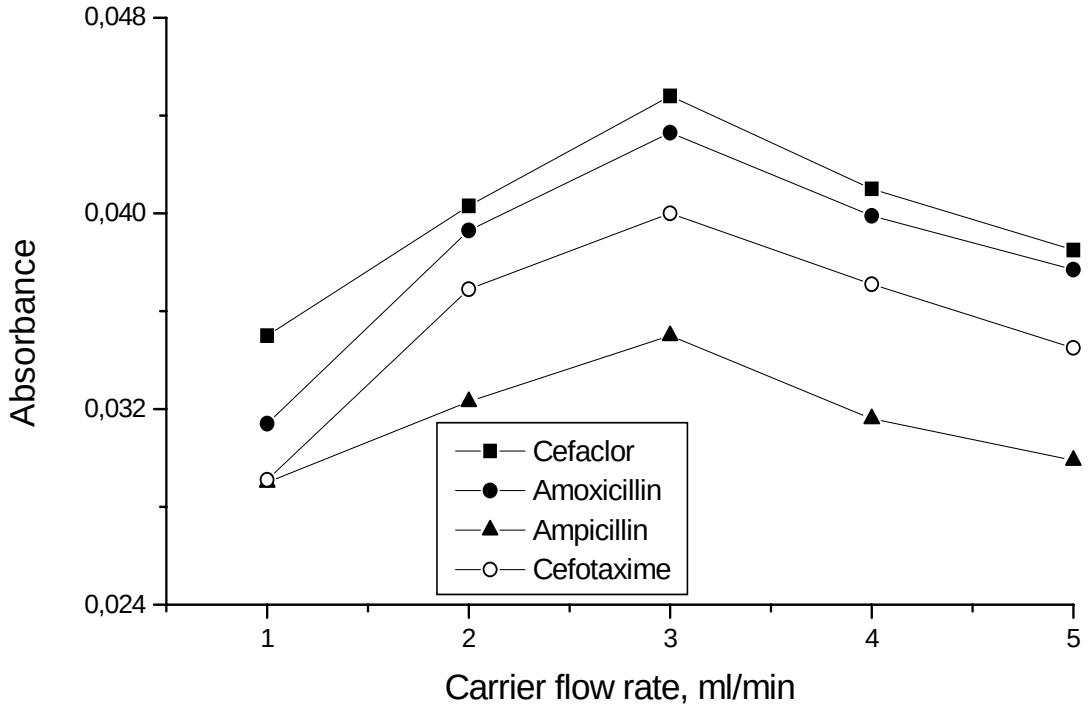


Fig. 5.29: Effect of the carrier flow rate on the signal of the BLAs compounds and (10 µg/ml) at 210 nm and 200 uL sample loop

5.2.5.3 Effect of the Sample Volume

The volume of the injected antibiotic sample was studied in order to establish the suitable sample loop that can be utilized to introduce the analyte into the carrier stream giving reasonable peak height absorbance. Therefore, volumes from 100 to 900 µl from 10 µg/ml each BLAs separately were injected at 3 ml/min carrier flow rate. The results depicted in figs (5.30 & 5.31) reveals that the antibiotic compounds can be injected from 100 µl up to 800, 500, 600 and 700 µl for cefaclor, amoxicillin, ampicillin and cefotaxime, respectively. The absorbance is linear in the estimated range due to the regular increase in the analyte quantity that reaches the flow cell. Beyond the higher limit, there is leveling off because of the saturation of the mobile phase with the analyte.

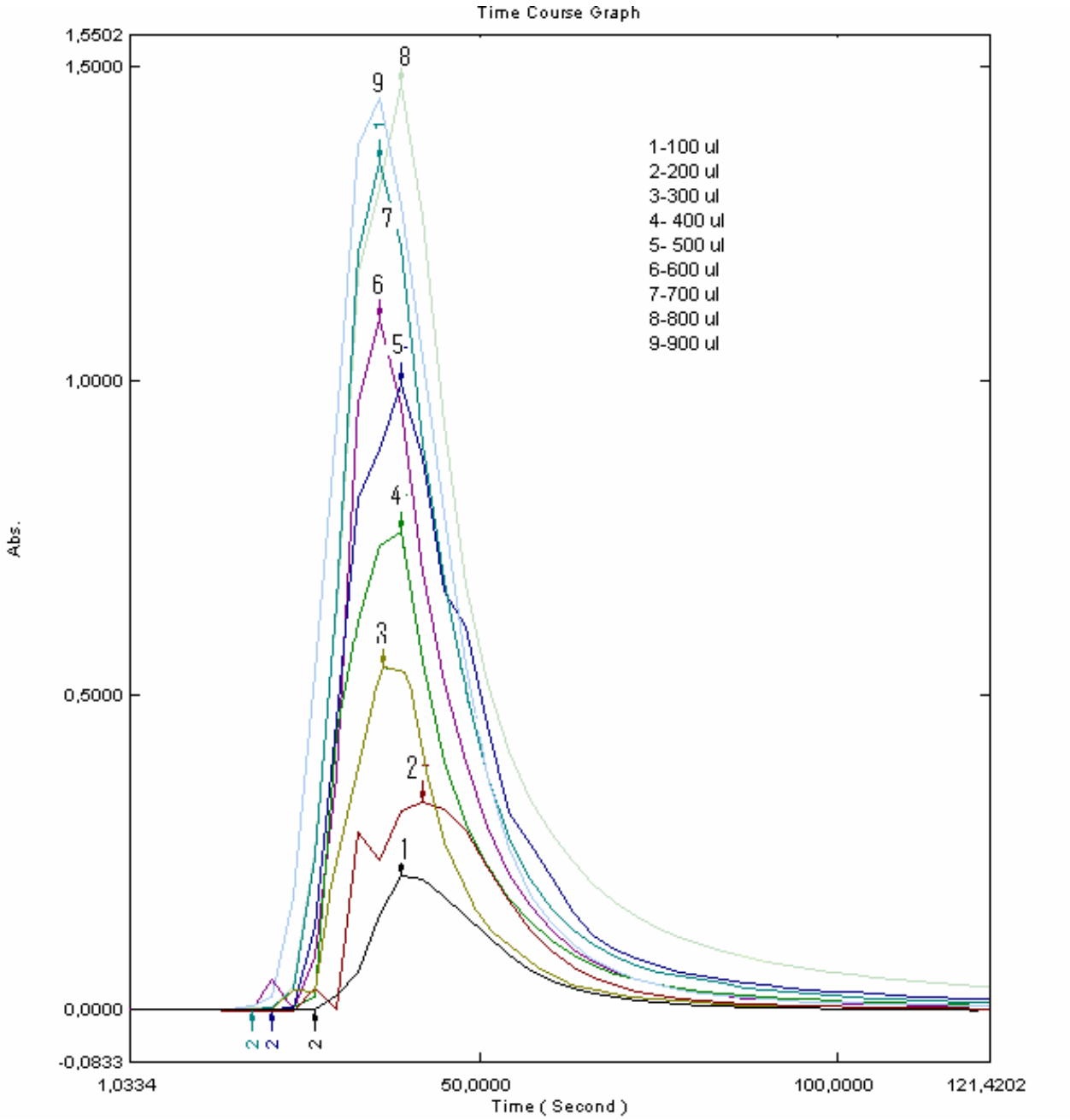


Fig. 5.30: Influence of the sample volume on the signal of cefaclor at 3 ml/min carrier flow rate and 210 nm

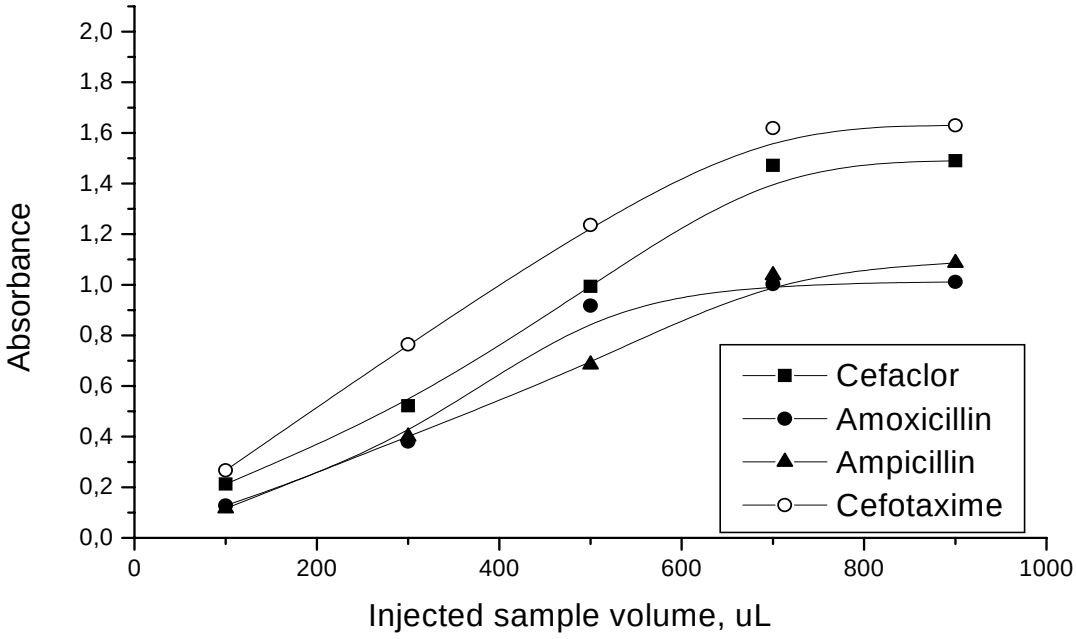


Fig. 5.31: Effect of the injected sample volume from 10 µg/ml BLAs on the signal height at 3.0 ml/min and 210 nm.

5.2.5.4 Eluent Concentration Effect on the Analyte Signal

The influence of the hydrochloric acid concentration on the signal height of the antibiotic was investigated. For this, 200 µl from the antibiotic solutions (10 µg/ml) were prepared in hydrochloric acid at concentrations from 0.05 – 0.30 mol/l were injected into the carrier at 3.0 ml/min. It was found an increase in the signal height of the analyte with increasing acid concentration till reaching a maximum value which is followed by a decrease as shown in Fig. 5.32. The increase in the first part of the curve can be due to the background signal of the acid. However, the decrease might be attributed to the decomposition of the analyte under such higher acidic conditions. Accordingly, the optimum acid concentration was chosen for cefaclor, ampicillin and cefotaxime is 0.15 mol /l. On the other hand, amoxicillin can withstand hydrochloric acid concentration up to 0.20 mol/l. Therefore, the concentration 0.15 is taken as the higher limit of the acid concentration that could be used in the elution step.

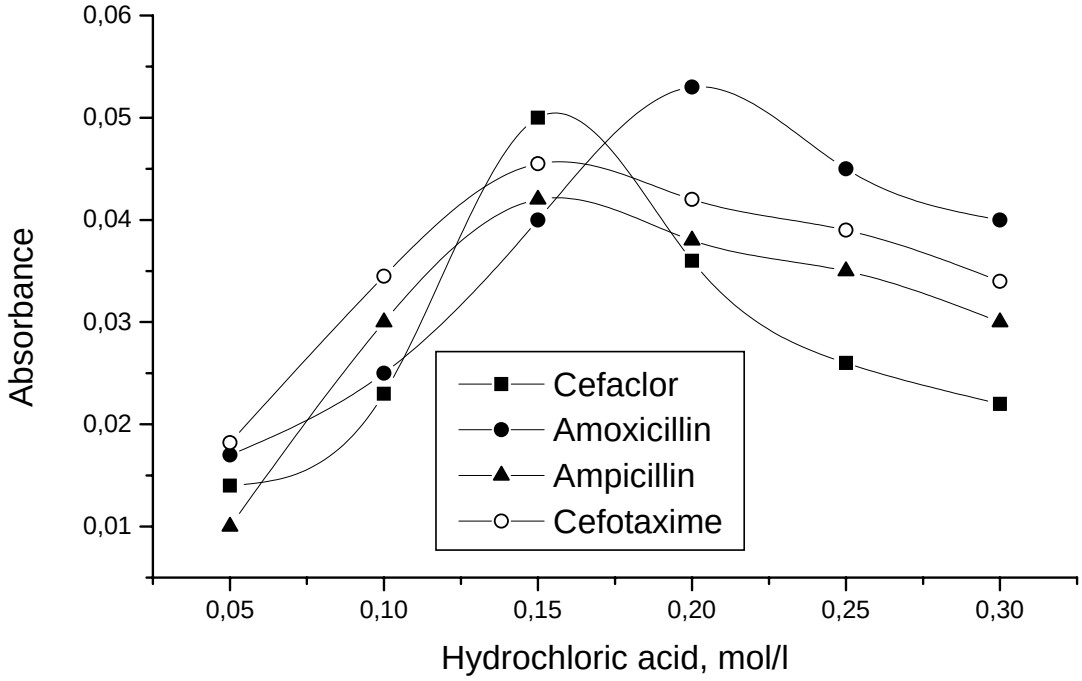


Fig. 5.32: Effect of hydrochloric acid concentration on the signal of the BLAs for 200 µl injection loop (10 µg/ml), 3.0 ml/min carrier flow at 210 nm.

5.2.6 Calibration Curves

In order to use the on-line SPE system for quantitative applications, the calibration lines were set up under the previously optimized conditions (measurement wavelength is 210 nm, carrier flow rate is 3.0 ml/min, sample loop is 200 µl and 0.15 mol/l hydrochloric acid as blank). Linear curves were obtained in the concentration range 5 – 25 µg/ml. The peak maximum linearly increases with increasing the concentration of the analyte in the injected sample. The calibration lines for the analytes within the range 0 – 25 µg/ml are plotted in Fig. 5.33 and the linear regression data are listed in Table 5.20. Figure 5.34 shows the obtained signal data at varying concentration for cefaclor.

Table 5.20: Linear Regression for data for BLAs with on-line SPE system

Analyte	Regression equation	R	SD	P
Cefaclor	A = (0.00667)C – 0.00347	0.99634	0.00518	<0.0001
Amoxicillin	A = (0.00594)C – 0.00356	0.99627	0.00466	<0.0001
Ampicillin	A = (0.00458)C – 0.00161	0.99586	0.00379	<0.0001
Cefotaxime	A = (0.00420)C – 0.00283	0.99617	0.00323	<0.0001

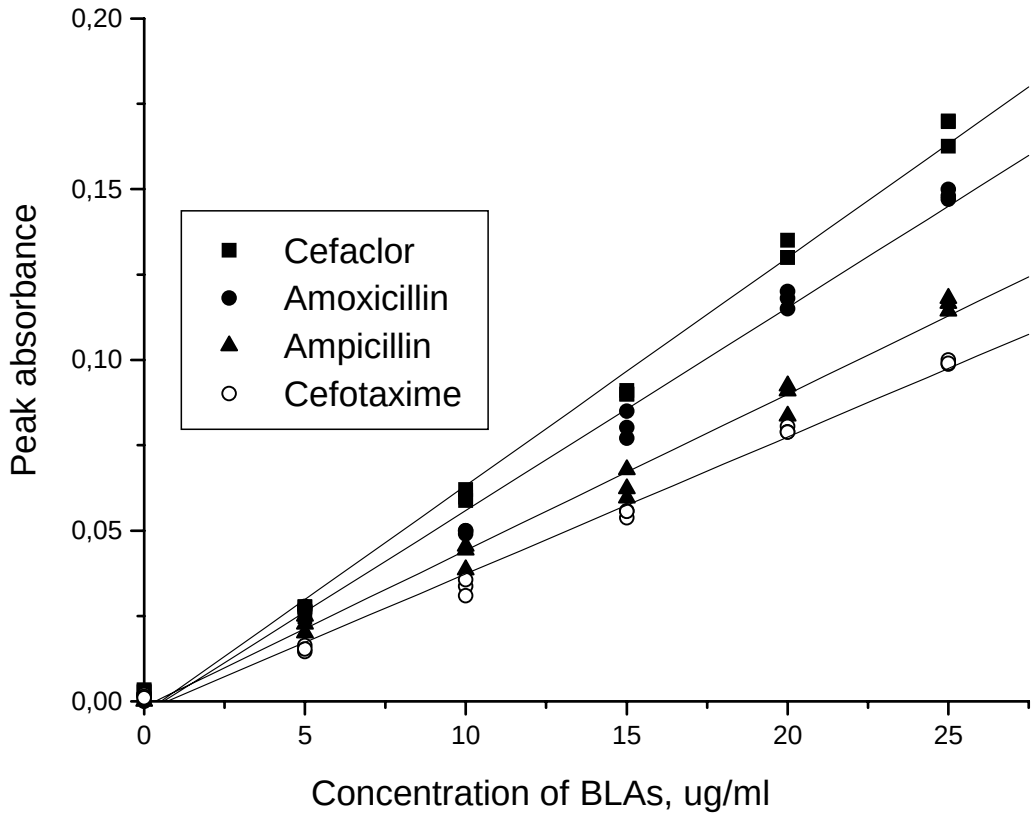


Fig. 5.33: Calibration curves for BLAs for the injection of 200 µl samples at 3.0 ml/min carrier flow rate, 0.15 M HCl blanck and 210 nm.

