

**USE OF AMINE- AND MERCAPTO-MODIFIED
SILICA AS SOLID PHASE EXTRACTION
SORBENT FOR SPECIATION OF INORGANIC
SELENIUM PRIOR TO DETERMINATION BY
ATOMIC SPECTROMETRIC TECHNIQUES**

**A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of**

**MASTER OF SCIENCE
in Chemistry**

**by
Esen DÖNERTAŞ**

**July 2010
İZMİR**

We approve the thesis of **Esen DÖNERTAŞ**

Prof. Dr. Ahmet E. EROĞLU
Supervisor

Prof. Dr. Emür HENDEN
Committee Member

Prof. Dr. Latif ELÇİ
Committee Member

Assoc. Prof. Dr. Durmuş ÖZDEMİR
Committee Member

Assist. Prof. Dr. Ali ÇAĞIR
Committee Member

Prof. Dr. Serdar ÖZÇELİK
Head of the Department of Chemistry

Assoc. Prof. Dr. Talat YALÇIN
Dean of the Graduate School of
Engineering and Science

ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof. Dr. Ahmet Emin EROĞLU for his support and encouragement throughout this thesis. I extremely appreciate his knowledge, kindness and guidance.

Sincere thanks to Assist. Prof. Dr. Ali ÇAĞIR for his valuable comments.

I would like to thank to Prof. Dr. Emür HENDEN, Prof. Dr. Latif ELÇİ, and Assoc. Prof. Dr. Durmuş ÖZDEMİR who agreed to be the members of my thesis committee.

I am deeply thankful to the research scientists at the Environmental Research Centre for ICP-MS analyses, especially to Sanem Ezgi KINAL for her endless patience and understanding, to Dr. Hüseyin ÖZGENER for elemental analyses, to the research scientists at the Center for Materials Research (IZTECH) for carrying out TGA, BET, and SEM analyses, and finally to Prof. Dr. Klaus ALBERT for providing help on solid-state NMR analysis.

I am very happy to work in a group of people full of integrity, solidarity, and understanding. My deepest gratitude belongs to my clever, hardworking friend Ezel BOYACI who shared her ideas with me all the time. I would also like thank to my lovely roommates Dilek ARICA ATEŞ, Meral KARACA, Semira ÜNAL, Ayşegül ŞEKER and to all my friends in chemistry department for their support.

Special thanks belong to my beloved friend Merve DEMİRKURT who showed me the meaning of friendship and supported me both in good and bad times.

Lastly I want to express my gratitude to my sister Elif YAVAŞ and her husband Mert YAVAŞ for sharing their experiences through all the stages of this study. Finally I dedicate this thesis to my parents, Hülya DÖNERTAŞ and İsmail DÖNERTAŞ.

.

ABSTRACT

USE OF AMINE- AND MERCAPTO-MODIFIED SILICA AS SOLID PHASE EXTRACTION SORBENT FOR SPECIATION OF INORGANIC SELENIUM PRIOR TO DETERMINATION BY ATOMIC SPECTROMETRIC TECHNIQUES

Silica-based sorbents containing amino-, mercapto-, and both functional groups (bifunctional) were prepared and used for the sorption of inorganic Se(IV) and Se(VI) species from waters prior to their determination by atomic spectrometric techniques, namely, inductively coupled plasma mass spectrometry (ICP-MS) and hydride generation atomic absorption spectrometry (HGAAS). The presence of the functional groups on the surface of the silica was demonstrated by using several characterization techniques such as scanning electron microscopy (SEM), solid-state NMR spectroscopy, thermo gravimetric analysis (TGA), elemental analysis and Brunauer-Emmett-Teller (BET) surface area analysis.

Amine-modified silica was found to be selective towards Se(VI) at pHs 2.0 and 3.0 whereas mercapto-modified silica retains Se(IV) over a wide range from acidic regions to pH 4.0. Bifunctional silica, on the other hand, possesses the good features of the two sorbents; it can be used in the separate sorption of Se(IV) or of both Se(IV) and Se(VI) species. Acidic pHs (<1.0) are convenient for the sorption of Se(IV) alone where no sorption is observed for Se(VI). Alternatively, pH can be adjusted to 2.0 or 3.0 and Se(IV) and Se(VI) can be retained simultaneously. Desorption of selenium species from the sorbents was realized with two different eluents; 0.2% (m/v) KIO₃ in 1 M HCl was used for Se(IV) and 2.0 M HCl for Se(VI). The efficiency of the proposed sorbents was demonstrated through spike recovery tests carried out with bottled drinking and tap water samples and the percentage recoveries were found to change between 82.2 ± 7.1 and 109.4 ± 3.6 for Se(IV). For Se(VI), mechanically mixed amino- and mercapto-modified (MIX) silicas in (1:1) ratio has shown the best performance with percentage recoveries of 87.1 ± 3.6 and 74.5 ± 6.6 for the spiked bottled drinking and tap water samples, respectively.

ÖZET

İNORGANİK SELENYUMUN ATOMİK SPEKTROMETRİK TEKNİKLERLE TAYİNİ ÖNCESİ TÜRLENDİRİLMESİ İÇİN AMİN- VE MERKAPTO- İLE MODİFİYE EDİLMİŞ SİLİKANIN KATI FAZ EKSTRAKSİYON SORBENTİ OLARAK KULLANILMASI

Amino-, merkapto-, ve her iki fonksiyonel grubu içeren (çift fonksiyonlu) silika bazlı sorbentler hazırlanmış ve indüktif eşleşmiş plazma kütle spektrometri (ICP-MS) ve hidrür oluşturmali atomic absorpsiyon spektrometri (HGAAS) gibi atomic spektrometrik tekniklerle tayin öncesi, anorganik Se(IV) ve Se(VI)'nın sorpsiyonunda kullanılmıştır. Silika yüzeyinde fonksiyonel grupların varlığı, taramalı elektron mikroskopi (SEM), katı-hal NMR spektroskopisi, termo gravimetrik analiz (TGA), elementel analiz ve Brunauer-Emmett-Teller (BET) yüzey alanı analizi gibi çeşitli karakterizasyon tekniklerinin kullanımıyla gösterilmiştir.

Amino- gruplarıyla modifiye edilen silikanın pH 2.0 ve 3.0'da Se(VI)'ya seçici davrandığı bulunurken, merkapto- ile modifiye edilen silikanın asidik bölgelerden pH 4.0'a kadar geniş bir aralıkta Se(VI)'yı tuttuğu belirlenmiştir. Çift fonksiyonlu silika ise her iki sorbentin de iyi özelliklerine sahiptir; sadece Se(IV)'ün veya Se(IV) ve Se(VI)'nın birlikte sorpsiyonunda kullanılabilir. Asidik pH'lar (<1.0) sadece Se(IV) sorpsiyonu için uygundur; Se(VI) için sorpsiyon gözlenmemektedir. Diğer yandan, çözelti pH'sı 2.0 veya 3.0'a ayarlanabilir, Se(IV) ve Se(VI) birlikte tutulabilir. Selenyum türlerinin sorbent yüzeyinden desorpsiyonu iki farklı eluent ile gerçekleştirilmiştir; Se(IV) için 1 M HCl içinde hazırlanmış 0.2% (m/v) KIO₃, Se(VI) içinse 2.0 M HCl kullanılmıştır. Önerilen sorbentlerin yetkinliği, şişelenmiş içme suyu ve musluk suyuna katım/geri kazanım testleriyle gösterilmiş, geri kazanım yüzdelerinin Se(IV) için 82.2 ± 7.1 ve 109.4 ± 3.6 arasında değiştiği bulunmuştur. Se(VI) için en iyi performansı, sadece amino- ve sadece merkapto- gruplarıyla modifiye edilmiş silikaların (1:1) mekanik karışımıyla elde edilen sorbent göstermiş, geri kazanım yüzdeleri, katılanmış şişeli içme suyu ve musluk suyu için sırasıyla 87.1 ± 3.6 ve 74.5 ± 6.6 olarak belirlenmiştir.

TABLE OF CONTENTS

LIST OF FIGURES	viii
LIST OF TABLES	ix
CHAPTER 1. INTRODUCTION	1
1.1. Essentiality of Selenium.....	1
1.2. Selenium Species in the Environment and in Biological Systems	2
1.3. Bioavailability of Selenium	3
1.4. Selenium Determination Methods	4
1.5. Speciation of Inorganic Selenium	5
1.6. Solid Phase Extraction	7
1.7. Silica as Solid Sorbent	9
1.8. Aim of This Work	11
CHAPTER 12. EXPERIMENTAL.....	12
2.1. Chemicals and Reagents	12
2.2. Instrumentation and Apparatus	12
2.3. Aqueous Calibration Plot.....	15
2.4. Synthesis of Sorbents with Different Functional Groups	15
2.4.1. Synthesis of (3-Aminopropyl)triethoxysilane (3-APTES)- modified Silica.....	16
2.4.2. Synthesis of (3-Mercaptopropyl)trimethoxysilane (3-MPTMS)- modified Silica.....	17
2.4.3. Synthesis of Mercapto- and Amine-modified Silica.....	17
2.5. Characterization of the Synthesized Sorbents.....	17
2.6. Sorption Studies	18
2.6.1. Effect of Solution pH	18
2.6.2. Effect of Shaking Time	18
2.6.3. Effect of Sorbent Amount	19
2.6.4. Effect of Sorption Temperature	19
2.6.5. Repetitive Loading	19
2.6.6. Effect of Initial Concentration	19

2.7. Desorption Studies	20
2.8. Method Validation	20
CHAPTER 3. RESULTS AND DISCUSSION.....	21
3.1. Characterization of the Sorbents	21
3.1.1. Solid State Nuclear Magnetic Resonance (SSNMR) Spectroscopy	21
3.1.2. Brunauer-Emmett-Teller (BET) Surface Area Analysis.....	23
3.1.3. Scanning Electron Microscopy (SEM)	24
3.1.4. Elemental Analysis.....	25
3.1.5. Thermo Gravimetric Analysis (TGA).....	26
3.2. Calibration Plots.....	27
3.2.1. Calibration Curve for Se(IV) and Se(VI) Using ICP-MS.....	27
3.2.2. Calibration Curve for Se(IV) Using HGAAS	27
3.3. Sorption Studies	28
3.3.1. Effect of Solution pH	28
3.3.2. Effect of Shaking Time	33
3.3.3. Effect of Sorbent Amount	35
3.3.4. Effect of Sorption Temperature	37
3.3.5. Repetitive Loading	37
3.3.6. Effect of Initial Concentration	40
3.4. Desorption Studies	41
3.5. Method Validation	42
CHAPTER 4. CONCLUSION	45
REFERENCES	46