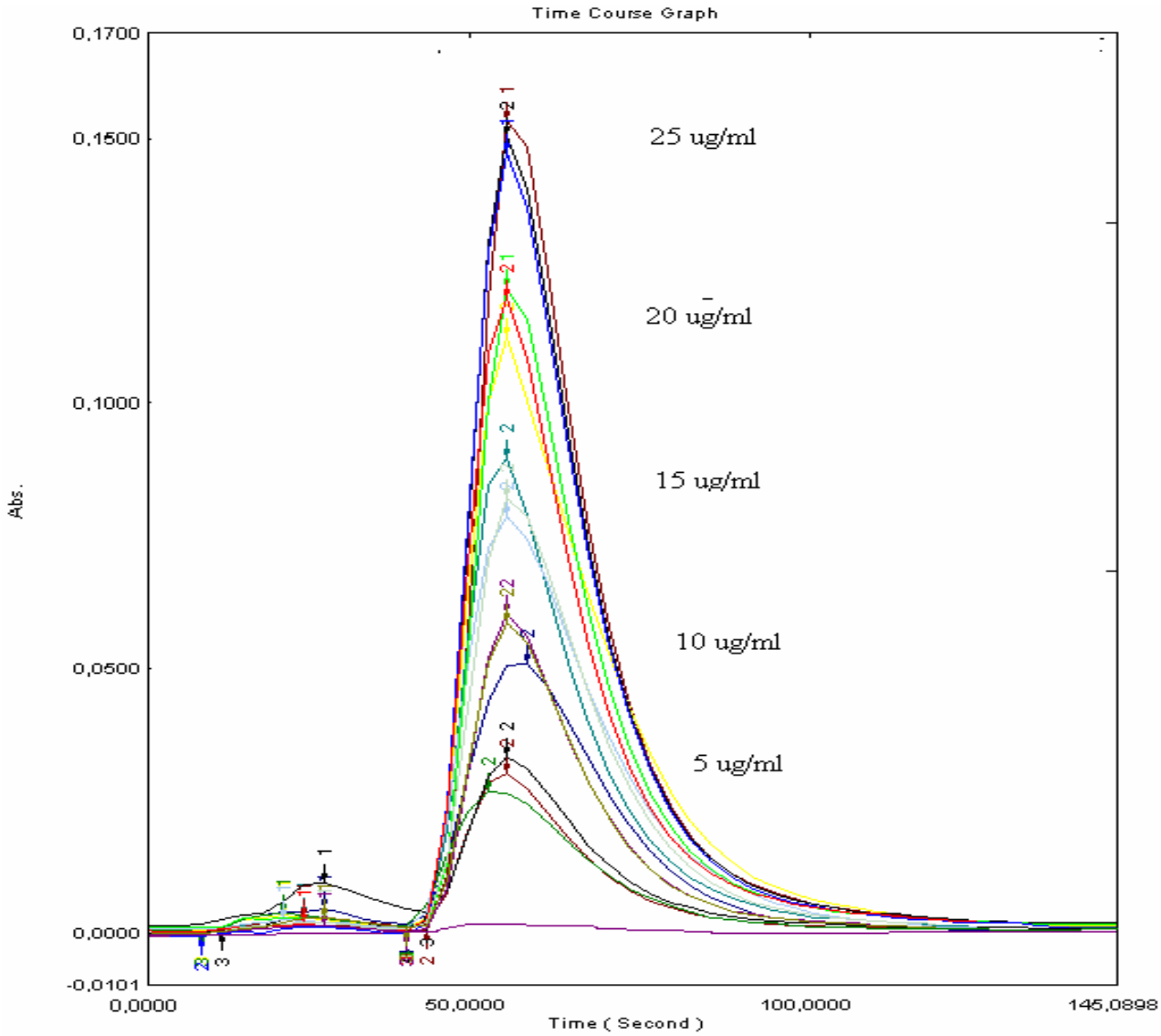


Fig.5.34: Absorbance signals for different concentrations of cefaclor at 210 nm, 3.0 ml/min carrier flow rate and 200 µL injection loop.

5.2.7 Chemical and Flow Optimizations

5.2.7.1 Effect of Sample pH

The first chemical parameter investigated was the sample pH, since the correct adjustment of this variable is necessary to improve the formation of the ion – associatation complexes between the antibiotic compound and the PCTDD which leads to better sorption process. The influence of this parameter on the height of response signals produced by the analyte was studied in the pH range 7–10. Buffer solutions of ammonia were used to adjust the varying sample pH values. The analytical signal reaches its maximum value in the narrow range of pH 8.5 – 9.0, 9.0 – 10.0, 9.0 – 9.5 and 8.5 – 9.0 for cefaclor, amoxicillin, ampicillin and cefotaxime, respectively. At pH values less than pH 7.0, a constant signal height was observed which might suggest no effect due to the ligand compound. The results obtained for the pH dependence of the cefaclor extraction with the on-line SPE system and PCTDD – PUF sorbent is shown in Fig. 5.35. As can be noted in the Fig. 5.36, when the sample pH is lower than 8.0, the absorbance signals decrease abruptly, probably due to the low availability of antibiotics in its ionized form which reduced drastically the complex formation to the sorbent and, consequently, the preconcentration efficiency. At pH ≥ 9.5 the analytical signal shows slight decrease (except with cefotaxime the decline is large) which might be attributed to the interference of the analyte from the sorbent due to competition between the negatively charged hydroxyl ion of the buffer and the caboxylate anion of the analyte with the positive centers on the ligand. So, for all subsequent experiments, ammonia buffer solution at pH 8.5 was employed to adjust the cefaclor and cefotaxime sample pH. While amoxicillin and ampicillin samples were adjusted with buffer solution with pH 9.0.

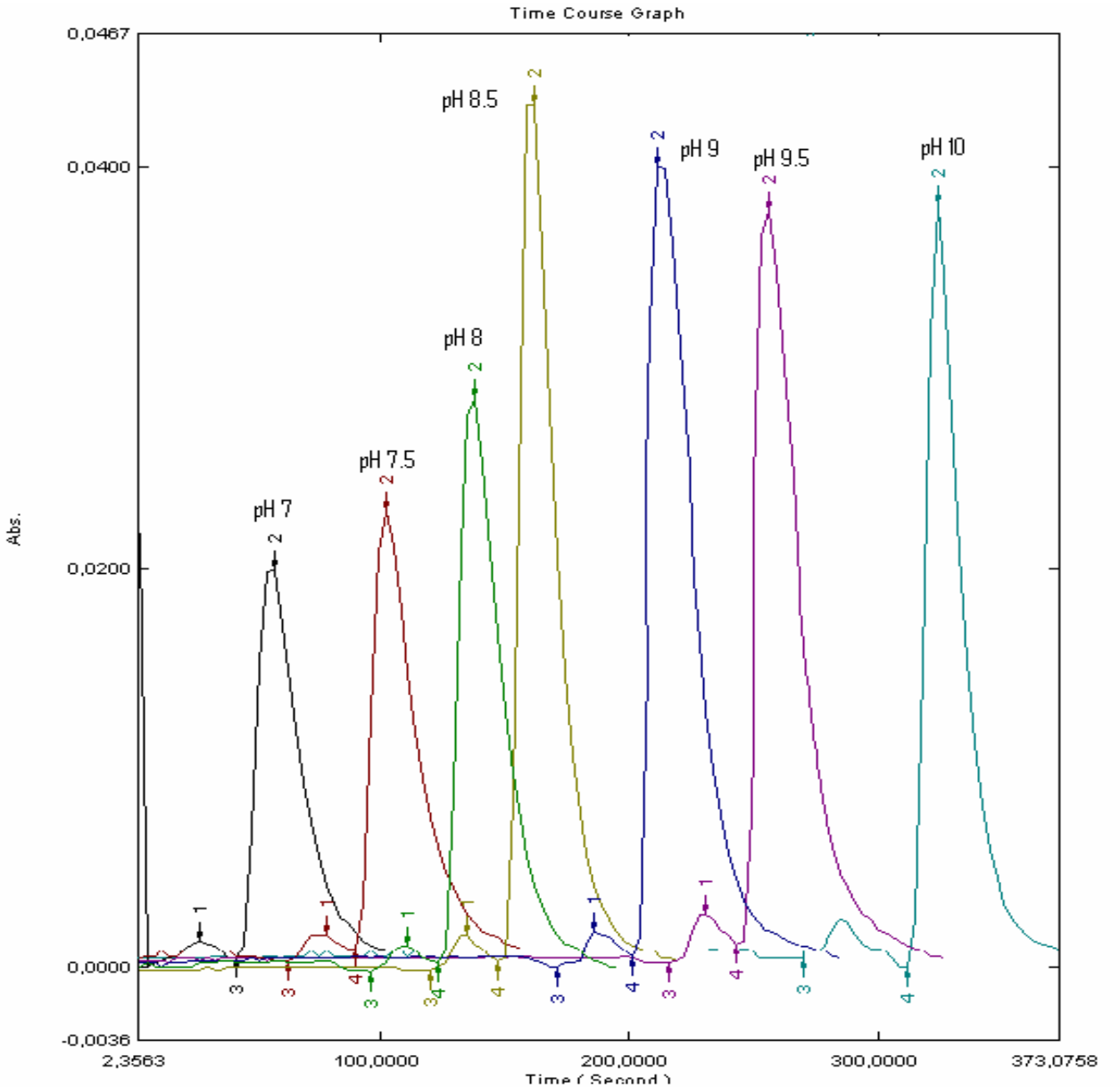


Fig. 5.35: Influence of the sample pH on the recovery of cefaclor (2 µg injected), 3 ml/min carrier flow rate and desorption with 0.15 M HCl at 210 nm.

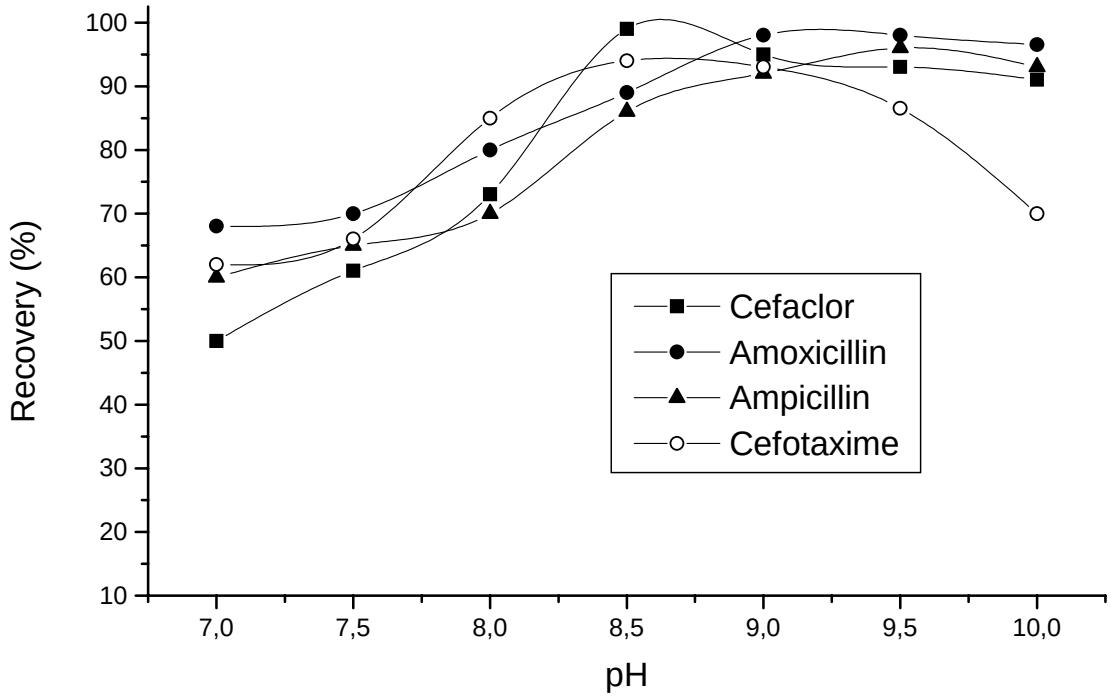


Fig. 5.36: Influence of sample pH on the sorption of BLAs: 2µg injected,200 ul loop, 210 nm, 0.15 M HCl for desorption at 1.5 ml/min

5.2.7.2 Effect of the preconcentration time

Preconcentration time in the on-line SPE system was studied in the range 10 – 300 seconds. In this investigation the volume of the injected sample into the PCTDD - PUF column will increase by increasing the preconcentration time. It was found that the height of the analytical signal of the antibiotic compound increases linearly up to 180s with cefaclor, amoxicillin, ampicillin and cefotaxime at sample concentration of 250 ng/ml and optimized pH. This indicates that practically no leaching of the retained antibiotics to the sorbent occurs. At prolonged preconcentration times the rate of increase in the analytical signal becomes lower or nonlinear (Fig 5.38). Thus, 120s preconcentration time was applied in this study to achieve relatively high sample throughput and moderate sample consumption. However, longer preconcentration time in case of dilute samples may be used when higher concentration factors are needed or if the sample concentration is too low to give relevant analytical signal. The dependence of the ampicillin peak height on the preconcentration time is illustrated in Fig. 5.37.

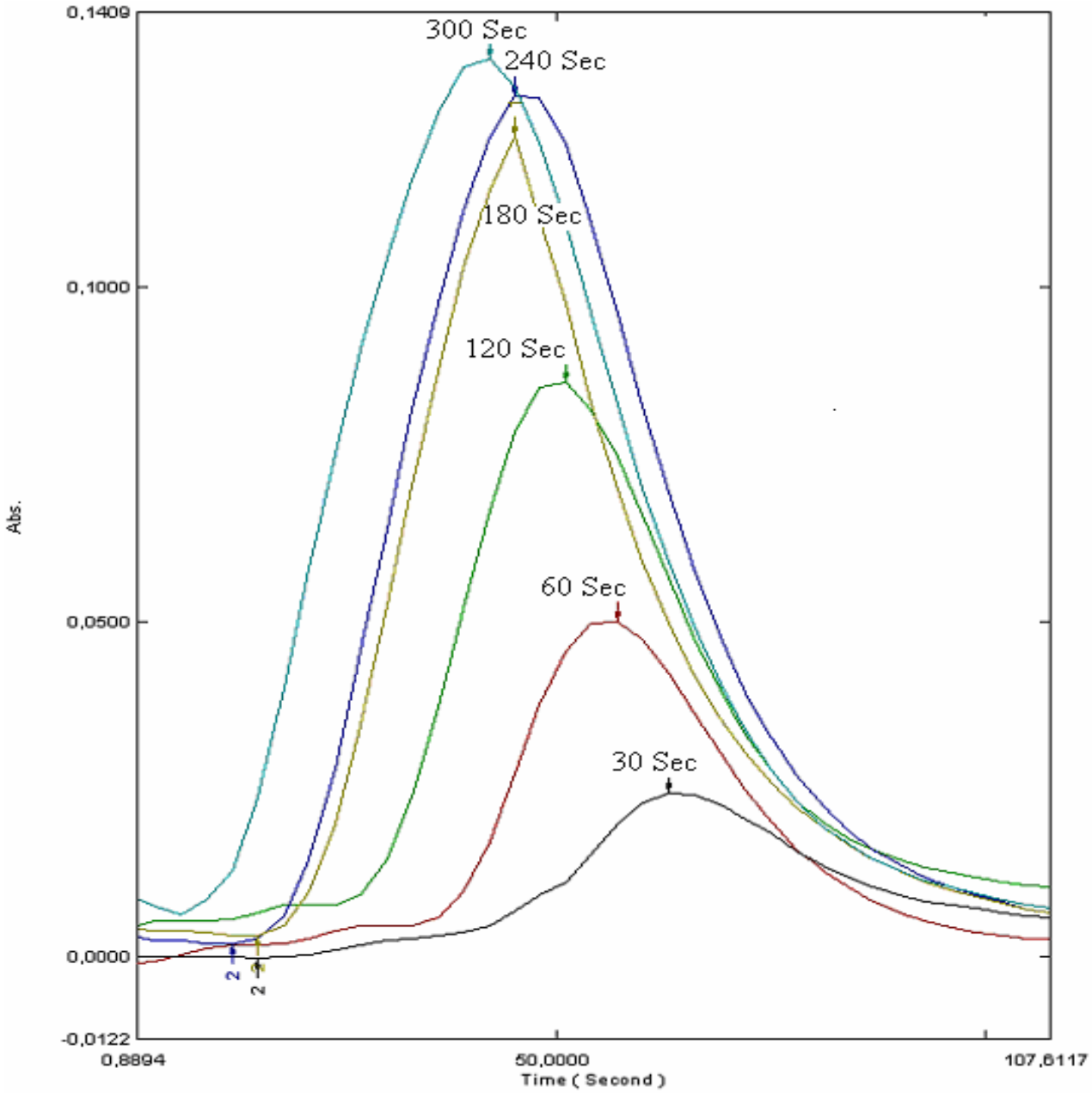


Fig. 5.37: Influence of the preconcentration time on the recovery of ampicillin (250 ng/ml), at 3.0 ml/min and desorption with 0.15 M HCl at 210 nm

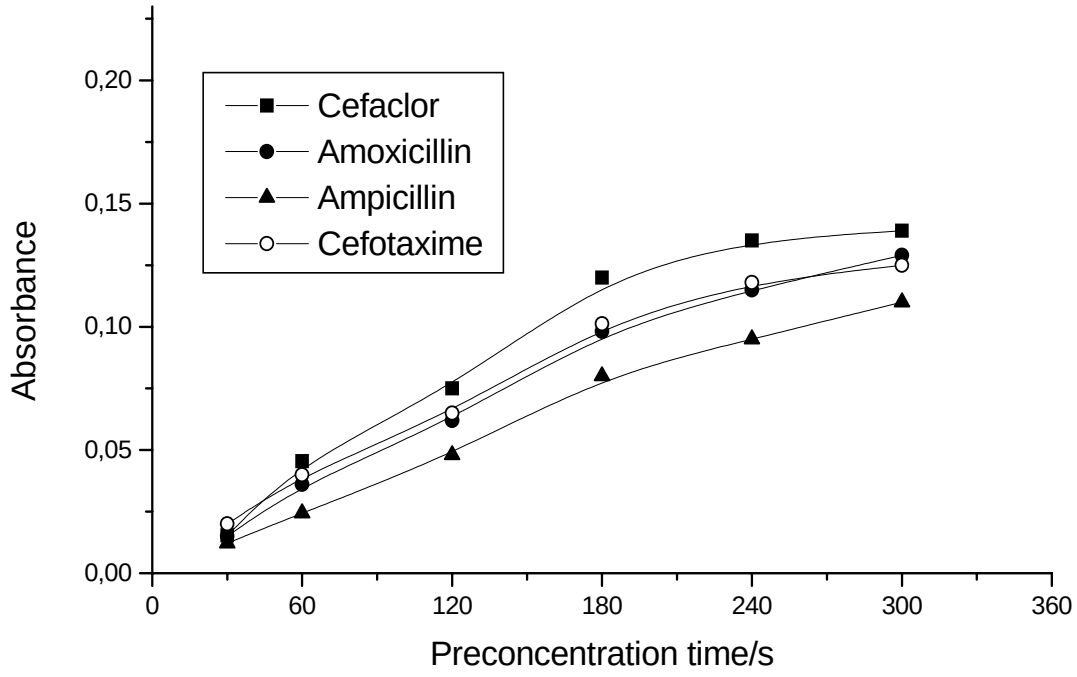


Fig. 5.38: Effect of the preconcentration time on the sorption of BLAs to the PCTDD - PUF minicolumn: 250 ng/ml antibiotic solution, 3.0 ml/min carrier flow rate and 0.15 M HCl for desorption at 210 nm

5.2.7.3 Eluent Concentration

Elution of BLAs from the PUF minicolumn was studied with hydrochloric acid solution at several concentrations. Other acids (nitric, sulphuric or acetic) were also tested as eluents but they gave a strong background signal than hydrochloric acid at the same concentration. Desorption with acids is fast, since it rapidly decrease the pH inside the minicolumn which reduce the complexing capacity of the analyte to the solid phase. Moreover, it may form chloro-complexes with the cationic copolymer, favouring the release of the analyte from the sorbent. To find the better concentration of the acid, hydrochloric acid solutions of concentration varying from 0.025 to 0.175 mol/l were examined using 200 µl injection loop and 250 ng/ml antibiotic sample at the optimum pH for extraction. The absorbance signals for the four antibiotic compounds are maximum and relatively constant when the acid concentration ≥ 0.15 mol/l as can

be seen in Fig. 5.40. The signal peak height of the desorbed amount of cefaclor at various hydrochloric acid concentrations are depicted in Fig. 5.39. The slight increase in the peak height at acid concentrations more than 0.15 mol/l can be due to the background absorbance of the acid itself. Therefore, the hydrochloric acid concentration 0.15 mol/l was chosen as the appropriate eluent for further desorption step.