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
Figure 1.1. Pe-pH diagram of selenium at 25 °C.	3
Figure 1.2. Schematic illustration of the SPE modes (a) column type, (b) batch type.	8
Figure 2.1. Segmented Flow Injection HGAAS system used in selenium determinations.	14
Figure 2.2. Apparatus used in the synthesis of the sorbents.	16
Figure 2.3. Amine modification of silica. (a) Silica, (b) Activated silica, (c) NH ₂	17
Figure 2.4. Mercapto modification of silica. (a) Silica, (b) Activated silica, (c) SH.	17
Figure 3.1. NMR spectrum of NH ₂ silica.	22
Figure 3.2. NMR spectrum of SH silica.	22
Figure 3.3. NMR spectrum of SN silica.	23
Figure 3.4. Characteristic SEM images of (a) unmodified silica, (b) NH ₂ silica, (c) SH silica, (d) SN silica.	24
Figure 3.5. TGA curves of (a) unmodified silica, (b) NH ₂ silica, (c) SH silica, (d) SN silica.	26
Figure 3.6. Calibration plots of Se(IV) and Se(VI).	27
Figure 3.7. Calibration plot of Se(IV).	28
Figure 3.8. Distribution diagram of (a) Se(IV) and (b) Se(VI) in aqueous solutions.	30
Figure 3.9. Effect of solution pH on the sorption of (a) Se(IV) and (b) Se(VI).	31
Figure 3.10. Effect of solution pH on the sorption of Se(IV) and Se(VI).	32
Figure 3.11. Effect of shaking time on Se(VI) sorption at pH 2.0.	34
Figure 3.12. Effect of shaking time on Se(IV) sorption at (a) pH 2.0, (b) pH 3.0.	33
Figure 3.13. Effect of the sorbent amount on Se(VI) sorption at pH 2.0.	36
Figure 3.14. Effect of the sorbent amount on Se(IV) sorption at (a) pH 2.0, (b) pH 3.0.	35
Figure 3.15. Effect of sorption temperature on (a) Se(IV), (b) Se(VI) sorption at pH 2.0.	38
Figure 3.16. Effect of repetitive loading on (a) Se(IV), (b) Se(VI) sorption at pH 2.0. ...	39

LIST OF TABLES

<u>Table</u>	<u>Page</u>
Table 2.1. ICP-MS operating parameters.....	13
Table 2.2. SFI-HGAAS operating parameters.	14
Table 2.3. The abbreviations of the synthesized sorbents.....	16
Table 3.1. BET analysis results of silica and modified silicas.....	23
Table 3.2. Elemental analysis results of the synthesized sorbents.....	25
Table 3.3. Elemental analysis results of the synthesized sorbents after desorption.....	25
Table 3.4. Effect of initial Se(IV) concentration on the sorption by SH silica at pH 2.0.....	40
Table 3.5. Effect of initial Se(IV) concentration on the sorption by SN and NH ₂ silica in 2 M HCl.....	41
Table 3.6. Effect of initial Se(IV) + Se(VI) concentration on the sorption by SN silica at pH 2.0 and 3.0.....	41
Table 3.7. Percentage desorption results of Se(IV) and Se(VI).....	42
Table 3.8. Percent sorption of spiked selenite in bottled drinking and tap water.	43
Table 3.9. Percent sorption of spiked selenate in bottled drinking and tap water.....	43
Table 3.10. Percent recoveries of spiked selenite in bottled drinking and tap water.....	44
Table 3.11. Percent recoveries of spiked selenate in bottled drinking and tap water.	44

CHAPTER 1

INTRODUCTION

1.1. Essentiality of Selenium

Selenium is both trace and essential element that has a lot of important biological functions in human body. In recent years, researchers have been interested in selenium speciation increasingly because of the uncertain nature of selenium. It can be a toxic or an essential element depending on its concentration. It has been stated that consumption of selenium at levels less than 0.1 mg kg^{-1} per day will cause deficiency, whereas more than 1 mg kg^{-1} will cause toxicity (Wada et al. 1993). Severe irritations on the respiratory system, a typical metallic taste in the mouth, pulmonary edema, the characteristic smell of garlic in the breath, and sweat due to dimethyl selenide are some symptoms of selenium overdose (Bedwall et al. 1993; Diaz et al. 1997).

In earlier times, only toxic effects of selenium were considered. Chronic poisoning has been reported from high levels of selenium exposure from some types of plants that have been grown in soils, which can accumulate high selenium contents, and from animals eating these plants (Levander and Burk 1994). In 1979, Keshan Disease Research Group (1979) from China has found some evidence regarding the disease called Keshan caused by the low selenium content in that zone. Keshan disease can cause cardiac muscle degeneration and affects children and premenopausal women. There are other problems that can be caused by selenium deficiency such as muscle problems, digestive alterations, cardiovascular disease and rheumatic disturbances (Neve et al. 1987; Ortuño et al. 1996).

The antioxidant behavior of selenocysteine that constitutes the active center of the glutathione peroxidase enzyme (GSH-Px) is one of the important functions of selenium (Chappuis and Poupon 1991; Levander and Burk 1994). The reduced form of this enzyme reduces the hydrogen peroxide and lipidic hydroperoxides at the level of the cytosol and mitochondrial matrix (Chappuis and Poupon 1991; Simonoff and Simonoff 1991). Selenium has also the ability to suppress the toxicity of other heavy metals such as mercury, lead, silver although the interaction mechanism has not been

completely understood and explained (Frost 1983; Cuvin-Aralar and Furness 1991; Ellingsen et al. 1993; Levander and Burk 1994).

1.2. Selenium Species in the Environment and in Biological Systems

Various forms of selenium are present in the environment and in biological systems; such as the inorganic Se(IV) (SeO_3^{2-} , selenite), Se(VI) (SeO_4^{2-} , selenate), and selenides (e.g. HgSe), and a bunch of organic forms such as selenomethionine, selenocysteine, selenocystathionine, and dimethylselenide. Species containing methyl groups are volatile. Hydrogen selenide is the most toxic form among all selenium compounds (Agency for Toxic Substances and Disease Registry 1996). Inorganic forms are mostly found in soils and natural waters, whereas organic forms can exist in yeast, broccoli, garlic, onion, mushrooms, wheat, and soybeans (Polatajko et al. 2006). Animals, especially fish, have higher selenium content than plants (Ari et al. 1991; Benemariya 1992; Diaz-Alarcón et al. 1995; Diaz-Alarcon et al. 1996b), and determination of protein content is important in order to decide the selenium amount in food. The highest selenium accumulation is found in marine products, especially in shrimps (Hershey et al. 1988; Zhang et al. 1993; Diaz-Alarcon et al. 1994). In acidic soils, selenite which is the less absorbable and insoluble form is predominant and therefore it is not available for plants. In alkaline soils, on the other hand, selenite is oxidized to selenate and becomes easier to be absorbed by plants (Gondi et al. 1992). Selenite and selenate absorbed by plants can be converted into organoselenium by replacing sulfur in the amino acids (Simonoff and Simonoff 1991; Favier 1993). Common forms are selenomethionine, selenocysteine and selenocystathionine.

Selenium is generally found in four oxidation states, -2 (H_2Se), 0 (elemental selenium), +4 (SeO_3^{2-}), and +6 (SeO_4^{2-}). The presence of the particular ions depends on the pH and the redox potential of the solution. Therefore Pe-pH stability field diagram of selenium can be used to predict the dominant forms. Figure 1.1 demonstrates that acidic and reducing conditions reduce inorganic selenites to elemental selenium, whereas alkaline and oxidizing conditions favor the formation of selenates. The pK value of H_2SeO_4 (selenic acid) can be estimated by thermodynamic calculations and it is negative. Thus this form of selenium is most likely not present under natural conditions.

The second dissociation of selenic acid needs to be taken into consideration and is reported to be ranging from 1.66 to 2.05 (Seby et al. 2001).

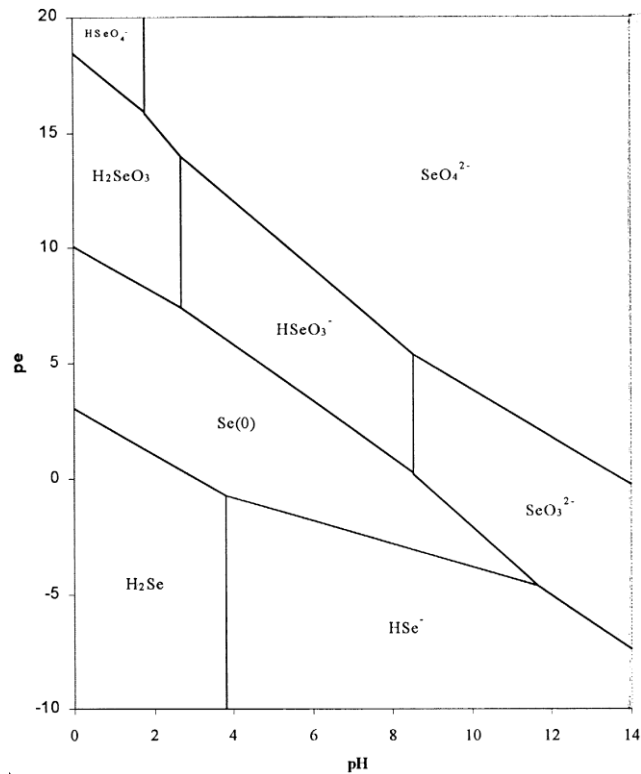


Figure 1.1. Pe-pH diagram of selenium at 25 °C.
(Source: Seby et al. 2001)

1.3. Bioavailability of Selenium

An essential element must be available from food and be absorbed by the gut and then used by the body. Experiments done both with animals and humans have shown that organic forms of selenium are more bioavailable than its inorganic forms (Dumont et al. 2006). The comparison made between selenite and selenate has demonstrated no observable distinction but since selenite is less stable and more toxic than selenate, the latter can be considered as a more convenient form for selenium intake.

The importance of selenium concentration in human, as mentioned before, originates from the fact that, it can be either toxic or necessary. In addition, the total concentration of selenium is not sufficient to understand the real effect of the element and therefore, it is necessary to consider the bioavailability of the nutrient. The amount

absorbed and used by the organism is required to be known because generally only a small portion can be transformed to a biologically available form (Cabañero et al. 2007). The most important selenium sources in diet are wheat and meat. Se-Cysteine and Se-Methionine forms are mostly found in meat and they are essential aminoacids (Dumont et al. 2006). Selenium content of fish is high but it is not highly bioavailable because selenium forms insoluble inorganic complexes (Pappa et al. 2006). Finley et al. (2004) stated that the chemical forms of selenium species also change among foods. For instance, although broccoli contains methylated forms of selenium, its bioavailability is low. On the other hand, red meats which can accumulate high selenium are also highly bioavailable for selenoprotein synthesis. The Food and Nutrition Board of the United States Research Council has suggested dietary limits as 70 μg Se/day for a healthy adult man and 55 μg Se/day for a healthy adult woman (Navarro-Alarcon and Lopez-Martinez 2000).

1.4. Selenium Determination Methods

There are various instrumental techniques to determine selenium concentration in different samples. Electrothermal atomic absorption spectrometry (ETAAS) was used by Izgi et al. (2005) to determine the concentration of selenium in garlic and onions after the application of a preconcentration step with 3,3-diaminobenzidine (DAB) reagent on the activated carbon. Saygi et al. (2006) used graphite furnace atomic absorption spectrometry (GFAAS) for the determination and speciation of Se(IV) and Se(VI) in environmental samples after the solid phase extraction of Se(IV)-ammonium pyrrolidine dithiocarbamate (APDC) chelate on Diaion HP-2MG. Valencia et al. (1999) developed a method based on solid-phase spectrophotometry to determine Se(IV) in natural waters. The principle of the method was the oxidation of potassium iodide by Se(IV) in acidic conditions and the formation of an ionic association complex of resulting I_3^- with Rhodamine B (RB) fixed on a dextran type lipophilic gel. Then the gel phase absorbance was measured at 590 and 800 nm.

Another method using spectrophotometry was proposed by Chand and Prasad (2009) for the determination of Se(IV), Se(VI) and total inorganic selenium in water. It was based on the catalytic effect of Se(IV) on the reduction of bromate by hydrazine dihydrochloride, the decolorization of methyl orange (MO) by the generated bromine,

and the monitoring of the absorbance spectrophotometrically at 507 nm as a function of time. Zhao et al. (2010) developed a method using a low volume microwave digestion procedure. The selenium content of the samples was determined by hydride generation atomic fluorescence spectrometry (HG-AFS), or voltammetry. All forms of selenium compounds in biological samples were decomposed to selenite. Microwave digestion conditions were optimized using concentrated HNO₃ and H₂SO₄. Panigati et al. (2007) employed differential pulse cathodic stripping voltammetry (DPCSV) on the hanging drop mercury electrode (HDME) in the presence of Cu(II) to determine the total selenium content in rice. A mixture of wet acid/dry ashing with Mg(II) salts were used to carry out the digestion in open vessel. Zhang et al. (2009) used nano-titanium dioxide particles for the removal of Se(IV) and Se(VI) from aqueous solutions. Selenium determinations were made by UV-VIS spectrometry using 1.0 mL 6.0 mol L⁻¹ HCl, 3.0 mL 0.05% malachite green, 3.0 mL 10% KI and 3.0 mL 10% Arabic gum solution.

Other methods such as inductively coupled plasma atomic emission spectrometry (ICP-AES) and hydride generation-inductively coupled plasma optical emission spectrometry (HG-ICP-OES) were also used in most of the studies (Harwood et al. 1997, Stripeikis et al. 2000). Hydride generation atomic absorption spectrometry (HGAAS) is widely used because it provides the advantage for the determination of the different forms of selenium. Maleki et al. (2005) introduced a HGAAS method for the determination of traces of selenium at ng mL⁻¹ concentration ranges using a solid mixture of tartaric acid and sodium tetrahydroborate. Inductively coupled plasma mass spectrometry (ICP-MS) is one of the most effective methods in order to detect very low concentrations (µg L⁻¹) of selenium with a proper linear dynamic range (Kápolna et al. 2006, Miekeley et al. 2005). Liu (2009) introduced a method based on nano-Al₂O₃ solid phase extraction technique for the determination of ultra-trace amounts of inorganic selenium species in aqueous systems and used IC-ICP-MS as the detection system.

1.5. Speciation of Inorganic Selenium

As mentioned in previous sections, determination of the total concentration of selenium will not be sufficient for the true evaluation of the toxicity, bioavailability, absorption, and emission of the element. The necessity of determining different

oxidation states and chemical forms has led the researchers to separation and speciation analyses.

Over years, quite a large number of articles have been published related to the speciation of inorganic selenium. Miekeley et al. (2005) used a new method for the speciation of Se(IV), Se(VI), and selenocyanate based on the ion chromatographic separation. An anion exchange column (Metrosep A Supp 1) was used with the mobile phase consisting of a solution of cyanuric acid (3 mmol L^{-1}), modified with acetonitrile (2% v/v) and perchlorate acid (2.5 mmol L^{-1}). Separated selenium species were determined with quadrupole ICP-MS. Another study made by Kápolna et al. (2006) investigated selenium species in chives using HPLC-ICP-MS. Selenium containing amino acids were separated with reversed-phase ion-pairing chromatography and detected with ICP-MS.

Chen et al. (2009) used a micro-column packed with lanthanum hydroxide precipitate coated onto cellulose fibre for the separation and speciation of inorganic selenium. The eluent was $\text{NaBH}_4\text{-NaOH}$. Hydride was generated with the mixing of eluate and hydrochloric acid followed by the detection with HG-AFS. Selenate was reduced to selenite and speciation is estimated by the difference of total inorganic selenium and selenite. A electrodeposition–electrothermal AAS method for speciation analysis of selenocysteine and Se(IV) in environmental water and agricultural soil samples has been developed by Najavi et al (2010). An uncontrolled applied potential (1.8 V) on a mercury coated electrode was applied for the selective reduction of selenium species. In acidic media (1.0 M HCl solution) the only inorganic selenium species electrodeposited was Se(IV), and organic selenium species was selenocysteine.

A new method was proposed by Li et al. (2008) for the simultaneous speciation of inorganic selenium in water by electrothermal vaporization ICP-MS following selective cloud point extraction (CPE). The principle of the method is based on the formation of Se(IV)-diethyldithiocarbamate (DDTC) complex at pH 6.00, and the quantitative extraction of the complex into the non-ionic surfactant-rich phase of octylphenoxypolyethoxyethanol (Triton X-114), whereas Se(VI) remained in aqueous solutions. The total selenium content was measured by ETV-ICP-MS after the reduction of Se(VI) using L-cysteine and concentration of Se(VI) was calculated from the difference. Lin (2007) used a Dowex 1X2 resin separation technique followed by analysis with AAS for the study of inorganic selenium speciation in groundwaters. The

species, Se(IV) and Se(VI), retained on the resin column were eluted with 0.1 M and 1 M nitric acid solutions.

Tüzen et al. (2007) presented a separation and speciation procedure for selenium in food and water samples prior to its determination with GFAAS. Magnesium hydroxide was used as the coprecipitating agent, and Se(VI) was reduced using hydrochloric acid and microwave digestion. The method was employed to determine the total selenium concentration in both natural waters and various food samples after microwave digestion.

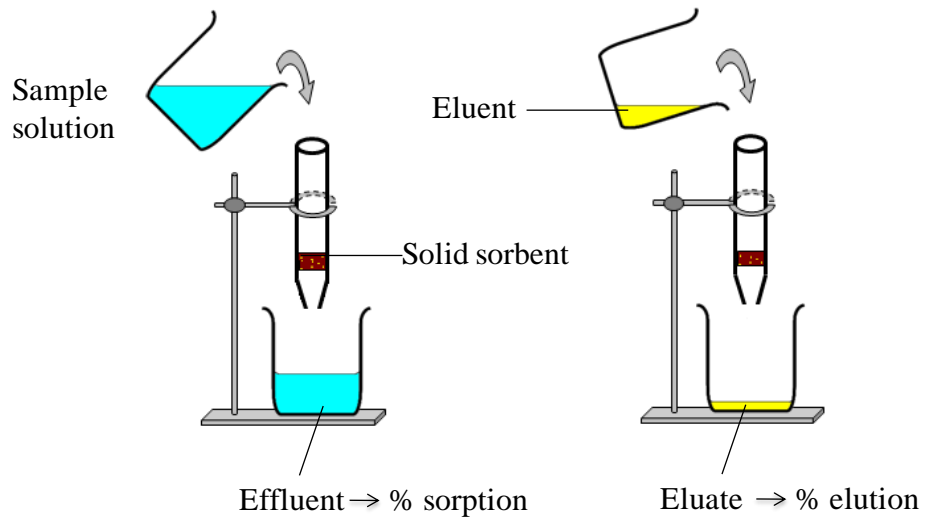
1.6. Solid Phase Extraction

Many separation and preconcentration methods can be found in literature for various purposes. For example, liquid-liquid extraction (solvent extraction) allows the separation of two or more components due to their unequal solubilities in two immiscible liquid phases. Electro-deposition is an electrolytic process in which a metal is deposited at the cathode from a solution of its ions. Coprecipitation is a simultaneous precipitation process in which a normally soluble component is precipitated with the use of a macro-component from the same solution by the formation of mixed crystals, adsorption, occlusion or mechanical entrapment. Ion exchange is a reversible chemical reaction wherein an ion (an atom or molecule that has lost or gained an electron and thus acquired an electrical charge) from solution is exchanged for a similarly charged ion attached to an immobile solid particle. In membrane filtration, there is a membrane which acts as a very specific filter that will let water flow through, while it catches suspended solids and other substances.