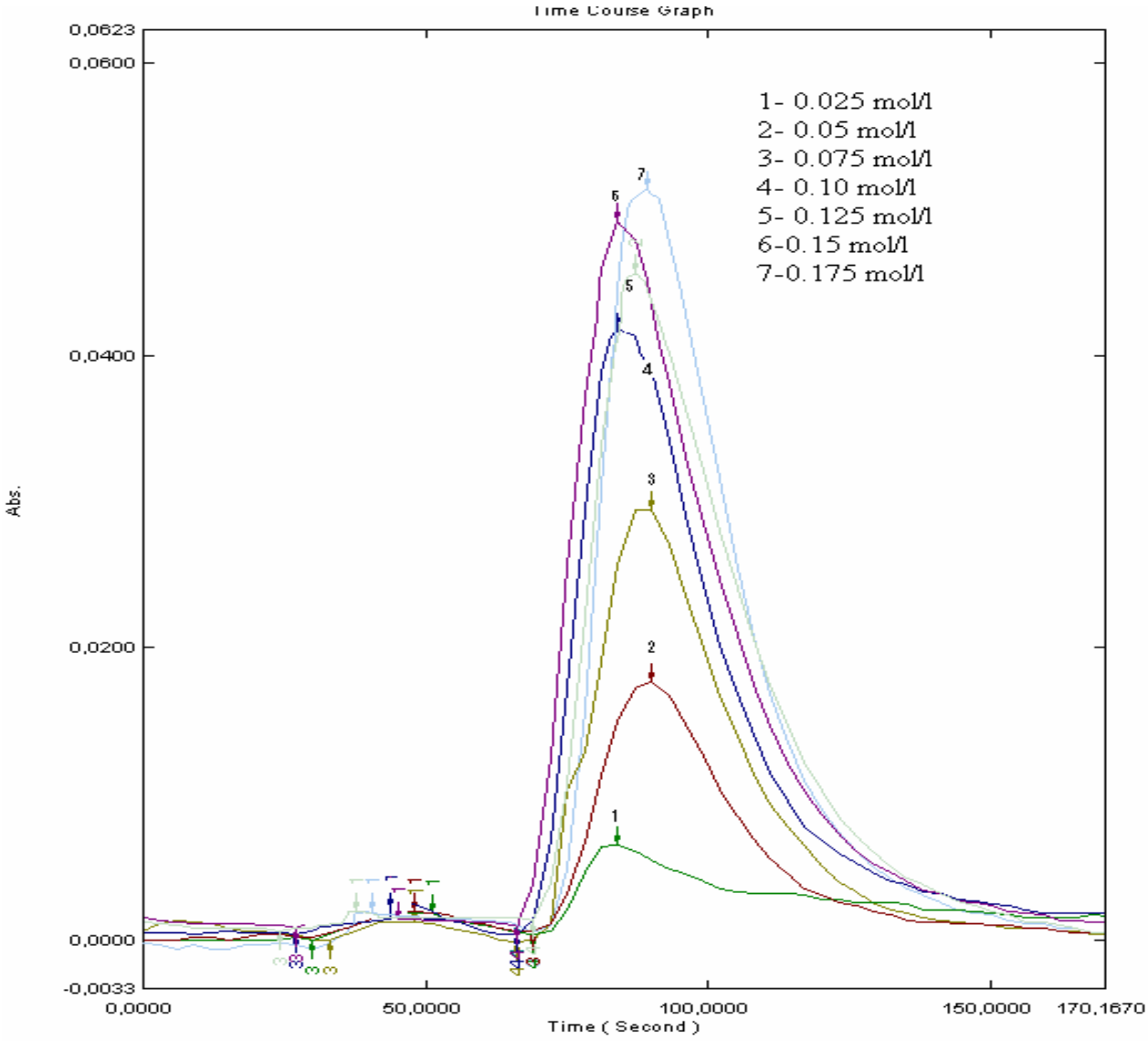


Fig. 5.39: Influence of the eluent (HCl) concentration on the recovery signal of cefaclor: 250 ng/ml, at pH 8.5,2 min preconcentration time carrier flow rate 3.0 ml/min, elution loop 200 µl at 210 nm.

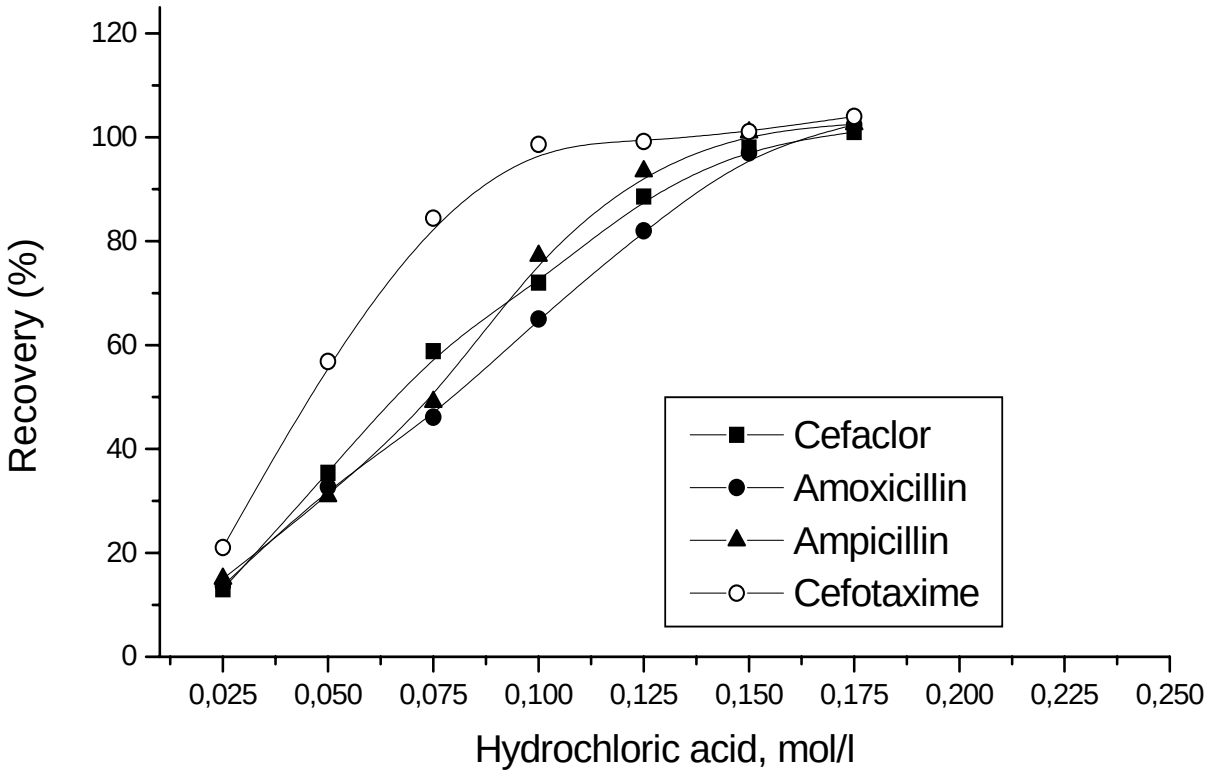


Fig. 5.40: Effect of the eluent (hydrochloric acid) concentration on the recovery of BLAs: 250 ng/ml, 2 min preconcentration time at 3 ml/min (6 ml sample injected), at 210 nm with PCTDD - PUF sorbent with on-line SPE system.

5.2.7.4 Effect of the Volume of the Eluent Loop

The influence of the injected volume of the eluent solution was tested in order to ensure that enough volume would be injected into the system to provide complete desorption of the antibiotic compound. Several eluent loops with volumes from 20 to 300 µl were applied. The derived signals showed regular increase in the absorbance maximum within the volume ranging from 20 to 100 µl. From 100 to 300 µl eluent volume, the analytical signal is nearly constant as shown in Fig. 5.41 for amoxicillin. This indicated that the 100 µl loop is enough to achieve complete recovery of the analyte. Therefore, in order to assess that the eluent is large enough, the 200 µl loop was selected as the eluent loop (see Fig 5.42).

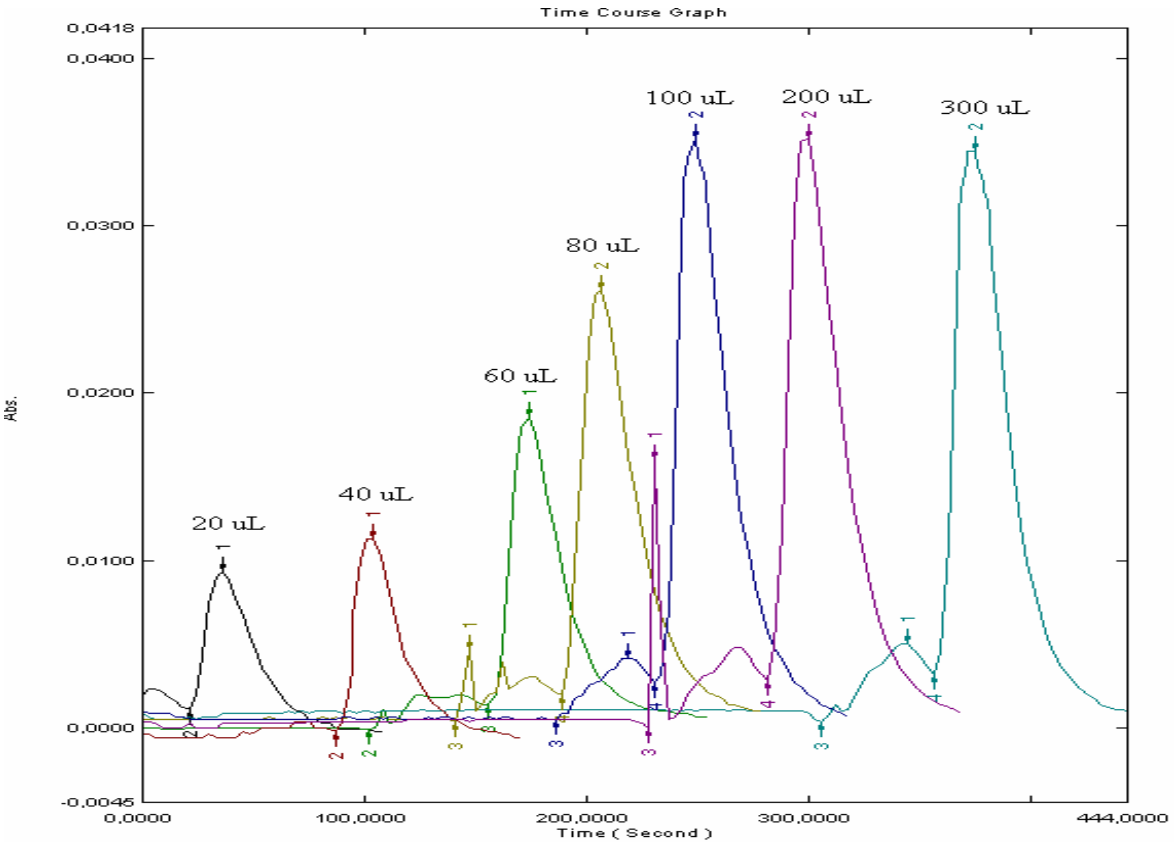


Fig. 5.41: Effect of the eluent volume on the recovery of amoxicillin (250 ng/ml), sample flow rate 3 ml/min,2 min preconcentration time, 0.15 mol/l HCl as eluent at 210 nm.

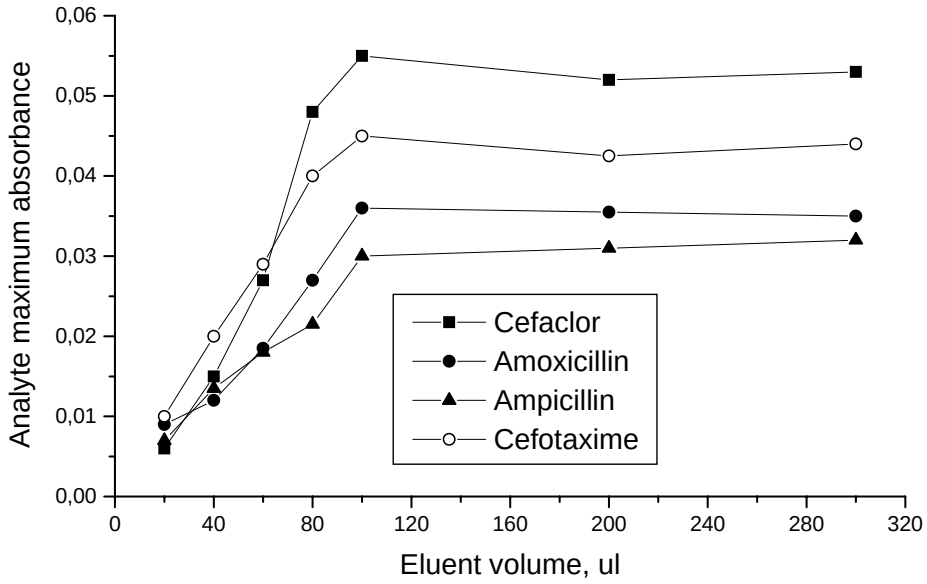


Fig. 5.42: Influence of the eluent (0.15 mol/l HCl) volume on the recovery of BLAs (250 ng/ml), 2 min preconcentration time, sample flow rate 3 ml/min, at 210 nm

5.2.7.5 Sample Flow Rate

In case of time – based on-line SPE systems, one of the most important parameters is the sample flow rate. This factor controls the amount of the sample that passes through the analytical minicolumn. Also, the sample flow rate should be carefully investigated to increase both analytical throughput and sensitivity. The optimum value of the sample flow rate should be set at the point which permits maximum mass transfer without loss of the analytical throughput. Another factor that must be taken into consideration is the backpressure or overpressure inside the separation column which probably affects the speed of the analysis and consequently the sample frequency. Actually, the physical shape of the PUF was studied comparatively using two forms of PUF; the first is the normal form as cylindrical plugs and the second as small particles and the same mass was used in packing the minicolumn. It was proved that the normal form is better because the second kind leads to an increase of the overpressure inside the minicolumn at higher flow rates. Therefore, we could not apply the blended form at higher carrier flow rates and the normal form was selected in all preconcentration experiments. It was also observed that even for high sampling flow rates, the PUF in its two physical forms caused very low overpressure in the closed system, unlike other sorbents commonly used under these conditions such as chelating resins or silica based solid sorbents. Moreover, the data have revealed the signal of the analyte is approximately constant and maximum within the range of sample flow rate from 0.5 to 3.0 ml/min as shown in Fig. 6.44. Lower flow rates than 0.50 ml/min were not studied because it would make the analysis much more time consuming. At higher flow rates than 3.0, there is a steady decrease in the absorbance as can be seen in Fig. 6.43 for the dependence of the analytical signal of ampicillin on the sample flow rate. Obviously, the sorption becomes lower at higher flow rates because the antibiotic compounds pass through the column too quickly and the contact time between the phases is not sufficient for complete retention of the antibiotics to the solid phase. Higher sample flow rate is recommended when the sorption of the analyte is complete since it enables achievement of larger concentration factors. Accordingly, a flow rate of 3.0 ml/min was recommended for subsequent experiments as a compromise between sensitivity and analytical efficiency.

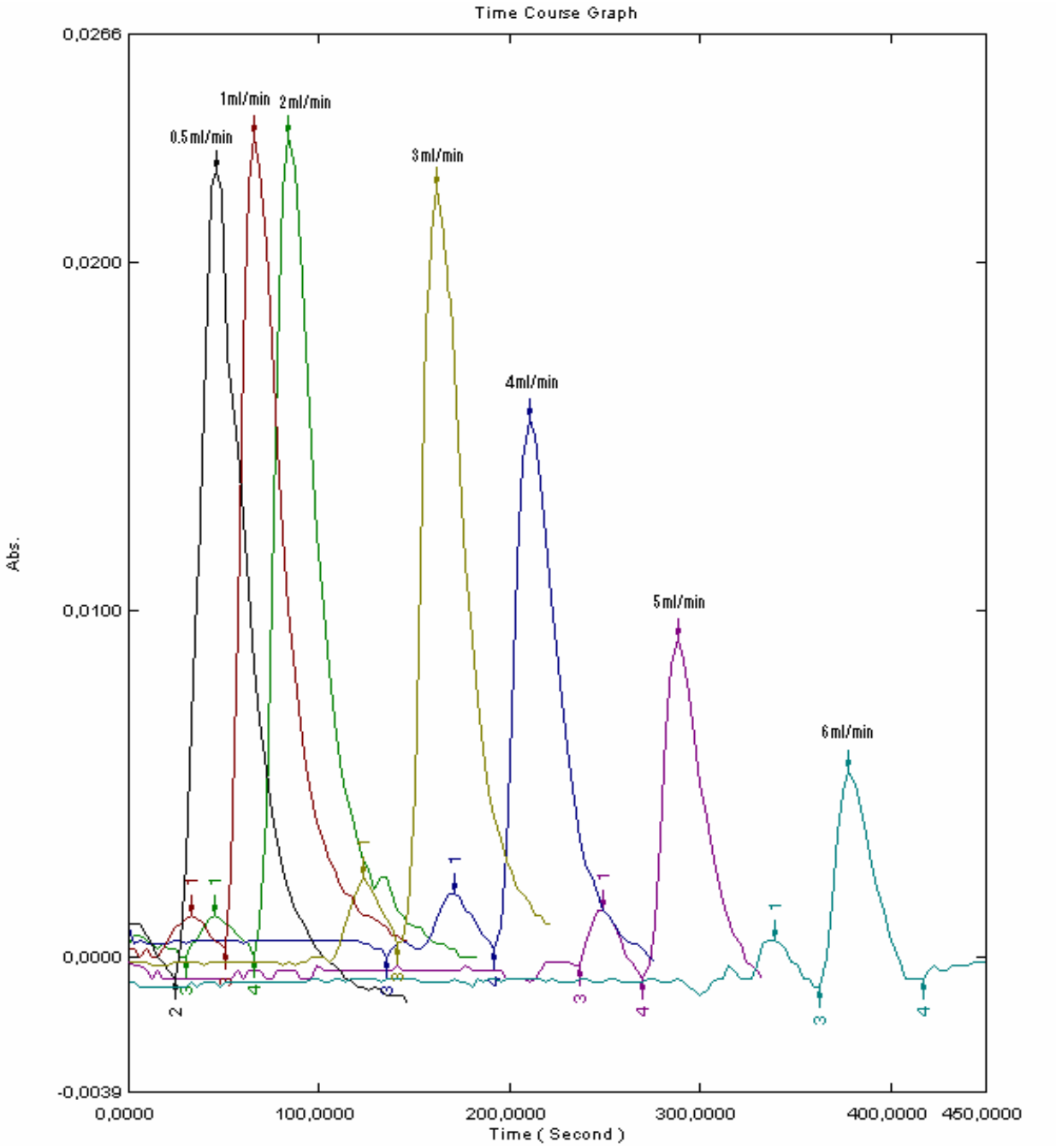


Fig. 5.43: Dependence of the analytical signal of ampicillin on the flow rate of the sample.

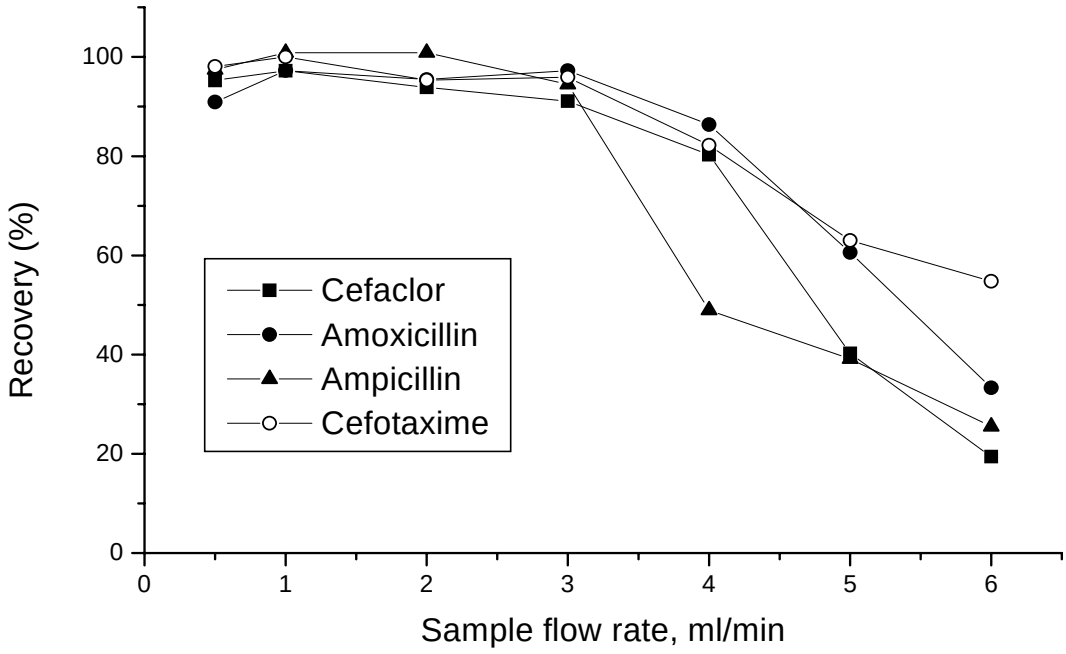


Fig.5.44: Effect of the sample flow rate on the recovery of BLAs using 2 µg injected antibiotic compound and desorption with 0.15 M HCl at 1.5 ml/min and 210 nm.

5.2.7.6 Eluent Flow Rate

The effect of the eluent flow rate was studied because this parameter has remarkable influence on the analytical throughput in case of continuous flow analysis. Thus experiments were carried out by pumping the eluent solution (0.15 mol/l HCl) at varying flow rates between 1.0 and 6.0 ml/ min. Figure 5.46 indicates that by increasing the eluent flow rate the recovery percentage of the analyte strongly decreases. Best analytical signals were verified at flow rate from 1 to 2 ml/min since the absorbance in this range is levelled-off. Above this range, the absorbance begins to slightly decrease up to 4 ml/min then a strong reduction in the signal when the flow rate is higher than 4 ml/min. The decrease in absorbance at higher eluent flow rates may be due to greater dispersion of the analyte. Finally, an elution flow rate of 1.5 ml/min was set up in any further desorption operations. The effect of the eluent flow rate on the signal height of cefotaxime is illustrated below in Fig. 5.45.

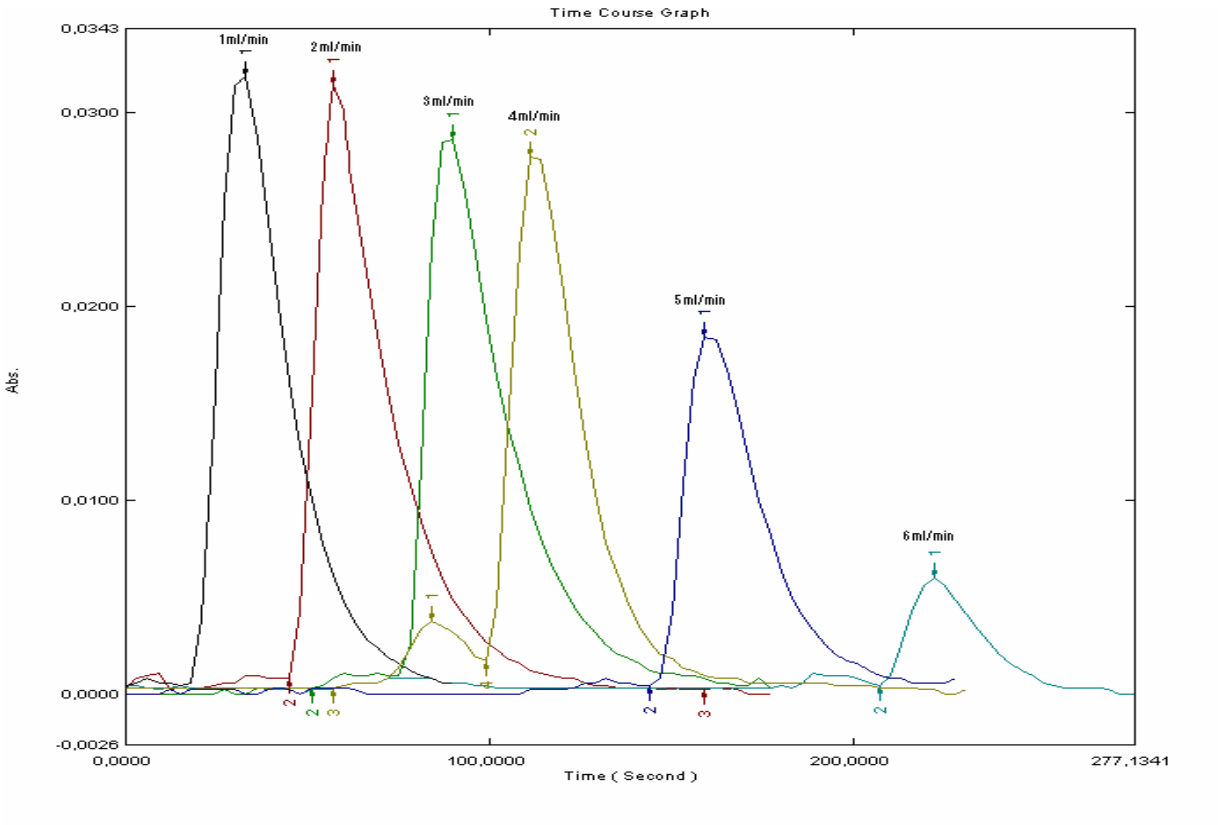


Fig. 5.45: Influence of the eluent flow rate on the analytical signal of Cefotaxime:250 ng/ml, pH 9.0, 2 min preconcentration time, desorption with 200 µL loop 0.15 mol/l HCl at 210 nm.

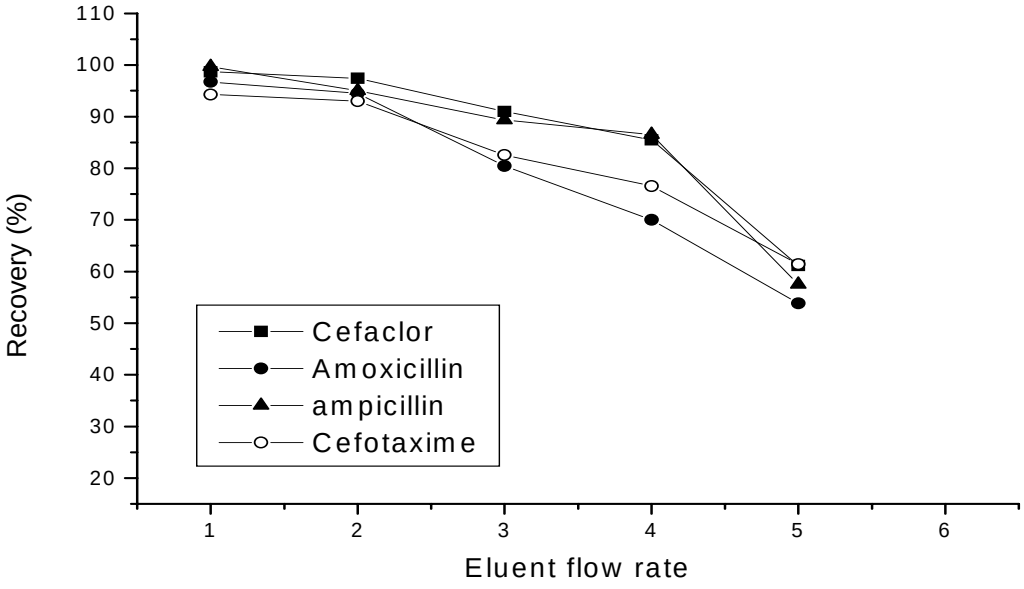


Fig. 5.46: Effect of the eluent flow rate on the recovery of the BLAs from the PCTDD - PUF: 250 ng/ml abtibiotic, pH 9, 2 min preconcentration time, 200 µL sample loop at 210 nm

5.2.7.7 Breakthrough Capacity

The capacity of the sorbent material is a very important parameter since it governs the maximum amount of analyte that could be completely retained in the analytical column. For this, solution for each antibiotic compound was prepared at concentration (500 ng/ml), adjusted to the optimized pH for maximum sorption and percolated the analytical column at suitable sample flow rate. Then, the analyte emerging in the effluent was measured against time. The time at which the antibiotic is detected in the effluent stream was applied to calculate the breakthrough capacity (μg/g or μmol/g). Figure 5.47 shows the breakthrough curves for the antibiotic compounds under the recommended conditions. It is clear from the data in Table 5.21 that the sorbent has equal capacity of retention towards the two penicillin compounds (amoxicillin and ampicillin) which is greater than those for the two cephalosporins (cefaclor and cefotaxime). Additionally, within the same group, the material showed superior capacity for cefaclor, which has slightly lower capacity than penicillins, than cefotaxime. So the capacity could be written in the order amoxicillin = ampicillin > cefaclor > cefotaxime. An interpretation of these capacity values may be due to the nature of side groups. The presence of similar side group (benzene or phenol) in case of amoxicillin, ampicillin and cefaclor may reveal the closer capacities. However, five member heterocyclic ring thiazole side groups exist in cefotaxime (Fig. 5.48). As we expect from the previous investigations, PUF has the preference to adsorb hydrophobic aromatic compounds and phenols than a heterocyclic one. In the case of higher analyte concentration, a greater amount of the sorbent material have to be used so that not to lose the analytical significance of the procedure.

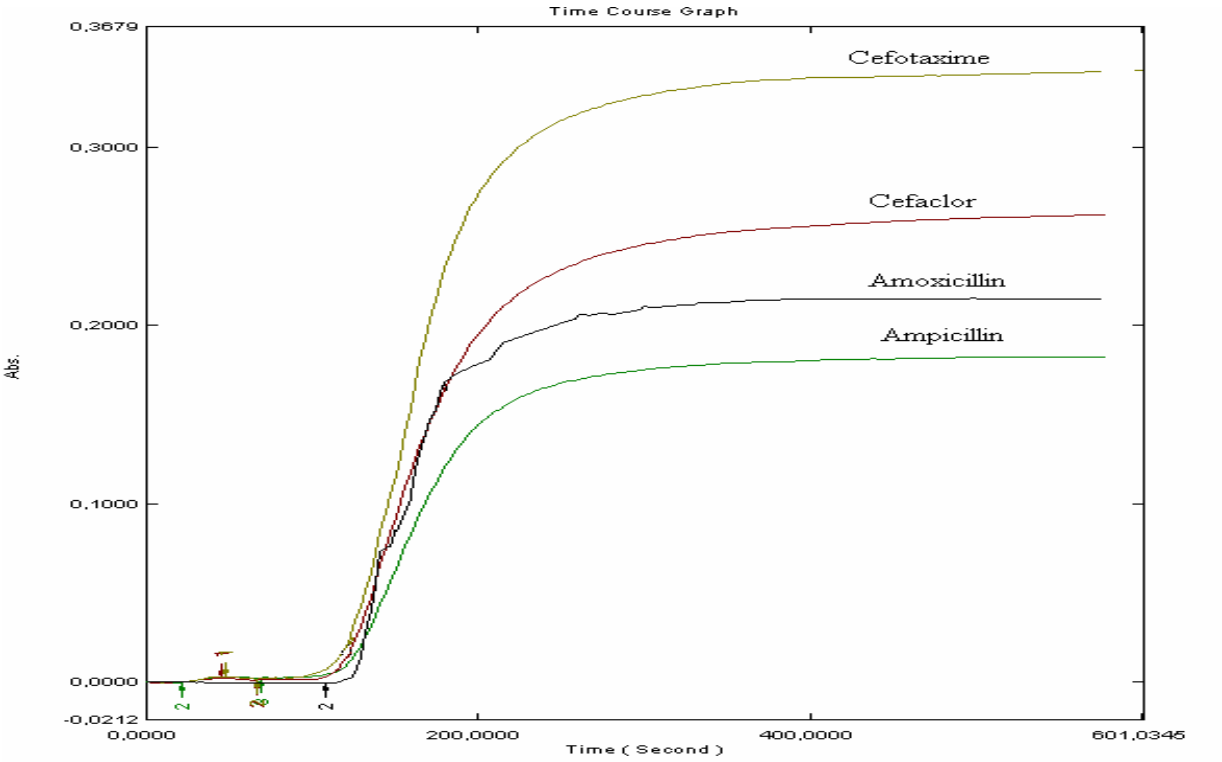
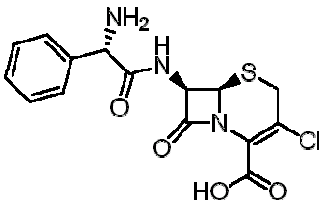
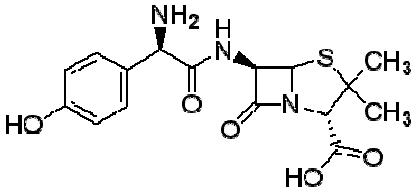


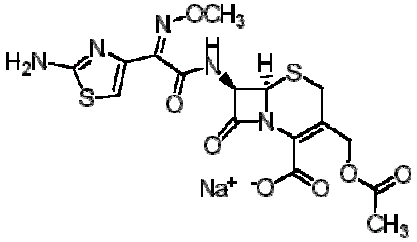
Fig. 5.47: Signal of BLAs in effluent solution function of time in the determination of break-through capacity for BLAs compounds (500 ng/ml) in ammonia buffer solution pH 9.0 and flow rate 3.0 ml/min. at 210 nm.



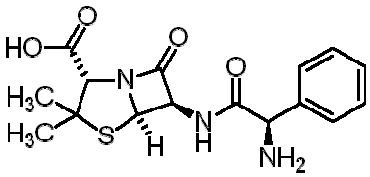
Cefaclor



Amoxicillin



Cefotaxime



Amopicillin

Fig. 5.48: Chemical structure of cefaclor, amoxicillin, ampicilln and cefotaxime.