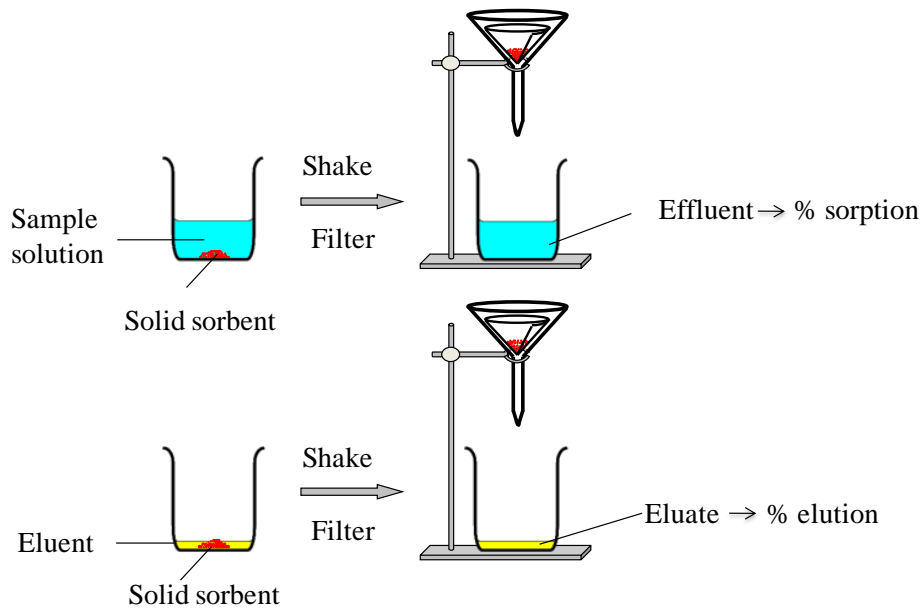
Solid phase extraction (SPE) is a non-equilibrium process and is based on the extraction of the desired species on a sorbent, and then elution of the retained species using a proper solvent. There are basically two application modes of SPE; namely, the column and the batch modes. In column type SPE, the column is loaded with the sample solution for the sorption of the analyte by the solid phase (Figure 1.2). The concentration of analyte in the effluent (non-sorbed fraction) is determined and used for the calculation of the percentage sorption. The recovery of the analyte (retained by the sorbent) is realized using, generally, a smaller volume of a proper eluent. The analyte

concentration in the eluate gives the percentage elution. Finally, total recovery calculations can be made according to Equation 1.1.

$$\text{Total Recovery} = \frac{[\text{analyte}]_{\text{eluate}}}{[\text{analyte}]_{\text{sample}}} \times 100 \quad (1.1)$$



(a)



(b)

Figure 1.2. Schematic illustration of the SPE modes (a) column type, (b) batch type.

In batch type SPE, a proper amount of solid sorbent is weighed and put into the sample solution. After shaking and filtering processes, the sorbent is taken into a small amount of eluent and similar shaking and filtration steps are applied (Figure 1.2). Following the analysis of the filtrate by a proper method, the percentage sorption, the percentage elution, and the total recovery can be calculated as in the case of column type SPE.

A special care must be given to the possible plugging problem that can be caused by the concentration, type, and size of the particulates in the sample, pore size of the sorbent and surface area of the sorbent bed. There is also a potential for the association of analyte with particulate and colloidal matter contamination in the sample. To avoid these problems sample particulate matter should be removed by filtration prior to SPE analysis. As any method, SPE has both advantages and disadvantages over the other methods mentioned above. The technique is simple, fast, does not require a large amount of solvent, and has ability to attain high preconcentration factors. In addition, the target species can be fixed in a more stable chemical form on the solid surface.

1.7. Silica as Solid Sorbent

Silica can be used in the sorption of quite a high number of analytes either directly or after the modification of its surface with proper functional groups. The sorption capacity of silica towards some metal ions, especially Cu, Ni, Co, Zn or Fe, has been reported to be very high (Sarkar et al. 1996). The silica surface consists of silanol groups and is suitable for modification with organic functional groups. The modification of the surface can be realized in basically two routes. In the first method, the organic reagent is adsorbed directly on the silanol group either by passing the solution through a column packed with silica (Liang et al. 2005) or by shaking silica in the reagent solution (Lorena et al. 1998). In the second method, the organic groups are bonded to the surface of silica covalently. The organic functional groups are expected to show high affinity to retain analyte species by forming chelates.

Silica has been used by many researchers as a starting material. After the successful modification of its surface, it becomes a very good sorbent for removal, speciation, preconcentration or separation of different species of an element such as arsenic, selenium, vanadium, etc. Burke et al. (2009) introduced aminopropyl and

mercaptopropyl functionalized and bi-functionalized mesoporous silica spheres to extract various metal ions such as Cr, Ni, Fe, Mn, Pd from aqueous solution. For the characterization of sorbents, elemental analysis and FTIR techniques were used. The principle of the synthesis was a post-synthesis reflux procedure. Co-functionalization was reported not to change the sorption capacities. AAS and ICP-AES were used to determine the extraction efficiencies of each metal ion species from solution at different pHs. Maximum sorption capacities were determined to be $384 \mu\text{mol g}^{-1}$ for Cr, $340 \mu\text{mol g}^{-1}$ for Ni, $358 \mu\text{mol g}^{-1}$ for Fe, $364 \mu\text{mol g}^{-1}$ for Mn and $188 \mu\text{mol g}^{-1}$ for Pd.

Zheng and Hu (2009) have developed a new method for inorganic selenium determination with dual silica monolithic capillary microextraction (CME) on-line hyphenated with ICP-MS. The microcolumn was composed of a 3-mercaptopropyl-trimethoxysilane (MPTS) modified silica monolithic capillary (C1) and a N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) modified silica monolithic capillary (C2). The silica supports were synthesized with sol-gel method. In another study by Tarafdar and Pramanik (2006), amino-functionalized mesostructured silica-zirconia mixed oxide has been synthesized through a synthesis path using water soluble sodium silicate, zirconium(IV) carbonate complex and 3-aminopropyltriethoxysilane in the presence of anionic surfactant sodium dodecyl sulphate. The sorbent has been reported to show excellent adsorption properties towards arsenate ions.

Blitz et al. (2007) introduced a group of newly synthesized sorbents based on silica modified with different functional groups such as amino-, cyano-, mercapto- and thiocyno- functional silanes. Nitrogen adsorption method was used to observe the difference in characteristics of the sorbents. Sorption studies made with Cu(II) and Pb(II) have demonstrated that narrow pore size resulted in a more favorable ΔG_{ads} , but a larger pore size assured greater access of surface groups to adsorbed ions, resulting in enhanced adsorption capacity.

1.8. Aim of This Work

The purpose of this study is to synthesize a sorbent that can be used in speciation of inorganic selenium, namely selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). For this reason, silica was selected as the solid sorbent and it was modified with amine- and mercapto-functional groups separately and together (bifunctional). After characterization of the sorbents with scanning electron microscopy (SEM), solid-state NMR, thermo gravimetric analysis (TGA), elemental analysis and Brunauer-Emmett-Teller (BET) surface area analysis, the experiments were focused on the optimization of the sorption performance of the sorbents. The parameters were the solution pH, the reaction temperature, the shaking time, and the sorbent amount. Possibility of repetitive loading of selenite and selenate was also investigated. Desorption of selenium species from the sorbents was realized with the use of several eluents. Proposed methodology was verified through the spike recovery tests carried out with bottled and tap water. Inductively coupled plasma mass spectrometry (ICP-MS) and segmented flow injection hydride generation atomic absorption spectrometry (SFI-HGAAS) were used for the determination of selenium.

CHAPTER 2

EXPERIMENTAL

2.1. Chemicals and Reagents

All the chemicals were of analytical reagent grade. Ultrapure water (18.2 MΩ, Millipore, Billerica, MA, USA) was used throughout the study. Glassware and plastic containers were soaked in 10% (v/v) nitric acid overnight and washed with distilled water before use.

Standard Se(IV) stock solution (1000.0 mg/L) was prepared by dissolving 0.833 g of Na₂SeO₃ · 5H₂O in 1.0% (v/v) HNO₃ and diluted to 250.0 mL with ultrapure water. Standard Se(VI) stock solution (1000.0 mg/L) was prepared by dissolving 0.598 g of Na₂SeO₄ in ultrapure water and diluted to 250.0 mL with ultrapure water. Calibration standards with lower concentrations were prepared daily by appropriate dilution of the stock standard. Sodium borohydride solution 1% (m/v) was prepared daily in 0.1% (m/v) NaOH for stabilization. Silica (Merck, Product Code: 1.10184, CAS no: [7631-86-9]), acetic acid (Riedel-de Haen, Product Code: 27225, CAS no: [64-19-7]), toluene (Riedel-de-Haen, Product Code: 24529, CAS no: [108-88-3]), acetone (Merck, Product Code: 1.00014, CAS no: [67-64-1]), 3-mercaptopropyltrimethoxysilane (3-MPTMS) (Fluka, Product Code: 63800, CAS no: [4420-74-0]), 3-aminopropyltriethoxysilane (3-APTES) (Merck, Product Code: 8.26619, CAS no: [14814-09-6]) were used for the synthesis of the sorbents.

2.2. Instrumentation and Apparatus

All pH adjustments and measurements were done using WTW InoLab pH 720 precision pH meter (Weilheim, Germany) using various concentrations of HNO₃, NH₃, HCl, and NaOH. Batch sorption studies were carried out using GFL 1083 water bath shaker (Burgwedel, Germany) equipped with a microprocessor thermostat for both efficient mixing and constant temperature during shaking. During repetitive loading

studies Hettich EBA 12 centrifuge (Tuttlingen, Germany) was used to separate the sorbent for reuse.

Agilent 7500ce Series (Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS) equipped with a high solid nebulizer, a Peltier-cooled spray chamber (2 °C), and an octopole collision/reaction cell with helium gas pressurization was used throughout the study for selenium determination. Although the most abundant isotope of selenium is ^{80}Se (49.60%), it could not be used in ICP-MS measurements because of the spectral interference of $^{40}\text{Ar}^{40}\text{Ar}$. Therefore, as an initial check, measurements were made with the three isotopes, namely, ^{78}Se , ^{80}Se , and ^{82}Se . Counts at $m/z = 80$ were enormously high which necessitated the use of collision cell. However, the signals from ^{78}Se and ^{82}Se counts were similar and sufficient; therefore, the measurements were done at $m/z = 78$ (^{78}Se , natural abundance of 23.50%). Operating parameters for ICP-MS system are given in Table 2.1.