Table 5.21: Capacity determination of the PCTDD – PUF sorbent for BLAs compounds

Parameter/ Antibiotic	Breakthrough time/s	Breakthrough Volume (ml)	Breakthrough Capacity (µg/100mg)	Breakthrough Capacity (µmol/g)
Cefaclor	120	3.000	3.0	0.82
Amoxicillin	129	3.225	3.2	0.88
Ampicillin	123	3.075	3.1	0.88
Cefotaxime	114	2.850	2.9	0.60

5.2.7.8 Interference Effect

In order to assess the possible application of the proposed on-line SPE methodology to natural samples, the influence of several possible interferent substances, especially those commonly exist along with the antibiotic compounds was investigated in order to verify the selectivity of the proposed sorbent. Solutions containing antibiotic compound (250ng/ml) and other ions were prepared and the developed procedure was applied. The effect of the species is considered as interference when the signal in the presence of the matrix ion results in a deviation in the recovery of the antibiotic compound by ≥ ± 5%. The results for single ratio of the ampicillin to the interferent (1: 100) are depicted in Fig. 5.49. In this figure, peak tailing appears due to the elution with higher volumes of the eluent than the recommended one but the peak height still proportional to the analyte concentration. The data show relevant selectivity of the sorbent to the antibiotic compound with most of the studied foreign ions. For example, the strongest effect on the cefaclor extraction takes place in presence of aspartic acid; it goes down to 50 %. Ampicillin showed the least interference in sorption percentage with all ions except glucose (78 %), aspartic acid (65%), caffeine (81%) and uric acid (70%). Therefore, it is very important to determine the tolerable concentration ratios with respect to specified concentration of β-lactam antibiotic for interference at ≥ ± 5% level. The data obtained are presented in Table 5.22. The tolerated ratios were always higher than those commonly found in the majority of real samples which confirm a satisfactory selectivity of the proposed procedure.

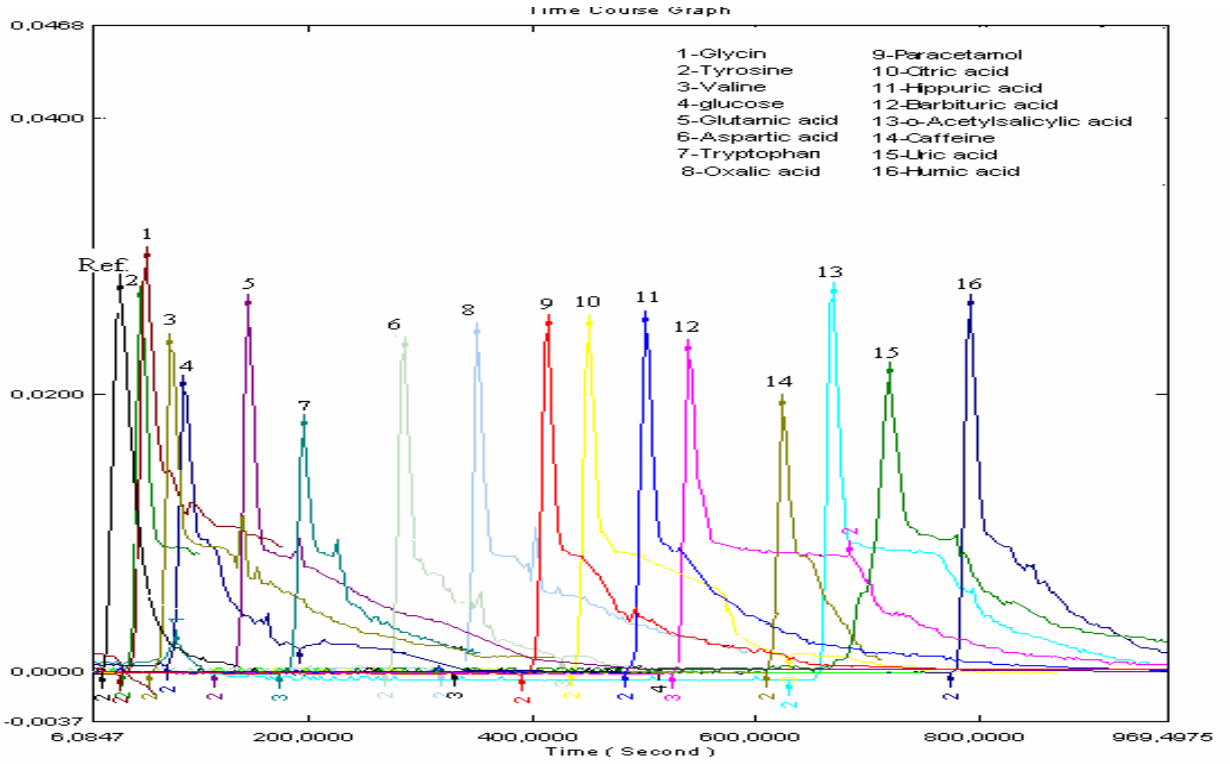


Fig. 5.49: Interference effect for ampicillin 250 ng/ml (at ratio 1:100), pH 8.5, 2 min sample preconcentration time, elution by 0.15 mol/l HCl at 210 nm.

Table 5.22: Interference effect on BLAs determination

Foreign Substance	Maximum acceptable ratio			
	Cefaclor	Amoxicillin	Ampicillin	Cefotaxime
Glycine	1:60	1:80	1:110	1:80
Tyrosine	1:60	1:60	1:90	1:80
Valine	1:70	1:60	1:80	1:90
Glutamic acid	1:70	1:50	1:100	1:60
Tryptophan	1:60	1:40	1:90	1:50
Glucose	1:80	1:70	1:70	1:70
Barbaturic acid	1:100	1:30	1:80	1:70
Acetylsalicylic acid	1:70	1:50	1:100	1:60
Aspartic acid	1:50	1:40	1:60	1:60
Paracetamol	1:100	1:30	1:90	1:90
Caffaeine	1:90	1:40	1:80	1:70
Oxalic acid	1:100	1:30	1:90	1:60
Citric acid	1:100	1:40	1:90	1:130
Hippuric acid	1:60	1:40	1:90	1:100
Uric acid	1:90	1:50	1:70	1:100
Humic acids	1:80	1:40	1:100	1:90

5.2.7.9 Precision and Sorbent Durabilty

The accuracy of the proposed flow analysis system was investigated in order to judge its feasibility in analytical applications. Therefore, the precision of the continuous preconcentration procedure, calculated as relative standard deviation in sample solutions containing antibiotics at 250 ng/ml and 2 min preconcentration time. In this consequence, the same antibiotic solution was preconcentrated and eluted successively several times and the signal height was recorded in each cycle. The data belongs to cefaclor and ampicillin shows that, the RSD are 2.3 and 4.5 % respectively by fifteen measurements which are considered satisfactory values (Fig. 5.50). Additionally, the life time of the mincolumn in the on-line SPE system was evaluated by comparing the analytical signal of the same BLAs concentration in different times. The result showed that the sorbent can be used for more than 15 times per day for about 6 months without any change in its retention capacity which indicates no loss in its analytical sensitivity. This suggests the unlimited use of the minicolumn under such conditions. Also, anchoring of the reagent in the support is the principle reason for the long lifetime of the column, because leaching of the reagent from the column is minimized. Another factor that can contribute to the long lifetime is the system design which permits washing with water before each preconcentration step.

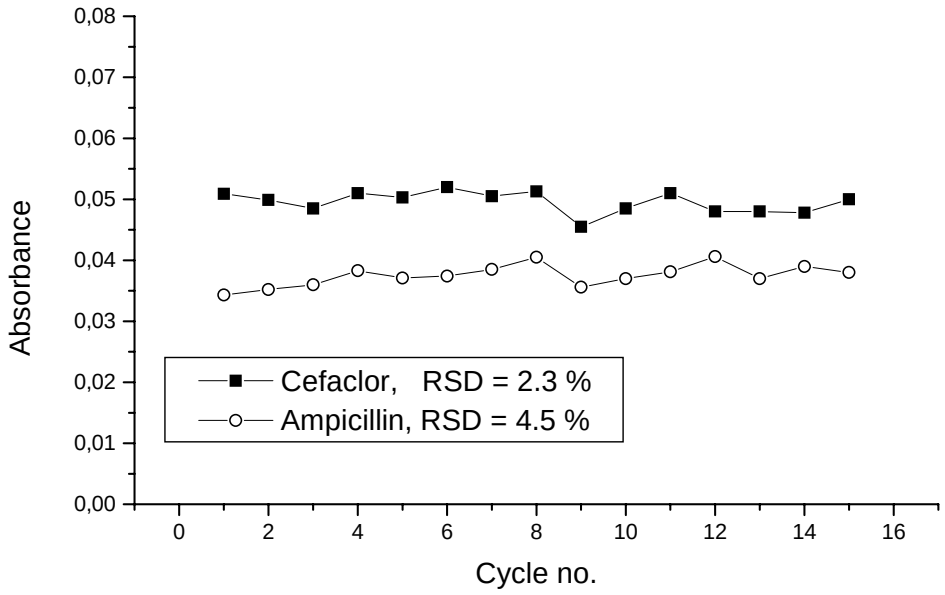


Fig. 5.50: Method precision for cefaclor and ampicillin (250 ng/ml), 2 min sample preconcentration time, 3 ml/min carrier flow and desorption with 200 uL 0.15 M HCl at 210 nm

5.2.7.10 Analytical Performance

The flow system methodology has been found to produce linear analytical fits in the range of concentration from 40 to 600, 50 to 500, 25 to 400 and 30 to 500 µg/ml of cefaclor, amoxicillin, ampicillin and cefotaxime, respectively, by employing 120 seconds preconcentration time. This fit could be well represented by the following equation for Cefaclor: A = (8.92156E-5) C+ 0.00157, R = 0.99513; For Amoxicillin: A = (5.87530E-5)C – 0.00334, R = 0.99317; for Ampicillin: A = (4.1675E-5)C – 0.00158, R = 0.99182; and for Cefotaxime: A = (7.5914E-5)C – 0.00228, R = 0.99415. The limit of detection (LOD) estimated from three times the standard deviation of five measurements of the blank divided by the slope of the calibration curve are 3.3, 5.1, 7.0 and 3.8 ng/ml for cefaclor, amoxicillin, ampicillin and cefotaxime respectively. Similarly, the limit of quantification (LOQ) is 11.0, 17.1, 23.6 and 12.8 ng/ml. The calibration graphs under optimized chemical and flow conditions for the antibiotic compounds are depicted in Fig. 5.51, and the corresponding analytical signals for amoxicillin is shown below in Fig. 5.52.

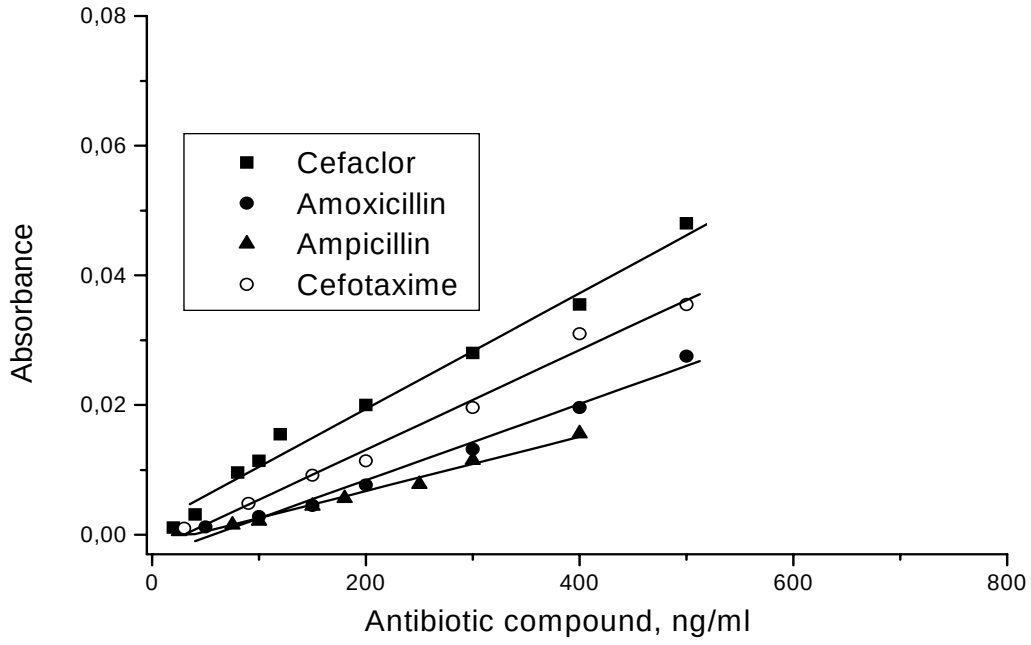


Fig. 5.51: Linear range of BLAs with PCTDD - PUF sorbent:2 min preconcentration time, 3 ml/min carrier flow rate, desorption with 0.15 mol/l HCl at 210 nm

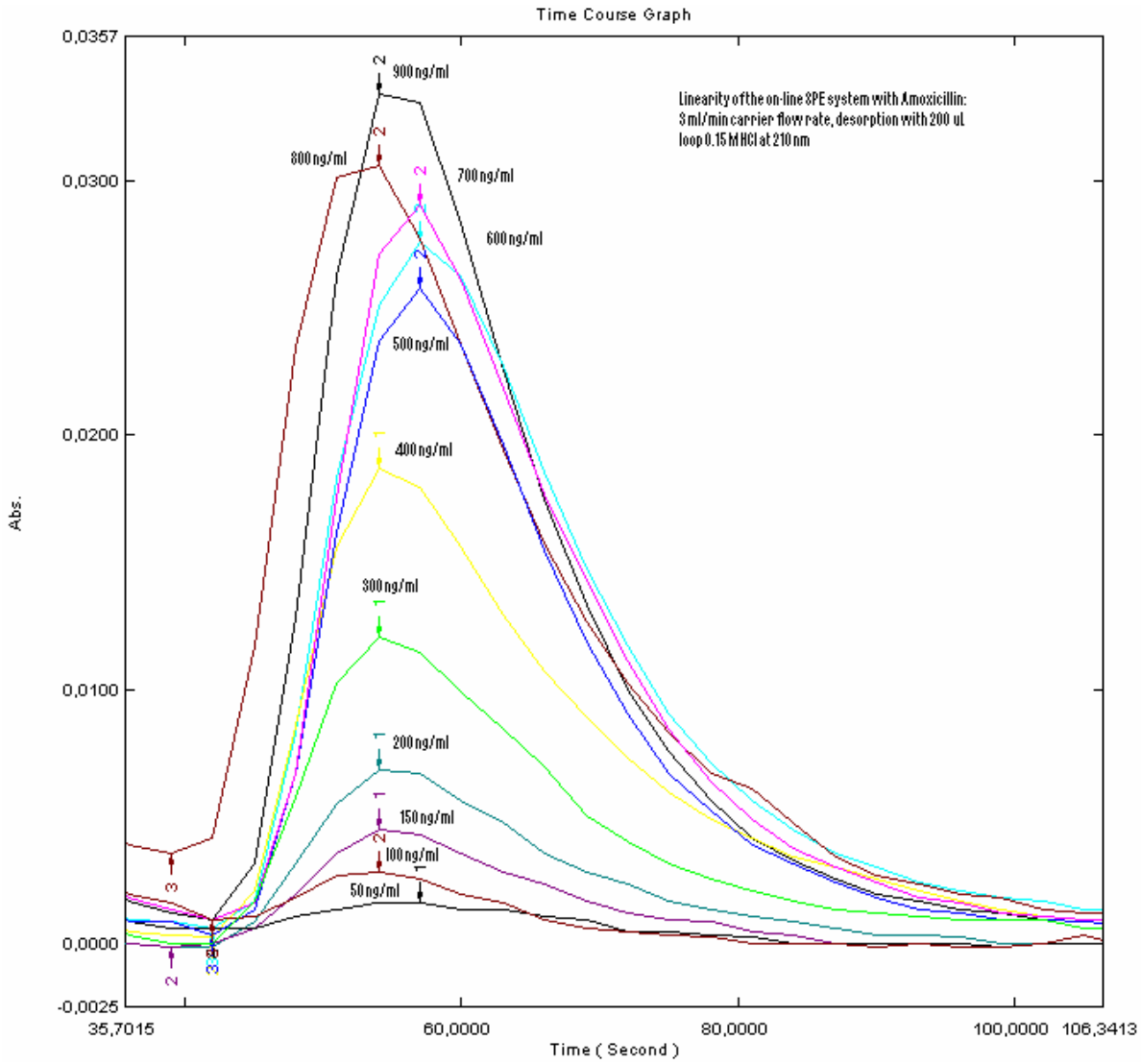


Fig. 5.52: Linearity range of analytical signals of amoxicillin at 3 ml/min sample flow rate, 2 min preconcentration time, and desorption with 200 µl 0.15 mol/l HCl at 210 nm.

5.2.7.11 Preconcentration

The preconcentration step is pivotal point in this study. Our attempt is to achieve as high CF as possible. Higher value of CF means the preconcentration system is able to detect the analyte at very low concentration levels with relevant accuracy by its accumulation in smaller eluate volume prior to the detection stage. The experimental CF [28] of the system under investigation calculated as the ratio of the slopes of the calibration graphs with and without

preconcentration. It was found to be 38, 39, 36 and 21 for cefaclor, amoxicillin, ampicillin and cefotaxime, respectively. Figures 5.53 and 5.54 show the analytical signals and the calibration curves in case of cefotaxime with and without preconcentration in the concentration range 200 – 500 ng/ml. Obviously, the highest value of CF is obtained for amoxicillin which is comparable to that for cefaclor while ampicillin indicated an intermediate value. On the other hand, cefotaxime has the lowest CF among those antibiotics. It is about 45, 46 and 41 % less than cefaclor, amoxicillin and ampicillin respectively. The CF is directly proportional to the retention affinity of the analyte to the sorbent. In other words as the antibiotics compound becomes strongly adsorbent in the column as higher CF will be achieved. An explanation of this may be attributed to the difference in the chemical structure of each compound and the diversity in the nature of the side group which was previously explained in section *5.3.7.7.*

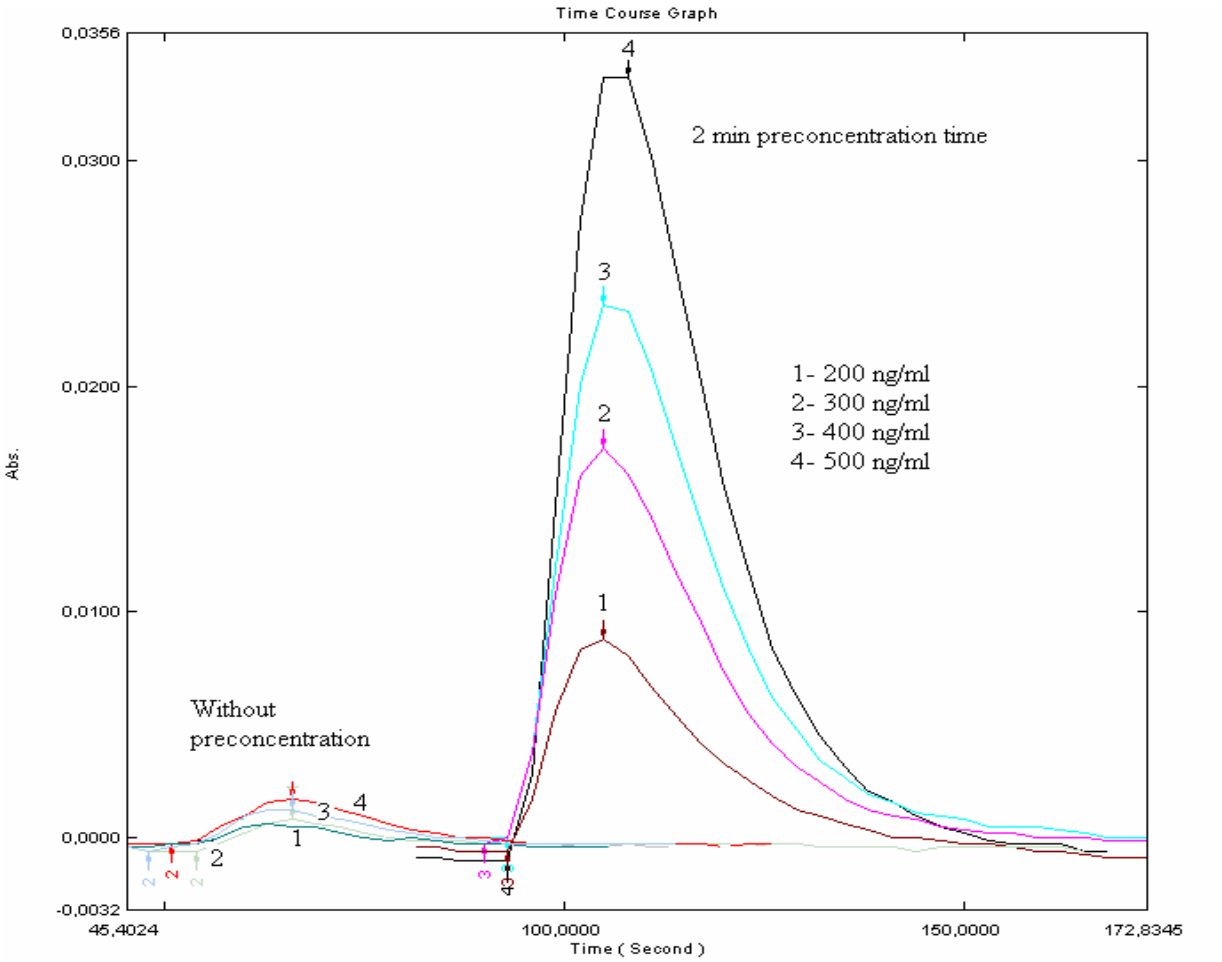


Fig. 5.53: Analytical signals of cefotaxime with and without preconcentration: 3 ml/min sample flow rate, desorption at 1.5 ml/min with 200µl 0.15 mol/l HCl at 210 nm.

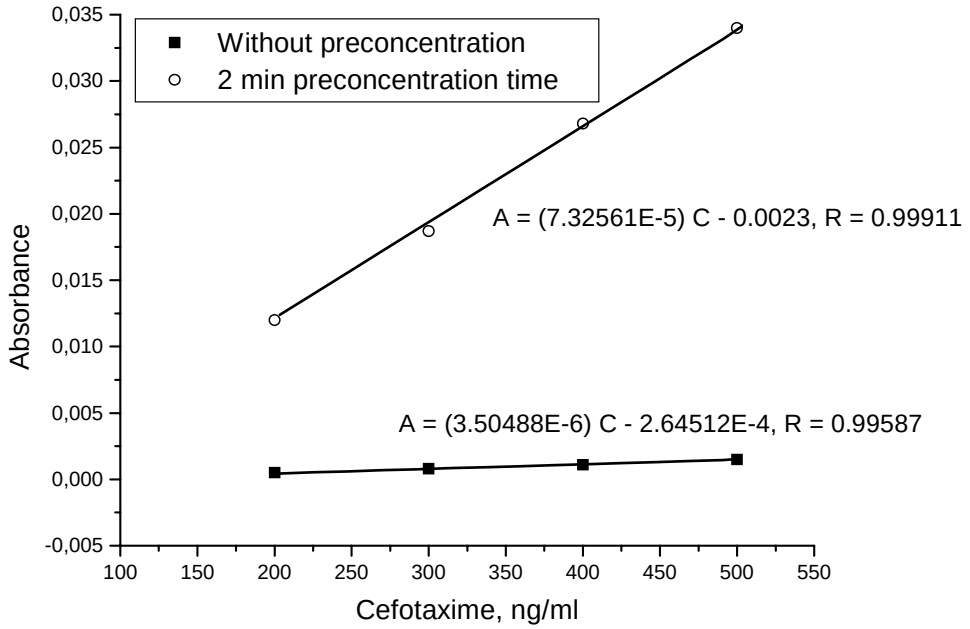


Fig. 5.54: Calibration graphs of cefotaxime with and without preconcentration:3 ml/min sample flow rate,desorption with 0.15 M HCl at 1.5 ml/min and 210 nm

Table 5.23: Calibration slopes with and without preconcentration and CFs for BLAs

Parameter/BLAs	Slope No preconcentration	Slope 2 min preconcentration	CF
Cefaclor	2.31 E-6	8.89 E-5	38
Amoxicillin	2.14 E-6	8.26 E-5	39
Ampicillin	1.16 E-6	4.19 E-5	36
Cefotaxime	3.50 E-6	7.33 E-5	21

Table 5.24: Analytical performance data of the on-line SPE preconcentration procedure

Parameter	*Cefaclor*	*Amoxicillin*	*Ampicillin*	*Cefotaxime*
Concentration factor	*38*	*39*	*36*	*21*
Preconcentration time (s)	*120*	*120*	*120*	*120*
Sample volume (ml)	*6*	*6*	*6*	*6*
Sample frequency (h^{-1})	*12*	*12*	*12*	*12*
Linear range (ng/ml)	*40-600*	*50-500*	*25-400*	*30-500*
LOD (ng/ml)	*3.3*	*5.1*	*7.0*	*3.8*
LOQ (ng/ml)	*11.0*	*17.1*	*23.6*	*12.8*
Precision (RSD%, n=15)	*2.3*		*4.5*	

5.2.8 Separation of the BLAs Using Micellar Mobile Phase

Separation of analytes is one of the goals to use the continuous flow system in the determination of these organic compounds. Although liquid chromatography is the most widely used technique in separation of these compounds, yet it is not known, till now, the utilization of such simple setup in this purpose.

Micellar mobile phases have some advantages over usual hydro-organic mobile phases; e.g. they permit: the unique separation selectivity, which is not obtained with usual hydro-organic mobile phases; stable detector responses, particularly for electrochemical detectors, against gradient elution; and direct injection of samples containing very complex matrices, such as urine and blood. From these view points, a number of papers have been published which focused on the separation of organic or inorganic compounds, the retention mechanisms, and/or the applicability [207]. Several ionic compounds such as phenols [207], sugars [208] and amino acids [209] have been separated using micellar mobile phases.

Herein, we attempt to achieve separation pattern based on the use of constant mobile phase (isocratic separation) that can distribute these compounds along longer analytical column. Thus, a 25 cm 3 mm i.d polyethylene column packed with the PCTDD – PUF sorbent was employed

where a micellar mobile phase constitutes from ammonia buffer pH 7 and CTAB (1 x 10^{-3} mol/l, CMC = 3x10^{-4}) passed through at 0.15 ml/min. Since the sorbent has positively charged active sites and the mobile phase is cationic surfactant (positively charged) while the antibiotic compound is negatively charged, the analyte will distribute itself between the stationary phase (sorbent) and the mobile phase according to the difference in attraction forces in both directions. Fig. 5.55 shows the chromatograms for each antibiotic compound under the recommended conditions for separation. Both the pH and CTAB concentration of the mobile phase was examined. It was found that the mobile phase of pH 7 and 1 x 10^{-3} mol/l CTAB gives the best results. The retention time (R_t) is reproducible in the mixture and it was matched to that for the single chromatogram as shown in Table 5.25. The recovery is calculated from the peak area of the antibiotic in the mixture divided by the peak area obtained by single antibiotic sample.

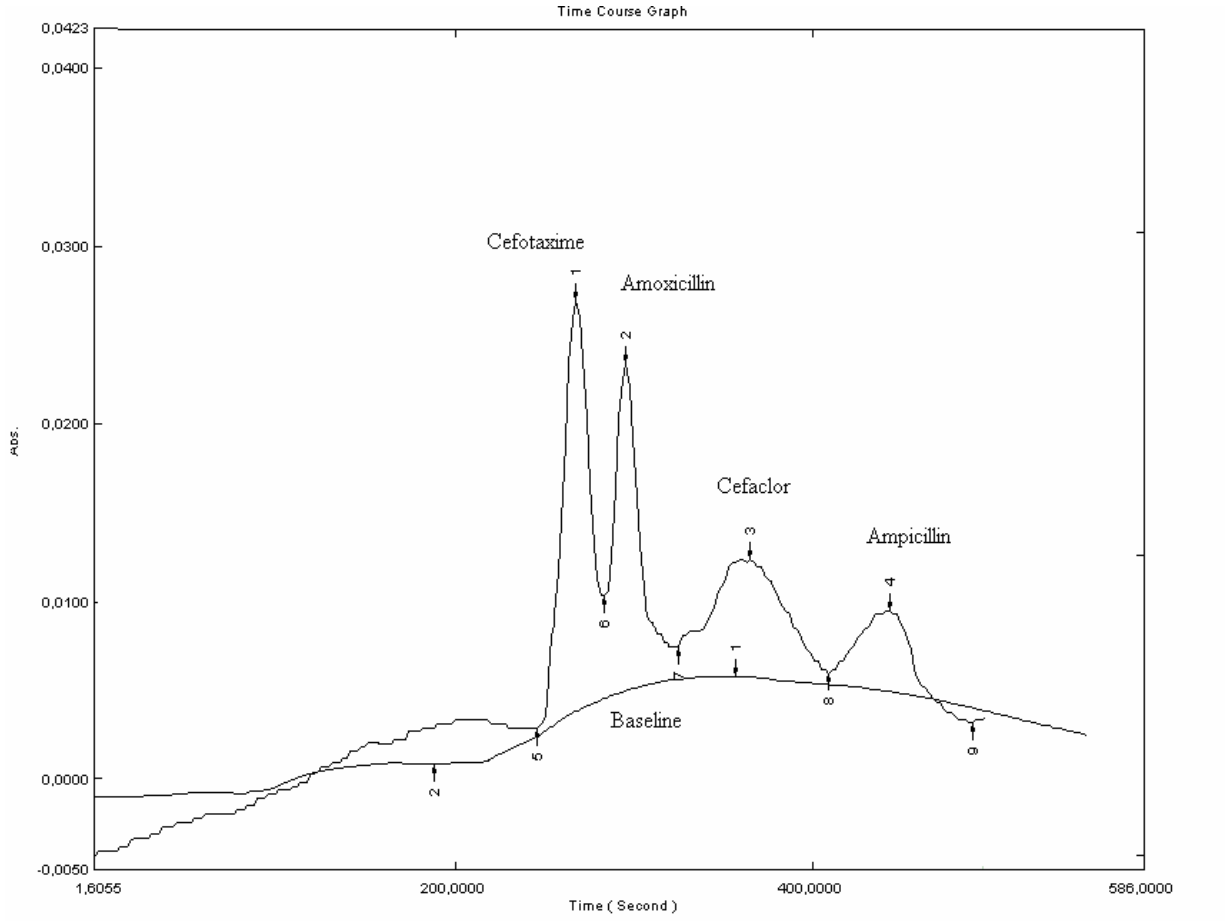


Fig.5.55: Separation of cefaclor, amoxicillin, ampicillin and cefotaxime using the PCTDD –PUF sorbent (25 cm x 3mm i.d) column, mobile phase consists of: 0.15 mol/l ammonia buffer pH 7 and 1x10^{-3} mol/l CTAB, at flow rate 0.15 ml/min and 210 nm.

Table 5.25: R_t and recovery (%) for BLAs

Parameter	Amoxicillin	Cefotaxime	Cefaclor	Ampicillin
R_t, (single) sec	272	308	370	449
R_t,(mixture, N=3) sec	270±7	300±10	380±15	450±5
Recovery (%)	90	86	92	96

The separation pattern indicated in Fig 5.55 shows some overlapping between the amoxicillin and cefotaxime signals which affects the recovery of these two compounds where it is 90 and 86 % respectively. On the other hand, cefaclor and ampicillin are better separated from each others and from the other two BLAs than the first two compounds. Their recoveries are quantitative; it is 92 and 96 % respectively.

The base line has the constant trend in the region of BLAs peaks but due to some technical reasons it is not quite stable. However, it approximately fits with the base line for the signals due to cefaclor and ampicillin. In case of overlapping peaks due to cefotaxime and amoxicillin the peak area was calculated by integration of the absorbance from point A till B and from B to C for cefotaxime and amoxicillin respectively as indicated in the model diagram in Fig. 5.56. Because the two peaks have relatively similar peak heights and band width, it could be expected there will be similar overlapping area on both sides of the vertical line BD. Therefore the point B was recognized the end point for cefotaxime signal and in the same time it is the starting point for amoxicillin signal. The peak area is dependent on both the peak height and the bandwidth at 50 % peak height [210].

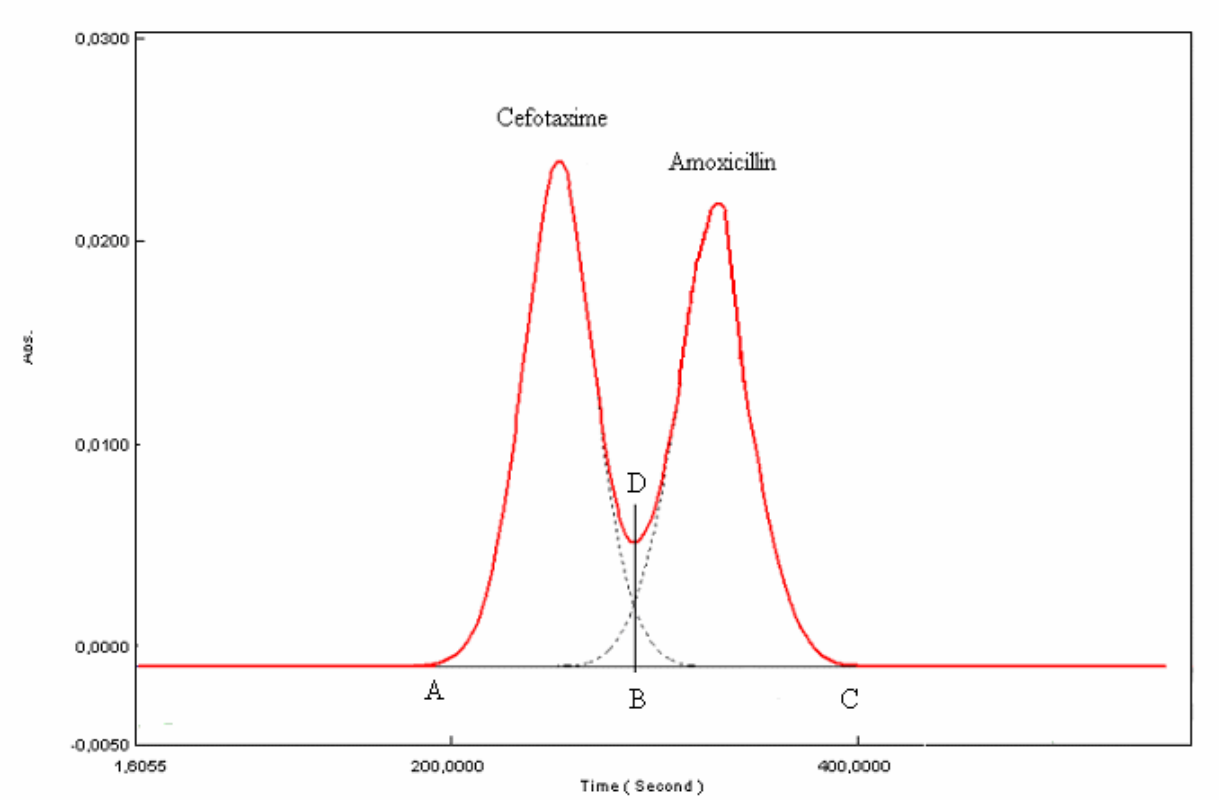


Fig.5.56: Model draw to show the influence of peak overlapping on the peak areas for cefotaxime and amoxicillin

5.2.9 Application to real samples

The developed FIA-PUF preconcentration system could preconcentrate and determine the antibiotics in aqueous and biological matrices. Two natural samples, namely human urine and cow milk, and pharmaceutical formulations were analyzed by this procedure. The results for three time analysis of urine and pharmaceuticals are listed in Tables 5.26 and 5.27 respectively. For spiked urine samples, it was found satisfactory results where the recovery varies from 95 to 109 % and RSD 2.1 – 5.4 %. Moreover, the amount of sorbent was increased in the analysis of pharmaceuticals since the sample concentration is too high. One gram sorbent column was employed to give efficient recovery. In Table 5.27, the recovery is found between 83 and 99 % and the RSD varies from 0.1 – 9.7 %. Although, high RSD % obtained in the analysis of Bacticlor but it is still less than the allowed value in the analysis of real samples (10 %).