For heavy matrix solutions such as the eluates after desorption studies, a Thermo Elemental Solaar M6 Series atomic absorption spectrometer (Cambridge, UK) with an air-acetylene burner with Segmented Flow Injection Hydride Generation (SFI-HGAAS) unit with continuous mode was used (Figure 2.1). A selenium hollow cathode lamp at 196.0 nm was used as the source, whereas a deuterium lamp was employed to correct background in all measurements. The quartz tube atomizer was 10 cm long, 8 mm in internal and 10 mm in external diameter with a 4 mm bore inlet tube fused at the middle for the introduction of sample. The quartz tube was heated externally with air-acetylene flame and nitrogen was used as the carrier gas. Operating parameters for SFI-HGAAS system can be seen in Table 2.2.

Table 2.1. ICP-MS operating parameters.

Forward power	1500 W
Reflected power	1 W
Coolant gas flow rate	15 L min ⁻¹
Auxiliary flow rate	0.90 L min ⁻¹
Sample uptake time	25 sec
Integration time	100 msec

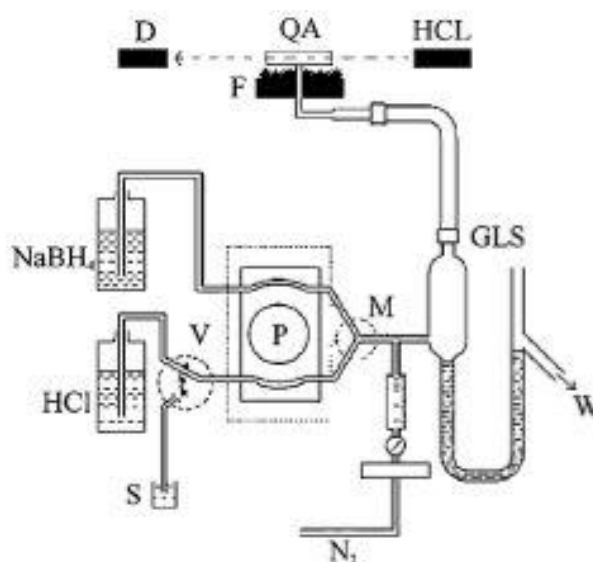


Figure 2.1. Segmented Flow Injection HGAAS system used in selenium determinations. D: deuterium lamp, QA: quartz atomizer, HCL: hollow cathode lamp, F: flame, GLS: gas-liquid separator, W: waste, P: peristaltic pump, V: three way valve and S: sample (Source: Yersel et al. 2005)

Table 2.2. SFI-HGAAS operating parameters.

Carrier gas (N ₂) flow rate	200 mL min ⁻¹
HCl flow rate	6.1 mL min ⁻¹
HCl concentration	3.0 % (v/v)
NaBH ₄ concentration	1.0 % (w/v) stabilized with 0.10 % (w/v) NaOH
NaBH ₄ flow rate	3.0 mL min ⁻¹
Sample flow rate	7-8 mL min ⁻¹

Several techniques were applied for the characterization of the sorbents. For example, a Philips XL-30S FEG scanning electron microscope (Eindhoven, The Netherlands) was used to take the images of the sorbents. A Perkin Elmer Pyris Diamond TG/DTA (Boston, MA, USA) was used to investigate the thermal properties of the sorbents. The compositions of synthesized sorbents were determined by LECO-CHNS-932 elemental analyzer (Mönchengladbach, Germany). Surface area measurements were performed with Micromeritics Gemini V Series Surface Area Analyzer (Norcross, USA). Solid-state ¹³C measurements were performed with an ASX 300 spectrometer (Bruker, Rheinstetten, Germany), using cross polarization and magic-angle spinning (CP/MAS) in the University of Tuebingen (Germany).

2.3. Aqueous Calibration Plot

Standard solutions from 0.0050 mg L⁻¹ to 0.10 mg L⁻¹ were prepared from 1000.0 mg L⁻¹ Se(IV) and Se(VI) respectively with simple dilution. All standards contained 1.0% (v/v) HNO₃ and measured with ICP-MS. For the desorption study of Se(IV), HGAAS was used as the detection method. Standard solutions from 0.0050 mg L⁻¹ to 0.050 mg L⁻¹ were prepared from 1000.0 mg L⁻¹ Se(IV). For the desorption of Se(IV), a strong oxidant, potassium iodate (KIO₃) was used and the oxidation of Se(IV) to Se(VI) has occurred. A reduction step was needed to observe selenium signal. Therefore, Se(VI) was reduced to Se(IV) by adding 6 M HCl into the sample solutions and standards and heating for 30 minutes at 80 °C. In addition the limit of detection (LOD) based on 3s (3 times the standard deviation above the blank value) and limit of quantification (LOQ) based on 10s were also evaluated for both detection methods.

2.4. Synthesis of Sorbents with Different Functional Groups

Activation of silica, the surface silanol groups, is necessary for the modification with a functional group. For this purpose, 5.0 g of silica was treated with 50.0 mL 0.010 M of CH₃COOH and left stirring for an hour. After that, it was washed with ultrapure water to neutralize the surface of silica. The pH of the solution was controlled with pH paper regularly to check if it became neutral. Finally it was dried in oven at 120.0 °C for 24 hours. The apparatus used in the synthesis is illustrated in Figure 2.2. To follow the text easily, the synthesized sorbents were abbreviated as given in Table 2.3.

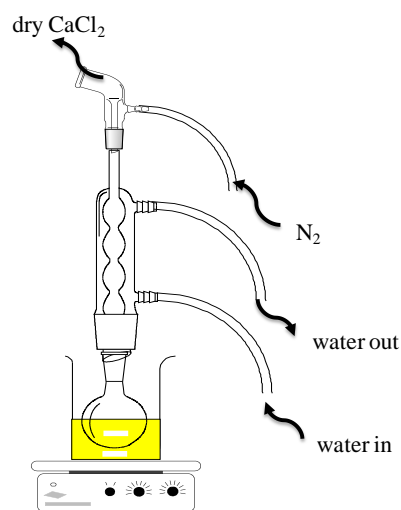


Figure 2.2. Apparatus used in the synthesis of the sorbents.

Table 2.3. The abbreviations of the synthesized sorbents.

Abbreviation	Sorbent
Silica	Activated silica (blank)
NH ₂	3-APTES-modified silica
SH	3-MPTMS-modified silica
SN	3-APTES- and 3-MPTMS-modified silica (bifunctional)
MIX	Physical mixture of 3-APTES- and 3-MPTMS-modified silica

2.4.1. Synthesis of (3-Aminopropyl)triethoxysilane (3-APTES)-modified Silica

In order to prepare NH₂ sorbent, 15.0 g of activated silica was stirred with 9.0 mL of 3-APTES in 30.0 mL of toluene at 110 °C for 24 hours under reflux. One end of the condenser had anhydrous CaCl₂ tube to prevent the moisture while the other end had N₂ gas connection to create an inert atmosphere. After the reaction was completed, the sorbent was washed with 20.0 mL of acetone and 10.0 mL of toluene and dried in oven at 60 °C for 24 hours. The schematic illustration of the modification of silica is shown in Figure 2.3.

2.4.2. Synthesis of (3-Mercaptopropyl)trimethoxysilane (3-MPTMS)-modified Silica

The same procedure was applied to prepare SH sorbent except 3-MPTMS was used instead of 3-APTES. The schematic illustration is shown in Figure 2.4.

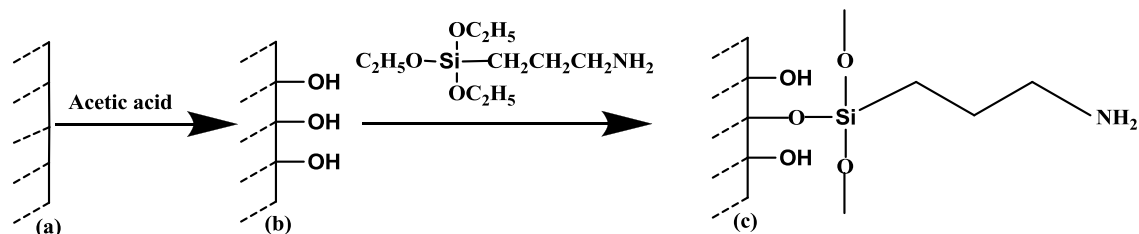


Figure 2.3. Amine modification of silica. (a) Silica, (b) Activated silica, (c) NH₂.

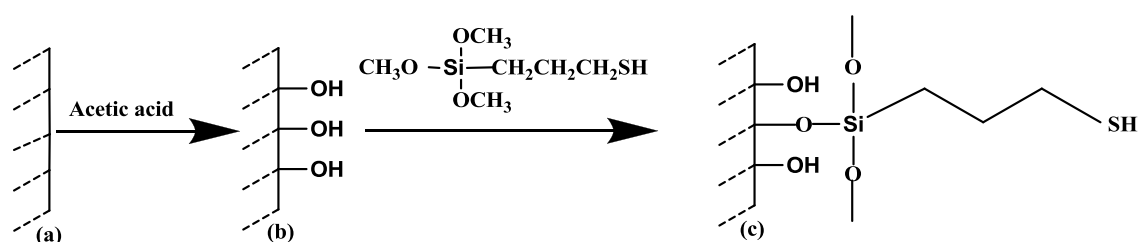


Figure 2.4. Mercapto modification of silica. (a) Silica, (b) Activated silica, (c) SH.

2.4.3. Synthesis of Mercapto- and Amine-modified Silica

The only change in the procedure was that, this time, 9.0 mL of 3-APTES and 9.0 mL 3-MPTMS were added to the mixture at the same time. Other steps were exactly the same.