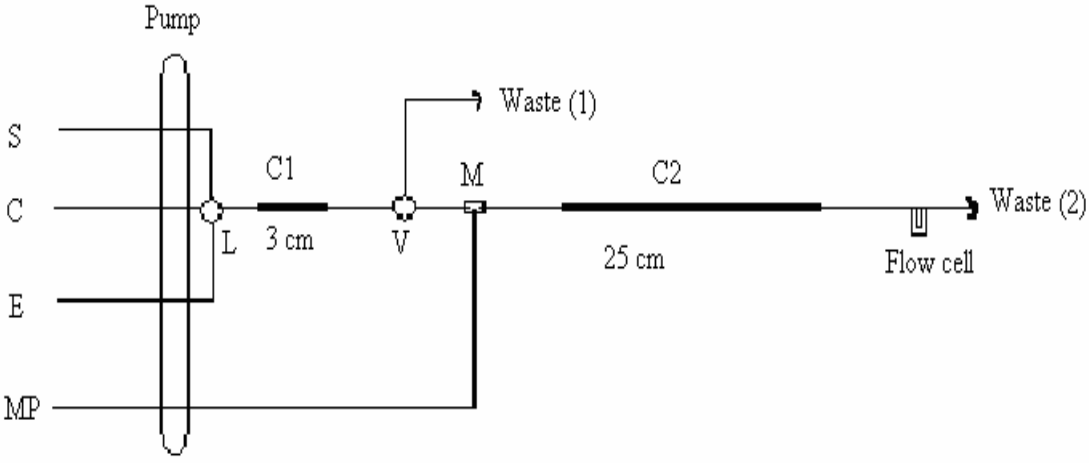
Table 5.26: Determination of BLAs in spiked human urine using the proposed procedure and 2 min preconcentration time.

BLAs	Spiked (ng/ml)	Absorbance	Found (ng/ml)	Found (ng/ml) (mean±S.D,n=3)	Recovery (%)	RSD (%)
Cefaclor	100	0.0100	94	95±5.1	95	5.4
	100	0.0106	101			
	100	0.0097	91			
	200	0.01957	202	198±5.0	99	2.6
	200	0.01937	199			
	200	0.01873	192			
Amoxicillin	100	0.0027	107	109±2.5	109	2.3
	100	0.0028	109			
	100	0.003	112			
	200	0.008	199	194±4.5	97	2.1
	200	0.0077	194			
	200	0.0075	190			
Ampicillin	100	0.0027	103	104 ±6.0	104	4.1
	100	0.0023	98			
	100	0.003	110			
	200	0.0064	191	197±7.1	99	3.6
	200	0.0070	205			
	200	0.0066	196			
Cefotaxime	100	0.006	109	106±3.5	106	3.3
	100	0.0058	106			
	100	0.0055	102			
	200	0.013	201	191±8.9	96	8.7
	200	0.012	188			
	200	0.0115	184			

Table 5.27: Analysis of pharmaceutical samples using the on-line SPE procedure with 1.0 g sorbent column and three replicate measurements.

Sample	Reported	Injected (µg)	Found, ng (Mean ±SD)	Recovery (%)	RSD (%)
Ampicillin	500 mg	5	4.94±0.40	99	8.7
Bacticlor	250 mg	2.5	2.32±1.20	93	9.7
Clorocef	250 mg	2.5	2.10±0.25	83	2.3
Cefaclor acis	500 mg	5	4.82±0.05	96	0.1
Amoxicillin acis	500 mg	5	4.61±0.06	92	1.4

In the analysis of cow milk, spiked samples were subjected to analysis according to the recommended continuous procedure and standard addition method. Since the sample might contain mixture of antibiotics, a separation step is necessary before detection of the analyte (all measurement is carried out at single wavelength). Accordingly, the separation column was connected along with the preconcentration column according to the setup illustrated in Fig. 5.57 shown below. The effluent of the sample is not allowed to pass through the separation column (C2) or the flow cell (this could be done by switching the valve (V) so that the effluent goes to waste route (1)) since it would require longer time for cleaning (more than one hour for the system to get the base line). Firstly, the sample was preconcentrated on the column C1. After this, the eluent E is injected at flow rate suitable for separation on C2 using the recommended mobile phase and the signal is recorded. Standard addition curves are obtained and the amount of antibiotics in the sample is calculated (Fig. 5.58) and the results are reported in Table 5.28.



S: Milk Sample at pH 9.0, C: Carrier (water), E: Eluent (0.15 mol/l HCl), MP: Mobile Phase C1: Preconcentration column, C2: Separation column, L: Eluent loop (200 uL), V: Three way valve, M: Manifold (Mixer).

Fig. 5.57: Preconcentration/separation system applied to the analysis of cow milk.

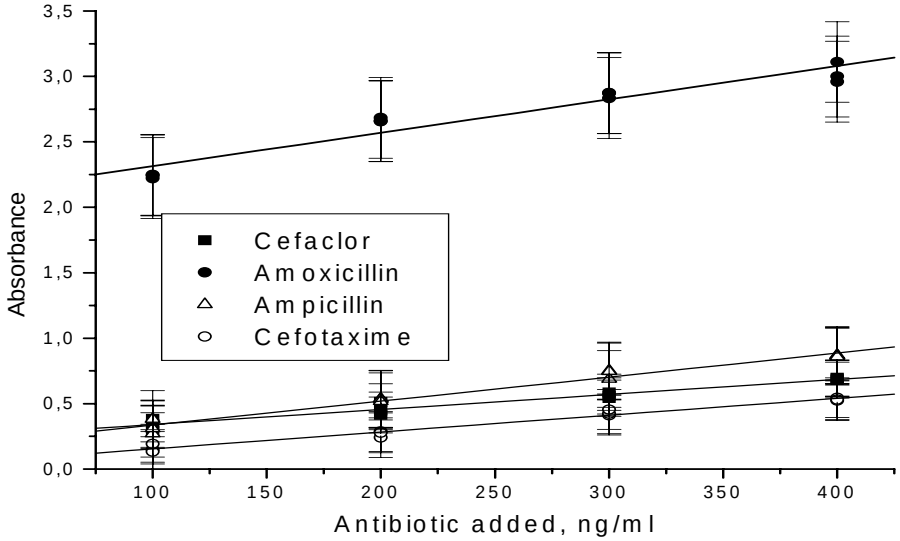


Fig. 5.58: Standard addition lines for determination of BLAs in cow milk sample using the recommended flow analysis procedure (vertical lines represent the error bars).

Table 5.28: Analysis of spiked cow milk using the proposed procedure.

Penicillin	Spiked Antibiotic (µg/l)	Recovery (%) (mean± S.D)	RSD%
Cefaclor	100	89±8.0	9.2
	200	102±7.9	8.1
	300	94 ±4.5	4.0
Amoxicillin	100	107±6.3	5.9
	200	93±5.1	5.4
	300	101±7.9	7.6
Ampicillin	100	92±8.0	8.8
	200	83±1.7	2.0
	300	90±4.3	4.8
Cefotaxime	100	94±7.5	8.6
	200	97±9.0	9.3
	300	88±4.5	5.2

Three replicates were carried out for each concentration

The proposed sorbent can preconcentrate and separate the examined antibiotics even in the presence of matrix compounds in milk. The chromatographic technique involved an isocratic mobile phase with UV detection. The recovery values were found to be within the range 83- 107 % and the RSD from 2.0 – 9.2 %. As a fact due to the strong background signal derived from the milk itself the increase in absorbance signal resulted from addition of antibiotic compound is very small. This leads to higher standard deviation in the obtained data as can be seen from the vertical lines due to the error bars in Fig. 5.58. However the method is considered satisfactory since a separation step was carried out prior to the determination to remove most interfering materials. Finally, the method under consideration is applicable in determination of these antibiotics in urine, pharmaceutical and milk samples where their concentration within the LOD of this procedure. However, BLAs in surface water exist at concentration down to ng/l therefore it could not be estimated by this on-line SPE procedure.

CONCLUSIONS AND FUTURE WORK

In this work, a fast and simple preconcentration/separation procedure was developed for the determination of heavy metals or β-lactam antibiotics. Functionalized sorbents, such as polyurethane foams, are very promising materials for application in on-line and off-line pre-concentration systems.

PUF has confirmed excellent properties as support for chemically modified sorbents using the pendant functional groups. In contrast to other organic polymers such as amberlite XAD-2 where the ligand size is a limiting factor, PUF provides large surface area so that ligands with large molecular weights (e.g. PCTDD) could be chemically immobilized.

The new developed sorbents were successfully employed for the determination of the studied analyte in natural and biological materials. The results obtained from the analysis of tap water, seawater apple leaves, urine, pharmaceuticals and milk samples has confirmed the reliability of the proposed method and its application to various samples. The synthesized foam columns were recycled several times without significantly affecting their uptake capacities.

The on-line procedure developed in this study is a precise and accurate alternative to conventional methods for determining BLAs in pharmaceutical and biological samples. The proposed method is very simple, sensitive, inexpensive and eco-friendly and it shows high tolerance to interference ions. Due to good analytical features such as detection limit, enrichment factor and precision the proposed procedure has been demonstrated to be applicable for trace analysis.

One of the most interesting in this work is the use of the simple on-line system for separation of antibiotics. Although, the separation pattern has not too smooth base line as in case of liquid chromatographic separations yet, it could be applied for simple analysis. The results showed relevant separation under the operated conditions. Besides, better separation can be achieved if more development of the system designs especially the inner dimensions of the flow cell. Some studies on this direction should be a focus of future work.

REFERENCES

[1] A. Raukema; Dynamics of chemisorption, *PhD thesis*, University of Amsterdam, The Netherlands, 13 April 1995.

[2] S. Mitra; *Sample preparation techniques in analytical chemistry*, Copyright 2003 by John Wiley & Sons, Inc. p77.

[3] A. D. Site; *J Phys Chem Ref. Data* 30 (2001)187.

[4] S. T. Bruhn, T. Seibicke, H. R. Schulten, P. Leinweber, *J Environ Qual* 33(2004) 1331.

[5] E. R. Graber; M. Borisover; *Geophysical Research Abstracts*, 5 (2003) 02196.

[6] F.L.Slejko, *Adsorption Technology*; Marcel Dekker, New York, 1985.

[7] G. Wedler; *Chemisorption: An experimental Approach*, Copyright 1976, p1.

[8] M. A. Anderson and A. J. Rubin; *Adsorption of Inorganics at Solid-Liquid Interfaces*, copyright 1981, p161.

[9] H. Wen; old e-ept, 1 (2004) 1.

[10] P. A. Webb; *Introduction to Chemical Adsorption Analytical Techniques and their Applications to Catalysis,* Micromeritics Instrument Corp., Norcross, Georgia 30093, January 2003.

[11] R. J. Lewis; *Sr. Hawley's Condensed Chemical Dictionary*, 13th Edition. Copyright 2000, John Wiley & Sons, Inc.

[12] K. B. Woodburn, P. S. C. Rao, M. Fukui, and P. Nkedi-Kizza; *J Contam Hydrol* 1 (1986) 227.

[13] P. S. C. Rao, A. G. Hornsby, D. P. Kilcrease, and P. N. Kizza; *J Environ Qual.*14 (1985)376.

[14] S. Karickhoff, D.S. Brown, and T. A. Scott; *Water Res* 13(1979)241.

[15] R. P. Schwarzenbach, P. M. Gschwend, and D. M. Imboden; *Environmental Organic Geochemistry*, Copyright 1993, John Wiley & Sons, Inc.

[16] C. T. Chiou, L. J. Peters, and V. H. Freed; *Science* 206 (1979) 831.

[17] IUPAC Compendium of Chemical Terminology, 2nd Edition (1997).

[18] N. J. K. Simpson; *Solid phase extraction, principles, techniques and application*s, copyright 2000, Marcel Dekker, Inc. pp2.

[19] P. M. Panagiotopoulou and M. Tsimidou; *Grasas Aceites,* 53 (2002) 84.

[20] V. Camel; *Spectrochimica Acta Part B: Atomic Spectroscopy*, 58 (2003) 1177.

[21] J. R. Dean; *Extraction Methods for Environmental Analysis*, Copyright © 1998 John Wiley & Sons Ltd.

[22] *Guide to solid phase extraction*, Supleco, 1998, pp3.

[23] D. Harvey; *Modern Analytical Chemistry*, Copyright 2000 by The McGraw-Hill, pp590.

[24] R. P. W. Scott; *The mechanism of retention*, Chrom-Ed Book Series. Copyright 2003 by library4science, LLC, pp 1.

[25] L. A. Berrueta, B. Gallo, F. Vicente, *Chromatographia* 40(1995) 7.

[26] J. B. B. da Silva, S. P. Quináia · M. C. E. Rollemberg; *Fresenius J Anal Chem* 369 (2001)657.

[27] A. N. Anthemidis, G. A. Zachariadis, J. A. Stratis; *Talanta* 58 (2002) 831.

[28] V.A. Lemos, S.L.C. Ferreira; Anal *Chim Acta* 441 (2001) 281.

[29] R. P. W. Scott; *Silica gel in LC*, copyright 2003 by Libraray4Science, pp1&102.

[30] L. I. Sokolova and A. P. Chernyaev; *J Anal Chem* 56 (2001) 1032.

[31] J. L. G. Ariza, J.A. Pozas, I. Giraldez and E. Morales; *Analyst* 124 (1999)75.

[32] O. Evans, B. J. Jacobs and A. L. Cohen; *Analyst* 116 (1991)15.

[33] W. Liu, Z. Zhang, and Z. Liu; Anal *Chim Acta* 592 (2007) 187.

[34] S. A. Nabi, E. Laiq, and A. Islam; *Acta chromatographica* 14 (2004)92.

[35] N. Masqué, R.M. Marcé and F. Borrull; *Trends Anal Chem* 17 (1998)384.

[36] L. Elci, L. M. Soylak, A. Uzun, E. Büyükpatir and M. Dogan; *Fresenius J Anal Chem* 368 (2000)358.

[37] O. Abollino, M. Aceto, M. C. Bruzzoniti, E. Mentasti and C. Sarzanini; *Anal Chim Acta* 375 (1998)293.

[38] K. Isshiki, Y. Sohrin, H. Karatani and E. Nakayama; *Anal Chim Acta* 224 (1989)55.

[39] A. G. Howard and M. H. Arbab-Zavar; *Talanta* 26 (1979)895.

[40] S. L. C. Ferreira, C. F. de Brito, A. F. Dantas, N. M. Lopo de Araujo and A.C.S. Costa; *Talanta* 48 (1999)1173.

[41] A. N. Masi and R. A. Olsina; *Fresenius J Anal Chem* 357 (1997)65.

[42] R. Saxena, A. K. Singh and S. S. Sambi; *Anal Chim Acta* 295 (1994) 199.

[43] R. Saxena, A. K. Singh and D. P. S. Rathore; *Analyst* 120 (1995)403.

[44] P. K. Tewari and A. K. Singh; *Analyst* 125 (2000)2350.

[45] R. Saxena and A. K. Singh; *Anal Chim Acta* 340 (1997)285.

[46] P. K. Tewari and A. K. Singh; Analyst 124 (1999)1847.

[47] P. K. Tewari and A. K. Singh; *Talanta* 53 (2001)823.

[48] C. W. Huck and G. K. Bonn; *J Chromatogr A* 885 (2000)5.

[49] J. M. Serrano, M. Silva; *Electrophoresis* 28 (2007)3242.

[50] J. W. Lee, H. J. Jung and H. Moon; *Korean J Chem Eng* 14(1997) 277.

[51] E. Benito-Peña, A.I. Partal-Rodera, M. E. León-González and M.C. Moreno-Bondi; *Anal Chim Acta* 556 (2006) 415.

[52] V. Porta, E. Mentasti, C. Sarzanini and M. C. Gennaro; *Talanta* 35 (1988)167.

[53] M. S.S. Curren, J. W. King; *J Chromatogr A* 954 (2002)41.

[54] S. L. C. Ferreira, D. S. Jesus, R. J. Cassella, A. C. S. Costa, M. S. Carvalho and R. E. Santelli; *Anal Chim Acta* 378 (1999)287.

[55] S. P. Quinaia, J. B. B. Da Silva, M. C. E. Rollemberg and A. J. Curtius; *Talanta* 54 (2001)687.

[56] I. A. Veselova and T. N. Shekhovtsova; *Anal Chim Acta* 413 (2000)95.

[57] C. Shuyu, Z. Zhifeng and Y. Huaming; *Anal Chim Acta* 451 (2002)305.

[58] S. Tsakovski, K. Benkhedda, E. Ivanova and F. C. Adams; *Anal Chim Acta* 453 (2002)143.

[59] I. A. Kovalev, L.V. Bogacheva, G. I. Tsysin, A. A. Formanovsky and Y. A. Zolotov; *Talanta* 52 (2000) 39.

[60] H. Kumagai, Y. Inoue, T. Yokoyama, T. M. Suzuki and T. Suzuki; *Anal Chem* 70 (1998) 4070.

[61] L. V. Dmitryenko and D. K. Hale; *J Chem Soc* 1965, 5570.

[62] M. N. Abbas; *Anal Lett* 36 (2003) 1231.

[63] S. M. A. El-Ola; *Polym plastics technol engineering* 46 (2007) 831.

[64] A. A. MacKay and B. Canterbury; *J Environ Qual* 34 (2005) 1964.

[65] H. Kumagai, T. Yokoyama, T.M. Suzuki*b* and T. Suzuki*; Analyst* 124 (1999) 1595.

[66] B. Wen, X. Q. Shuan and J. Lian; *Talanta* 56 (2002) 681.

[67] Q. Tian, C. D. Gomersall, A. Wong, P. Leung, G. Choi, G. M. Joynt, P. Tan and J. Lipman; *Inter J Antimicrob Agents* 28 (2006) 147.

[68] F. Sinner, M. R. Buchmeiser, R. Tessadri, M. Mupa, K. Wurst and G. K. Bonn; *J. Am. Chem. Soc.* 120 (1998) 2790.

[69] C. A. Masce, Y. Wang, K. Loftin, and M. Meyer; *J. Envir. Engrg.* 128 (2002) 253.

[70] G. H. Wagman, J. V. Bailey, and M. J. Weinstein; *Antimicrobial Agents and Chemotherapy* 7(1975) 316.

[71] B. B. Prasad, B. Shouri; *React funct Polym* 55 (2003) 159.

[72] O. Brüggemann, K. Haupt, L. Ye, E. Yilmaz and K. Mosbach; *J. Chromatogr A* 889 (2000)15.

[73] P. Vermette, H. J. Griesser, G. Laroche and R. Guidoin; *Biomedical Applications of Polyurethanes,* copyright 2001 Eurekah.com.

[74] I.G. Farben; *German Patent* 728 (1937) 981.

[75] R. B. Seymour, G. B. Kauffman*; J. Chem. Ed.* 69 (1992) 909.

[76] B. Feske, N. V. Las Vegas; Alliance *for the Polyurethane Industry Technical Conference* (October 2004) Retrieved on 2007-08-01.