2.5. Characterization of the Synthesized Sorbents

Characterization is very important to enlighten the structure and properties of the synthesized sorbents. The structural details may also help understand the possible

interaction/sorption mechanisms between the selenium species and the solid sorbents. Solid state NMR results give an idea about how the functional groups are bonded on silica surface. In addition, several other characterization methods were used such as Brunauer-Emmett-Teller (BET) surface area analysis to get information about the area covered by the functional groups, elemental analysis to find out the percentages of nitrogen, sulfur, hydrogen and carbon in the sorbents, TGA measurements to understand their thermal degradation behavior, and, scanning electron microscopy (SEM).to obtain the images of the sorbents.

2.6. Sorption Studies

2.6.1. Effect of Solution pH

Solution pH is one of the most important factors to understand the interaction between the forms of selenium and the functional groups on the sorbents. For this reason, initial experiments were carried out to find the optimum pH for sorption. Dilute HNO_3 and NH_3 were used to adjust the pH at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 10.0. Additional experiments were also done in 0.10 M, 0.50 M, and 2.0 M of HCl. During the pH optimization studies, other sorption parameters were kept constant at 50.0 mg sorbent amount, 20.0 mL solution volume of 100.0 $\mu\text{g/L}$ Se(IV) or Se(VI) solution. Sample solution was put in a 50 mL centrifuge tube, and the thermostated water bath shaker was set to 25.0 $^\circ\text{C}$ for 30 min shaking. Finally, the mixture was filtered to separate the sorbent, and the selenium concentration in the remaining solution was determined with ICP-MS or HGAAS after it was acidified with HNO_3 or HCl, respectively.

2.6.2. Effect of Shaking Time

Samples were shaken for 1, 5, 15, 30, 60, 120 minutes to understand the effect of shaking time on sorption. Other parameters were kept constant at 100.0 $\mu\text{g/L}$ selenium concentration, 20.0 mL solution volume, 50.0 mg sorbent amount, and 25.0 $^\circ\text{C}$ sorption temperature. Sorption experiments were carried out at pH 2.0 and 3.0. In this and also

all the following optimization studies, SH silica, SN silica and MIX were used for Se(IV) whereas NH₂ silica, SN silica and MIX were employed for Se(IV) sorption.

2.6.3. Effect of Sorbent Amount

The effect of sorbent amount was investigated with the use of 5.0, 10.0, 25.0, 50.0, and 100.0 mg while the other parameters were fixed at 100.0 µg/L selenium concentration, 30 min shaking time, 20.0 mL solution volume, and 25.0 °C sorption temperature. Experiments were carried out at pH 2.0 and 3.0.

2.6.4. Effect of Sorption Temperature

Effect of sorption temperature on the sorption of selenium was examined at 25.0 °C, 50.0 °C, and 75.0 °C. Other parameters were fixed at 100.0 µg/L selenium concentration, 30 min shaking time, 20.0 mL solution volume and 50.0 mg sorbent amount. Experiments were carried out at pH 2.0 and 3.0.

2.6.5. Repetitive Loading

To test the reusability of the sorbents, 20.0 mL of 100.0 µg/L selenium has been shaken with 50.0 mg of sorbent for 30 minutes at 25.0 °C. After shaking, the solution was centrifuged for 5 minutes at 6000 rpm, and the filtrate was analyzed by ICP-MS for selenium determination. Another 20.0 mL of selenium solution was put into the tube containing the sorbent and shaking/centrifugation steps were repeated as before. This procedure was applied 10 times sequentially. Other parameters were fixed at 100.0 µg/L selenium concentration, 30 min shaking time, 20.0 mL solution volume, and 25.0 °C sorption temperature. Experiments were carried out at pH 2.0.

2.6.6. Effect of Initial Concentration

The sorption efficiency of NH₂ silica, SH silica and SN silica was investigated for Se(IV) alone and Se(IV)+Se(VI) together, at the initial concentrations of 0.10, 1.0,

10.0, 50.0 and 100.0 mg L⁻¹. For this purpose, 20.0 mL of these solutions were shaken with 50.0 mg of sorbent at 25.0 °C for 30 min. The sorption studies with Se(IV) were carried out at pH 2.0 for SH silica and in 2.0 M HCl for NH₂ and bifunctional silicas, while for Se(IV)+Se(VI), the solution pH was kept at 2.0 and 3.0 for the bifunctional sorbent. The filtrates were analyzed with ICP-MS.

2.7. Desorption Studies

For Se(IV), a strong oxidant, potassium iodate (KIO₃); and for Se(VI), 2.0 M HCl was used for desorption. In the case of Se(IV), following the sorption step with the optimized conditions, the sorbent was shaken for an hour in a solution of 0.2% (m/v) KIO₃ in 1 M HCl and then was removed from the mixture by filtration. The selenium concentration in the eluate was determined by SFI-HGAAS. In the case of Se(VI), the desorption step was realized with the use of 2.0 M HCl in a similar procedure. The sorption parameters were as follows; 100.0 µg/L concentration of Se(IV) or Se(VI), shaking time of 30 min, solution volume of 20.0 mL, sorption temperature of 25.0 °C, solution pH of 2.0. The sorbents used were SH silica, SN silica and MIX for Se(IV) and NH₂ silica, SN silica and MIX for Sr(VI).

2.8. Method Validation

The sorption performance of the proposed sorbents was first investigated using the spiked samples of bottled drinking and tap water. The spike concentration was 100.0 µg L⁻¹ and the other parameters were as follows; solution pH of 1.0, 2.0, 3.0 for Se(IV) and 2.0, and 3.0 for Se(VI), shaking time of 30 min, solution volume of 20.0 mL, reaction temperature of 25.0 °C. As mentioned before, SH silica, SN silica and MIX were used for Se(IV), and NH₂ silica, SN silica and MIX were used for Se(VI). The methodology was also verified through spike recovery tests with the use of the related sorbents. During these studies the solution pH was 2.0 and the eluent volume was 20.0 mL while the other experimental parameters were kept constant as above.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Characterization of the Sorbents

3.1.1. Solid State Nuclear Magnetic Resonance (SSNMR) Spectroscopy

Solid state NMR spectra were obtained to elucidate the structure of the synthesized sorbents. Figures 3.1, 3.2 and 3.3 indicate the NMR spectra of NH₂ silica, SH silica, and SN (bifunctional) silica, respectively. On the ¹³C NMR spectrum of NH₂ silica, the peak numbered as 1 belongs to the carbon atom which is bound to silicon atom because it is the farthest carbon to the amine group and the electron density on this carbon is higher than the other carbons. It is relatively shielded and gives the closest peak to 0 ppm. The peak at 24.37 ppm is a bit closer to amine group which means electron density on this carbon is higher than the first carbon which leads a shift to the downfield region. The third peak at 44.95 ppm belongs to the carbon atom attached to the amine group, having the lowest electron density. This carbon is deshielded and has the highest chemical shift.

On the ¹³C NMR spectrum of SH silica (Figure 3.2), similar peaks are observed except there is a fourth peak at 52.15 ppm which belongs to the carbon atom attached to the oxygen atom. It has the lowest electron density and it is deshielded by highly electronegative oxygen, thus it gives the highest chemical shift. SN spectrum (Figure 3.3) has many peaks regarding both functional groups. Peaks numbered as 3 and 3' point out the carbons which are attached to amine and mercapto groups. Since nitrogen is more electronegative than sulfur, carbon numbered as 3 has lower electron density than 3', therefore has a higher chemical shift. Peaks numbered as 4 and 4' belong to carbons attached to the oxygen. Finally there is a fifth peak in this spectrum belongs to the β carbon of oxygen.

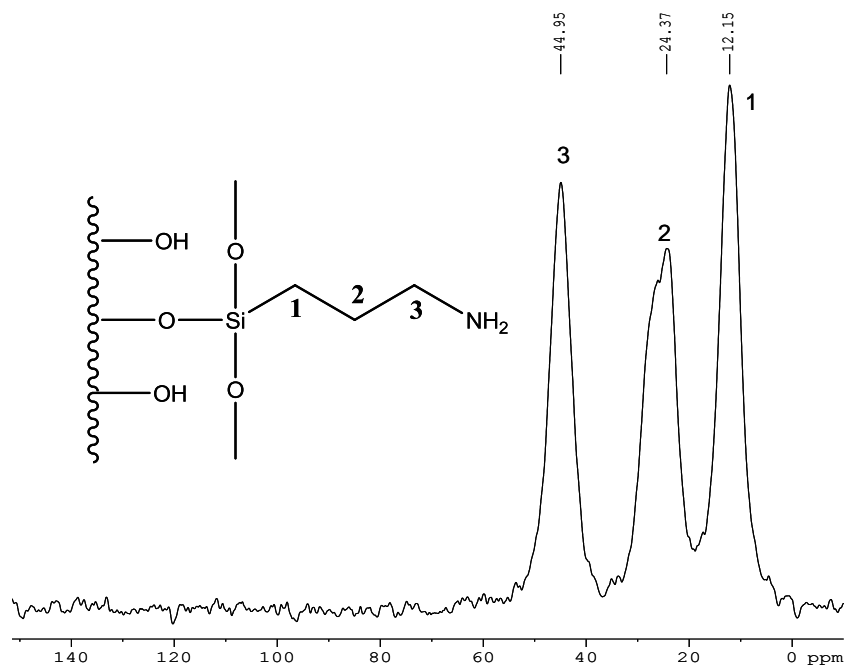


Figure 3.1. NMR spectrum of NH₂ silica.

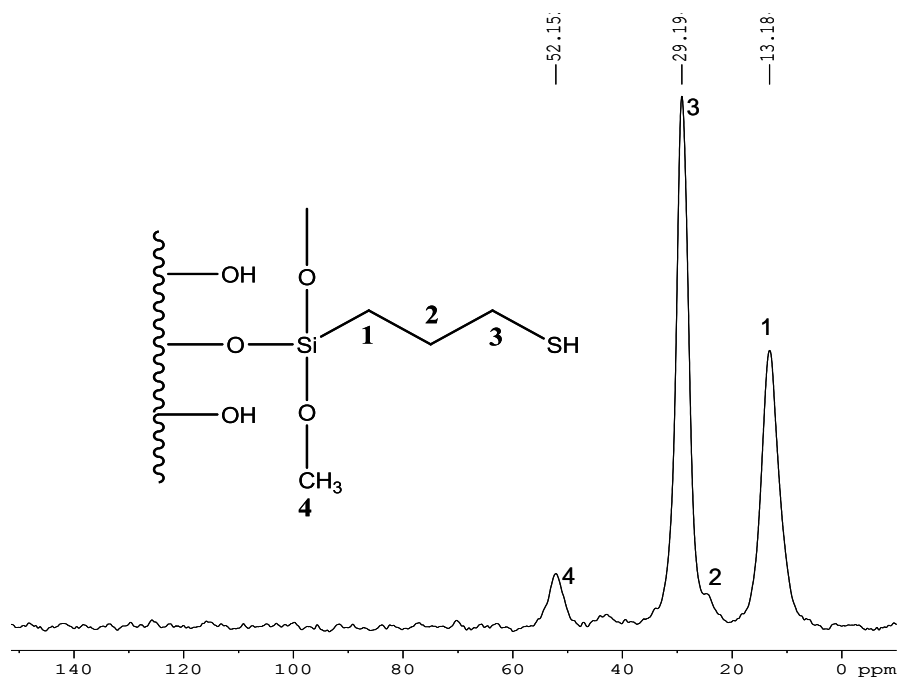


Figure 3.2. NMR spectrum of SH silica.

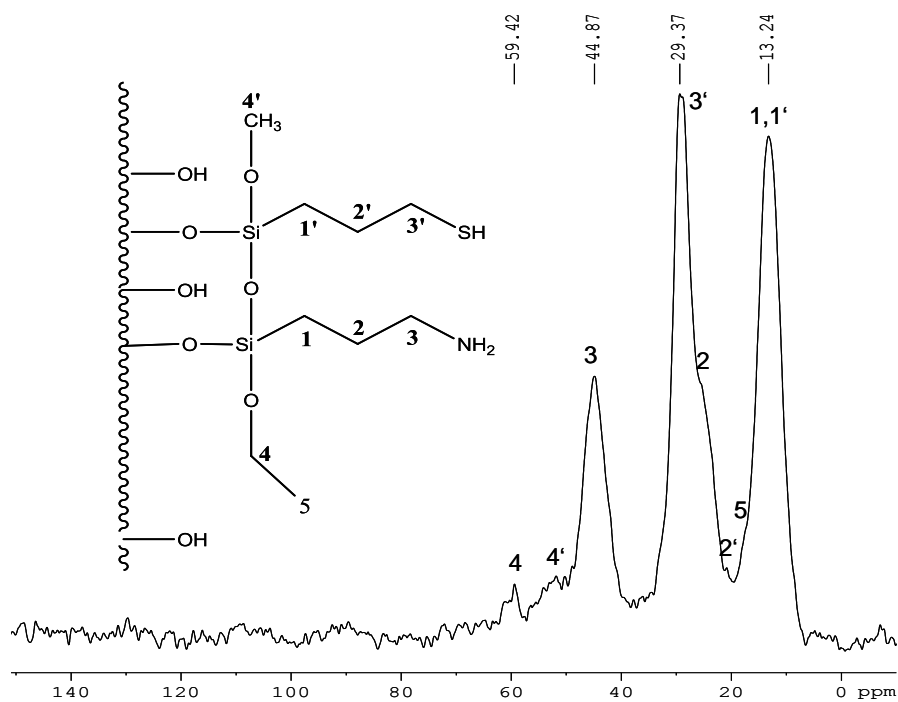


Figure 3.3. NMR spectrum of SN silica.

3.1.2. Brunauer-Emmett-Teller (BET) Surface Area Analysis

Table 3.1 indicates that modified silicas have lower surface areas and smaller pore widths than silica. The reason for these results must be the coverage of the silica surface with the functional groups through the formation of bonds between the silanol groups on the surface and the functional silanes.

Table 3.1. BET analysis results of silica and modified silicas.

Sorbent	BET surface area (m^2g^{-1})	Average pore width (\AA°)
Silica	253.9	17.3
NH_2 silica	168.9	14.1
SH silica	189.6	14.1
SN silica	183.9	14.1

3.1.3. Scanning Electron Microscopy (SEM)

The surfaces of synthesized sorbents were investigated using scanning electron microscopy. Characteristic SEM images of unmodified and modified silicas are shown in Figure 3.4. From the figure, it can be concluded that the unmodified silica has bigger particles than the others. Rather than being a real difference, this observation can be related to the synthesis procedure in which the mixture is refluxed and the silica particles become smaller during the reaction.

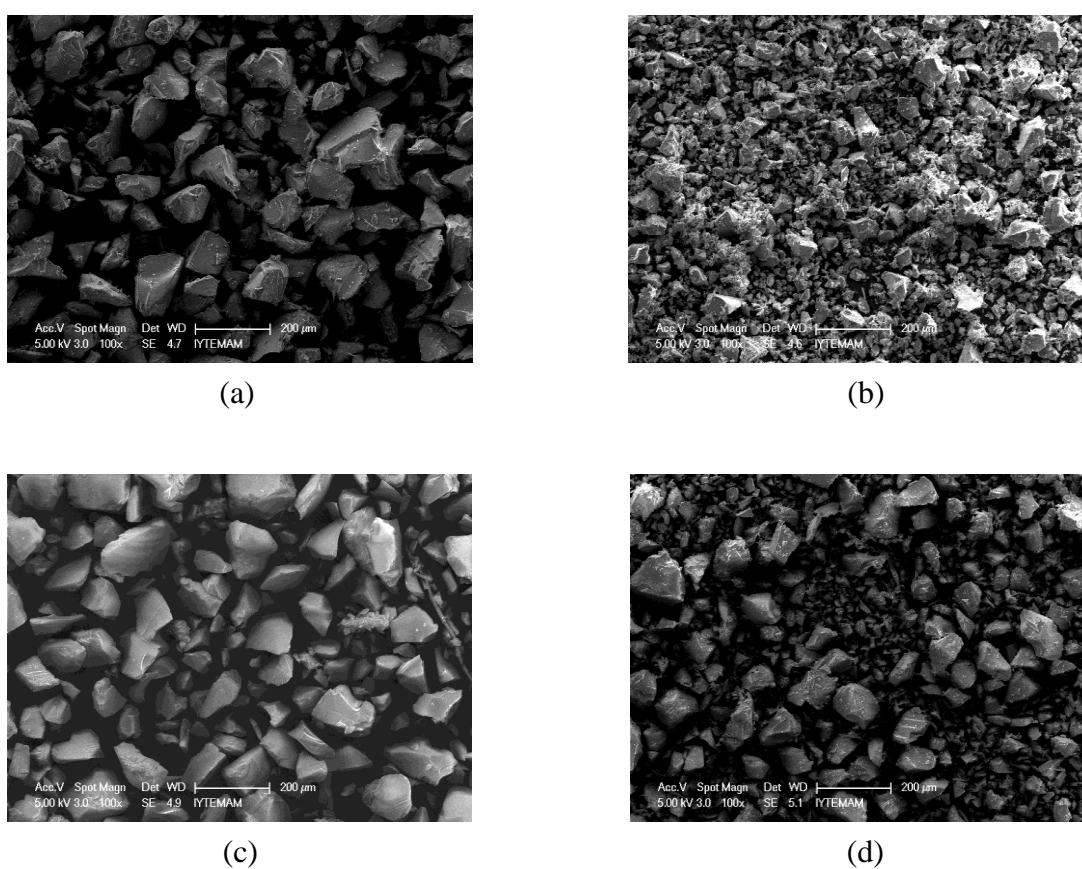


Figure 3.4. Characteristic SEM images of (a) unmodified silica, (b) NH₂ silica, (c) SH silica, (d) SN silica.

3.1.4. Elemental Analysis

The percentages of C, H, N, and S of synthesized sorbents were determined by elemental analysis and the results are given in Table 3.2. The percentages of N and S of modified silicas demonstrate that the functional groups are bonded onto the silica surface. This is also confirmed by the increase in the percentages of C and H indicating the attachment of 3-APTES and 3-MPTMS on silica. NH₂ silica has 2.10% N and SH silica has 3.72% S. On the other hand, the N and S percentages of the bifunctional silica are 1.62% and 2.62%, respectively. It appears that mono functionalized silicas have higher percentages of N and S than bifunctional silica. However, in the end, the total percentage of these elements refers to a higher percentage of modification. Additional measurements were done after desorption to see whether the eluents deteriorated the silica surface by removing the functional groups or not and it was found that the eluents did not cause a big change in the percentages of N and S. (Table 3.3) Although this observation has indicated the presence of N and S on the surface, it does not confirm the availability of the functional groups unless repetitive use of the sorbents has been demonstrated.

Table 3.2. Elemental analysis results of the synthesized sorbents.

Sorbent	C %	H %	N %	S %
Silica	0.16	0.88	ND	ND
NH ₂ silica	6.25	1.86	2.10	ND
SH silica	5.23	1.44	ND	3.72
SN silica	6.45	1.65	1.62	2.62

ND: Not detected

Table 3.3. Elemental analysis results of the synthesized sorbents after desorption.

Sorbent	C %	H %	N %	S %
NH ₂ silica	4.64	2.89	2.41	ND
SH silica	4.74	1.49	ND	3.91
SN silica (Se(IV))	6.16	1.79	1.23	2.75
SN silica (Se(VI))	6.34	1.72	1.75	2.96

ND: Not detected

3.1.5. Thermo Gravimetric Analysis (TGA)

Thermo gravimetric analysis was applied to the unmodified silica and the synthesized sorbents to investigate the extent of modification by monitoring the change in percent weight of the samples with increasing temperature. The graphs are given in Figure 3.5. Unmodified silica reveals approximately 4% weight loss between 25-200 °C due to the removal of water adsorbed on the surface. NH₂ and SH modified silica sorbents show similar thermal characteristics in the same temperature range; 4.6% and 1.5% weight loss, respectively because of the same reason. The thermal behavior of the bifunctional sorbent is between NH₂ and SH modified silicas, showing 3.0% loss at around 200 °C. Figure 3.5 also gives information about the weight loss between 200-600 °C as 7.1% for NH₂ silica, 10.2% for SH silica, and 9.5% for bifunctional silica which can be explained by the degradation of organic functional groups.