[77] T. Niemeyer, M. Patel, and E. Geiger; Alliance *for the Polyurethane Industry Technical Conference* (September, 2006) Retrieved on 2007-08-01.

[78] R. Giuffria; *J polym Sci* 60 (1962) 91.

[79] S. Paliigyi, and T. Braun; Analyst 106 (1981) 1326.

[80] M. Szycher; *Szycher's Handbook of Polyurethane*, CRC Press LLC, Florida (1999).

[81] D. Randall and S. Lee; *The Polyurethane Book*, Wiley, NY (2002).

[82] T. Romaškevič1, S. Budrienė, K. Pielichowski and J. Pielichowski; *Chemija*. 17 (2006) 74.

[83] *Cellular plastics,* Proc. Conf., Natick, Massachutessetts, 1996, National Academy of sciences, National research Council,Washington, D.C., 1967.

[84] C. J. Benning; *Plastic Foams: the Physics and Chemistry of Product Performance and Process Technology,* Wiley and Sons NY, Vol.1 (1969).

[85] R. J. Cassella, R. E. Santelli, A.G. Branco, V.A. Lemos, S. L. C. Ferreira, M. S. Carvalho; *Analyst* 124 (1999) 805.

[86] T. Braun, J. D. Navratil, A. B. Farag; *Polyurethane foam Sorbents in Separation Science*, CRC Press Inc, Boca Raton (1985).

[87] V. A. Lemos, M. S. Santos, E. S. Santos, M. J. S. Santos, W. N. L. dos Santos, A. S. Souza, D. S. de Jesus, C. F. das Virgens, M. S. Carvalho, N. Oleszczuk, M. G. R. Vale, B. Welz, S. L. C. Ferreira; *Spectrochim Acta Part B* 62 (2007) 4.

[88] V. A. Lemos, M. de la Guardia, S. L. C. Ferreira; *Talanta* 58 (2002) 475.

[89] H. C. dos Santos, Desenvolvimento de métodos analíticos de separação e pré concentração para determinação de molibdênio por técnicas espectroanalíticas; *PhD thesis*, Federal University of Bahia, Brazil, 2002.

[90] P. Fong and A. Chow; *Talanta* 39 (1992) 497.

[91] D. S. de Jesus, M. S. de Carvalho, A. C. S. Costa, S. L. C. Ferreira; *Talanta* 46 (1998) 1525.

[92] E. M. M. Ferreira, R. J. A. L'Amoura, J. M. N. Carmob, J. L. Mantovanoa, M. S. Carvalho; *Microchem J* 78 (2004) 1.

[93] M. S. Carvalho, J. A. Medeiros, A. W. Nobrega, J. L. Mantovano and V. P. A. Rocha; *Talanta* 42 (1995) 45.

[94] N. Chakraeiorti and S. K. Roy; *Talanta* 40 (1993) 1499.

[95] A. Raychaudhuri, S. K. Roy, A. K. Chakraburtty; *Talanta* 39 (1992) 1377.

[96] A. Raychaudhuri, S. K. Roy, A. K. Chakraburtty; *Anal Chim Acta 257* (1992) 123.

[97] S. Bhattachary, S. K. Roy, A. K. Chakraburtty; *Talanta* 37(1990) 1101.

[98] R. Caletka, R. Hausbeck and V. Krivan; *Talanta* 33(1986) 315.

[99] A. Chow, G. T. Yamashita and R. F. Hamon; *Talanta* 28 (1981) 437.

[100] M. S. Carvalho, I. C. S. Fragab, K. C. M. Neto, E. Q. S. Filho; *Talanta* 43 (1996) 1675.

[101] D. S. de Jesusa, M. G. Kornb, S. L.C. Ferreira, M. S. Carvalho; *Spectrochim Acta Part B* 55(2000)389.

[102] A. Raychaudhuri, S. K. Roy; *Talanta* 41(1994) 171.

[103] D. kundu and S. K. Roy; *Talanta* 39 (1992) 415.

[104] M. S. Carvalho, M. L. F. Domingues, J. L. Mantovano, E. Q. S. Filho; *Spectrochim Acta Part B* 53 (1998) 1945.

[105] R. N. Rodriguez, New method for determination of β – lactam antibiotics by means of diffuse reflectance spectroscopy and PUF as sorbent, University Duisburg- Essen, Germany; *phD thesis*, 2005,

[106] H. D. Gesser, A. Chow, F. C. Davis, J. F. Uthe and J. Reinke; *Anal Lett 4* (1971) 883.

[107] H. D. Gesser, A. B. Sparling, A. Chow and C. W. Turner; I. *Am Wafer Works Assoc 65 (1973)220.*

[108] B. K. Afghan, R. J. Wilkinson, A. Chow, T. W. Findley, H. D. Gesser and K. I. Srikameswaran; *Water Res 18* (1984) 9.

[109] P. R. Musty, G. Nickless ;. *J Chromatogr* 120 (1976) 369.

[110] K. M. Gough and H. D. Gesser; *J Chromatogr* 115 (1975) 383.

[111] L. Schumack and A. Chow; *Talanta* 34 (1987) 957.

[112] A. Chow, W. Branagh and J. Chance; *Talanta* 37(1990) 407.

[113] R. J. Cassella, S. Garrigues, R. E. Santelli, M. de la Guardia; *Talanta* 52 (2000) 717.

[114] J. Saxena, J. Kozuchowski, and D. K. Basu; *Environ Sci Technol* 11(1977) 682.

[115] E. V. Kuzmina, L. N. Khatuntseva, and S. G. Dmitrienko; *J Anal Chem* 63(2008) 34.

[116] M. S. El-Shahawi, A. M. Kiwan, S. M. Al-Daheri and M. H. Saleh; *Talanta* 42(1995)1471.

[117] M. S. El-Shahawi; *J Chromatogr A* 760 (1997) 176.

[118] M. S. El-Shahawi; *Talanta* 41(1994)1481.

[119] S. G. Dmitrienko, E. N. Shapovalova, E. Ya. Gurarii, M. V. Kochetova, O. A. Shpigun, and Yu. A. Zolotov; *J Anal Chem* 57(2002) 1009.

[120] S. G. Dmitrienko, O. M. Medvedeva, A. A. Ivanov, O. A. Shpigun, Yu. A. Zolotov; *Anal Chim Acta* 469 (2002) 295.

[121] A. B. Farag, M. S. El-Shahawi; *J Chromatogr* 552 (1991) 371.

[122] T. Braun and A. B. Farag; *Anal Chim Acta* 73 (1974) 301.

[123] D. Atanasova , V. Stefanova, E. Russeva; *Talanta* 45 (1998) 857.

[124] A. B. Farag, A. M. EL-Wakil and M. S. EL-Shahawi; *Talanta* 29 (1982) 789.

[125] W. N.L. dos Santos, C. M.C. Santos, S. L.C. Ferreira; *Microchem J* 75 (2003) 211.

[126] M. N. Saeed, M. Ahmed, A. Ghaffar; *Adsorption Sci and Tech* 21(2003) 67.

[127] W. N. L. dos Santos, J. L.O. Costa, R.G. O. Araújo, D. S. de Jesus and A. C. S. Costa; *J Hazard. Mater* 137 (2006) 1357.

[128] W. L. dos Santos, C. M. M. dos Santos, J. L.O. Costa, H. M.C. Andrade, S. L. C. Ferreira; *Microchem J* 77 (2004) 123.

[129] V. A. Lemos, R. E. Santelli, M. S. Carvalho and S. L. C. Ferreira; *Spectrochim Acta Part B* 55 (2000) 1497.

[130] V. A. Lemos, A. A. de Jesus, E. M. Gama, G.T. David and R. T. Yamaki; *Anal Lett* 38 (2005)683.

[131] E. M. Gama, A. S. Lima and V. A. Lemos; *J Hazard Mater* 136 (2006) 757.

[132] V. A. Lemos, W. N. L. dos Santos, J. S. Santos and M. B. de Carvalho; *Anal Chim Acta* 481 (2003)283.

[133] C. R.T. Tarley and M. A. Z. Arruda, *Anal. Sci.* 20 (2004) 961.

[134] C. R. T. Tarley and M. A. Z. Arruda; *Anal Lett* 38 (2005) 1427.

[135] A. Alexandrova, S. Arpadjan; *Anal Chim Acta* 307 (1995) 71.

[136] J. K. Mondal, D. Kundu; *Anal Sci* 21(2005)1491.

[147] P. T. Sukhanov, S. P. Kalinkina, and Ya. I. Korenman; *J Anal Chem* 62 (2007)1122.

[138] S. G. Dmitrienko, L.V. Goncharova, A.V. Zhigulev, R. E. Nosov, N. M. KuzÁmin, Yu. A. Zolotov; *Anal Chim Acta* 373 (1998) 131.

[139] A. A. Menegário, A. J. Silva, E. Pozzi, S. F. Durrant, C. H. A. Jr; *Spectrochim Acta Part B* 61 (2006) 1074.

[140] I. A. Veselova, T. N. Shekhovtsova; *Anal Chim Acta* 392 (1999) 151.

[141] M. S. Alhakawati, C. J. Banks; *J Envir Management* 72 (2004) 195.

[142] M. F El-Shahat, E. A Moawed and M. A. A Zaid; *Talanta* 59 (2003) 851.

[143] M. F. El-Shahat, E. A. Moawed, and A. B. Farag; *Talanta* 71 (2007) 236.

[144] S. W. Myung, Y. H. Yeom, Y. M. Jang, H. S. Choi, D. Cho; *J Materials Sci: Materials in Medicine* 16 (2005)745.

[145] G. A. Meligi; *Polymer-Plastics Technol and Engineering* 47(2008) 106.

[146] S. G. Dmitrienko, O. A. Sviridova, L. N. Pyatkova, V. M. Senyavin; *Anal Bioanal Chem* 374 (2002) 361.

[147] E. A. Moawed, M. A. A. Zaid and M. F. El-Shahat; *J Anal Chem* 61(2006)458.

[148] E. A. Moawed, and M. F. El-Shahat; *React Funct Polym* 66 (2006) 720.

[149] E. A. Moawed,; *J. Ind Chem Society* 81(2004)395.

[150] N. Burham, S. M. Abdel-Azeem and M. F. El-Shahat; *Anal Chim Acta* 579 (2006) 193.

[151] V. A. Lemos, A. S. dos Passos, G. S. Novaes, D. A. Santana, A. L. Carvalho, D. G. Silva; *React Funct Polym* 67 (2007) 573.

[152] D.S. Jesus, R. J. Cassella, S. L.C. Ferreira, A. C. S. Costa, M. S. de Carvalho, R. E. Santelli; *Anal Chim Acta* 366 (1998) 263.

[153] W. N. L. dos Santos; Aplicação de Matriz de Doehlert na Otimização de sistemas de pré-concentração em linha para determinação de zinco e chumbo em águas naturais e efluentes de refinaria de petróleo, *PhD thesis*, Federal University of Bahia, Brazil, 2004.

[154] V. A. Lemos; Sistema em linha para pré-concentração e determinação de cádmio e chumbo por Espectrometria de Absorção Atômica com chama utilizando minicoluna de espuma de poliuretano impregnada com 2-(2′- benzotiazolilazo)-p-cresol, *PhD thesis*, Federal University of Bahia, Brazil, 2001.

[155] R. J. Cassella; *J Environ Monit* 4 (2002) 522.

[156] T. Braun, M. N. Abbas, S. Torok, Z. Szokefalvinagy; *Anal Chim Acta* 160 (1984) 277.

[157] A. M. El-wakil, M. S. Elsahawi, A. B. Farag; *Anal Lett* 23 (1990) 703.

[158] I.E. De Vito, R. A. Olsina and A. N. Masi; *Fresenius J Anal Chem* 368 (2000) 392.

[159] P. F. Marchisio, A. Sales, S. Cerutti, E. Marchevsky, L. D. Martinez; *Instrum Sci. Technolog* 33 (2005) 449.

[160] P. F. Marchisio, A. Sales, S. Cerutti, E. Marchevsky, L. D. Martinez; *J Hazard Mater* 124 (2005) 113.

[161] O. Zhang, H. Minami, S. Inoue, I. Atsuya; *Anal Chim Acta* 407 (2000) 147.

[162] S. Akman, N. Tokman; *Talanta* 60 (2003) 199.

[163] S. Gupta, B. B. Prasad, *J Pharmaceut and Biomed Anal* 23 (2000) 307.

[164] B.A.Chowdhury, R.K. Chandra, *Prog Food Nutr Sci.* 11(1987) 55.

[165] L. Hejazi, D. E. Mohammadia, Y. Yamini, R. G. Brereton; *Talanta* 62 (2004) 185.

[166] S. L. C. Ferreira, J. R. Ferreira, A. F. Dantas, V. A. Lemos, N. M. L. Araujo, A. C. S. Costa; *Talanta* 50 (2000) 1253.

[167] A.R. Ghiasvand, R. Ghaderi, A. Kakanejadifard; *Talanta* 62 (2004) 287.

[168] P.K. Tewari, A. K. Singh, *Talanta* 56 (2002) 735.

[169] M. Zougagh, A. G. de Torres, E. V. Alonso, J. M. C. Pavón; *Talanta* 62 (2004) 503.

[170] M. H.A. Meloa, S. L.C. Ferreira, R. E. Santelli; *Microchem J* 65 (2000) 59.

[171] S. L. C. Ferreira, W. N. L. dos Santos, V. A. Lemos*; Anal Chim Acta* 445 (2001) 145.

[172] M. S. El-Shahawi, H. A. Nassif; *Anal Chim Acta* 481 (2003) 29.

[173] H. E. Fierz-David, Fundamental process of dye chemistry, Translated from the Fifth Austrian Edition, *Rochester, New York,* Copyright 1949, pp250.

[174] M. Tuzen and M. Soylak; *Anal Chim Acta* 504 (2004) 325.

[175] M. E. Malla, M. B. Alvarez, D. A. Batistoni; *Talanta* 57 (2002) 277.

[176] X. Wang, R. M. Barnes; *J Anal Atom Spectrom* 4(1989) 509.

[177] W. X. Ma, F. Liu, K. A. Li, W. Chen, S. Y. Tong; *Anal Chim Acta* 416 (2000) 191.

[178] A. Goswami, A. K. Singh and B. Venkataramani; *Talanta* 60(2003)1141.

[179] M. Kumar, D.P.S. Rathore, Ajai K. Singh; *Talanta* 51 (2000) 1187.

[180] R. Hirsch, T. A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, K. L. Kratz; *J Chromatogr A* 815 (1998) 213.

[181] I. Liska, *J Chromatogr A* 885 (2000) 3.

[182] M. C. Carson, *J Chromatogr* 885 (2000) 343.

[183] K. Kummerer and A. Henninger, *Clin Microbial Infect* 9 (2003)1203.

[184] P. Janos, K. Stulik, V. Pacakova; *Talanta* 39 (1992) 29.

[185] R. E. Majors; *LG-GC* 4 (1989) 972.

[186] P. Edder, A. Cominoli, C. Crovi; *Mitteilungen aus lebensmitte-luntersuchng and Hygiene* 90 (1999) 291.

[187] S. Reidiker, R. H. Stadler, *Anal Chem* 73 (2001) 1614.

[188] A. F. M. El Walily, A. A. K. Gazy, S. F. Belal, E. F. Khamis; *J Pharm and Biomed Anal* 20 (1999) 643.

[189] B. B. Prasad, S. Gupta, *J Pharmaceut Sci* 62 (2000) 261.

[190] M. Kai, H. Kinoshita, K. Ohta, S. Hara, M. K. Lee; *J Pharm Biomed Anal* 30 (2003) 1765.

[191] M. G. San, M. Halide, L. Alpizar, M. Jesus, V. Creda; *Anal and bioanal Chem* 387(2007) 663.

[192] H. Salem, H. Askal; *J Pharm Biomed Anal* 29 (2002) 347.

[193] H. Salem, G. A. Saleh; *J Pharm Biomed Anal* 28 (2002) 1205.

[194] M. T. Rasseel, K. H. Vandeqoude, *J Chromatogr B* 811 (2004) 159.

[195] A. S. Amin, A. L. El-Ansary, Y. M. Issa; *Talanta*, 41(1994)691.

[196] L. R. P. de Abreu, R. A. M. Ortiz, S. C. de Casso, J. P. Jr; *J Pharm Pharmaceut Sci* 6 (2003) 223.

[197] A. S. Amin; *Farmaco* 56 (2001) 211.

[198] K. L. Tyczkowska, R. D. Voyksner, R. F. Straub, A. L. Aronson; *J AOAC International* 77 (1994) 1122.

[199] J. M. Cha, S. Yang, K.H. Carlson; *J Chromatogr A* 1115 (2006) 46.

[200] T. Nagata, E. Ashizoawa, H. Hashimoto; *Shokuhin Eiseigaku Zasshi* 45 (2004)161.

[201] L.K. Sorensen, T.H. Elbaek, *Chromatographia* 60 (2004) 287.

[202] S. Bompadre, L. Ferrante, L. Leone; *J Chromatogr A* 812 (1998) 191.

[203] O. J. Pozo, C. Guerrero, J. V. Sancho, M. Ibanez, E. Pitarch, E. Hogendoorn, F. Hernandez; *J Chromatogr A* 1103 (2006) 83.

[204] N. Snippe, N.C. van de Merbel, F. P. M. Ruitera, O. M. Steijgerb, H. Lingemana, U.A.Th. Brinkman; *J Chromatogr B* 662 (1994) 61.

[205] R. J. Cassella, V. A. Salim, L. S. Jesuino, R. E. Santelli, L. C. Frerreira, M. S. de Carvalho; *Talanta* 54 (2001) 61.

[206] W. dos Santos, C. M. M. Santos, J. L. O. Costa, H. M. C. Andrade, S. L. C. Ferreira; *Microchem J* 77 (2004) 123.

[207] T. Okada; *Analytical Sciences* 9 (1993) 59.

[208] F. Momenbeik and J. H. Khorasani, *Acta Chromatographica* 16 (2006) 58.

[209] S. L.Gro, J. R. T. Lapasio, J. J. B. Baeza, M. C. G. A. Coque; *Anal Chim Acta* 418 (2000) 153.

[210] F. B.Marti, L. C.Bosch, M. G.Matas; *Chromatographia* 1(1968)5.