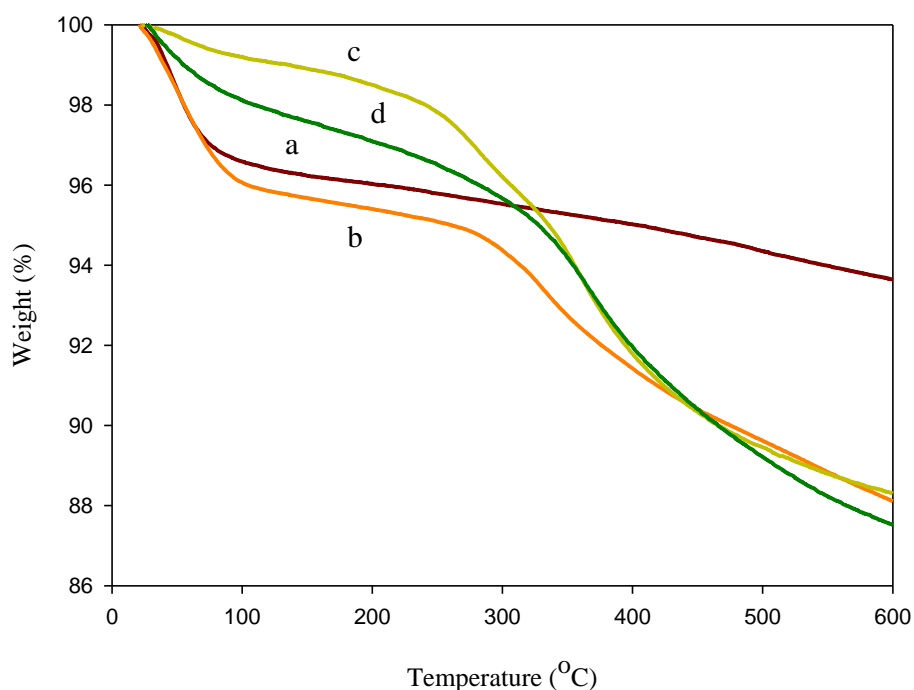


Figure 3.5. TGA curves of (a) unmodified silica, (b) NH₂ silica, (c) SH silica, (d) SN silica.

3.2. Calibration Plots

3.2.1. Calibration Curve for Se(IV) and Se(VI) Using ICP-MS

Plots of signal versus concentration constructed for Se(IV) and Se(VI) using ICP-MS can be seen in Figure 3.6. The limit of detection (LOD) based on 3s was calculated as $0.016 \mu\text{g L}^{-1}$ and limit of quantification (LOQ) based on 10s was $0.052 \mu\text{g L}^{-1}$.

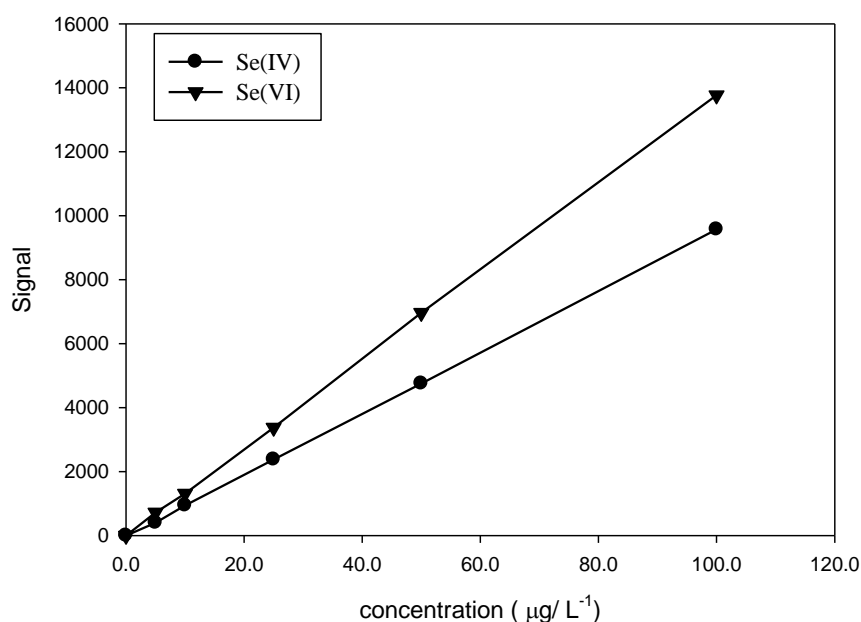


Figure 3.6. Calibration plots of Se(IV) and Se(VI). (●) Se(IV) ($y = 95.998x - 32.3$, $R^2 = 0.9999$), (▼) Se(VI) ($y = 138.01x - 12.65$, $R^2 = 0.9999$)

3.2.2. Calibration Curve for Se(IV) Using HGAAS

Plot of absorbance versus concentration constructed for Se(IV) using HGAAS can be seen in Figure 3.7. The limit of detection (LOD) based on 3s was calculated as $0.297 \mu\text{g L}^{-1}$ and limit of quantification (LOQ) based on 10s was $0.991 \mu\text{g L}^{-1}$.

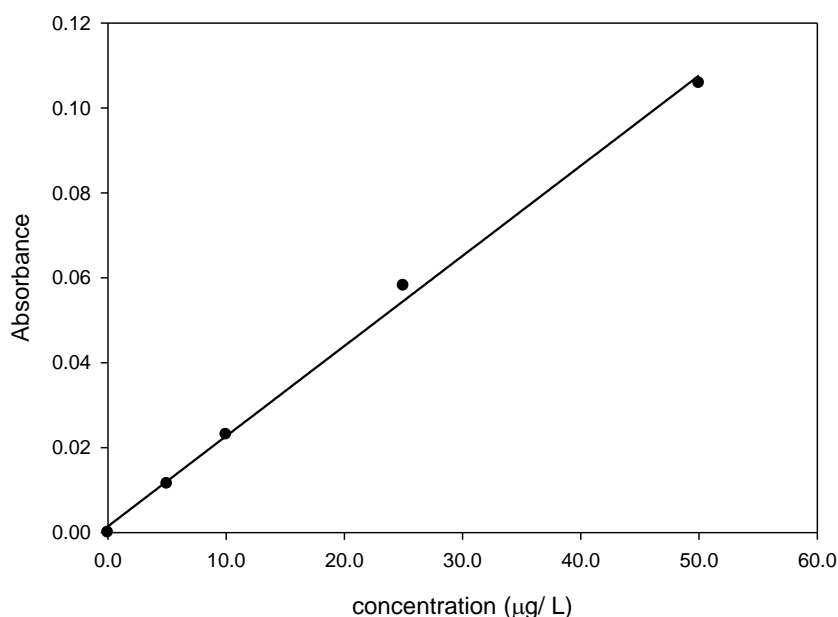


Figure 3.7. Calibration plot of Se(IV). ($y = 0.0021x + 0.0015$, $R^2 = 0.9975$)

3.3. Sorption Studies

3.3.1. Effect of Solution pH

The solution pH is one of the most important parameters which have a direct influence on the sorption. It both determines the form of the analyte in the solution and may also change the surface properties of the sorbent. The form of the analyte can be estimated from the distribution diagrams. The distribution diagrams for Se(IV) and Se(VI) are shown in Figure 3.8. Depending on the solution pH, Se(IV) can be in three different forms in aqueous solutions; namely, H_2SeO_3 is predominant in pHs lower than 2, HSeO_3^- between pH 2 and 8, and SeO_3^{2-} above pH 8 (Figure 3.8(a)). When Se(VI) is considered, SeO_4^{2-} and HSeO_4^- are the predominant forms above and below pH 2, respectively (Figure 3.8(b)).

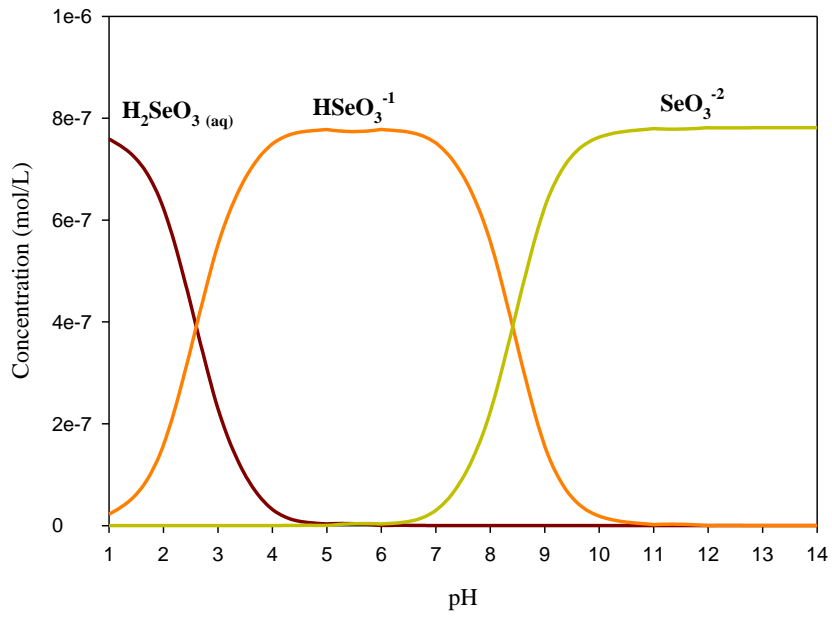
The effect of solution pH on the sorption of Se(IV) and Se(VI) is shown in Figure 3.9. Separate graphs were also drawn to better see the efficiency of each sorbent in the sorption of both species (Figure 3.10). As can be seen from Figure 3.8(a), NH_2 silica showed weak sorption towards Se(IV) except for 2.0 M HCl (>95%). At first look, this high acidity region appears to be a convenient medium for Se(IV) sorption by

NH₂ silica; however, as will be shown later, the efficiency of the sorbent is open to environmental affects and is not robust. On the other hand, this sorbent displays almost a quantitative (>95%) sorption to Se(VI) at pHs 2.0 and 3.0. The sorption mechanism can be explained by the electrostatic interaction between the protonated amine groups on the silica surface and the negatively charged forms of Se(VI) in the solution, predominantly, SeO₄²⁻. These results demonstrate that NH₂ silica is an efficient sorbent for Se(VI) at the indicated pH values under the experimental conditions applied.

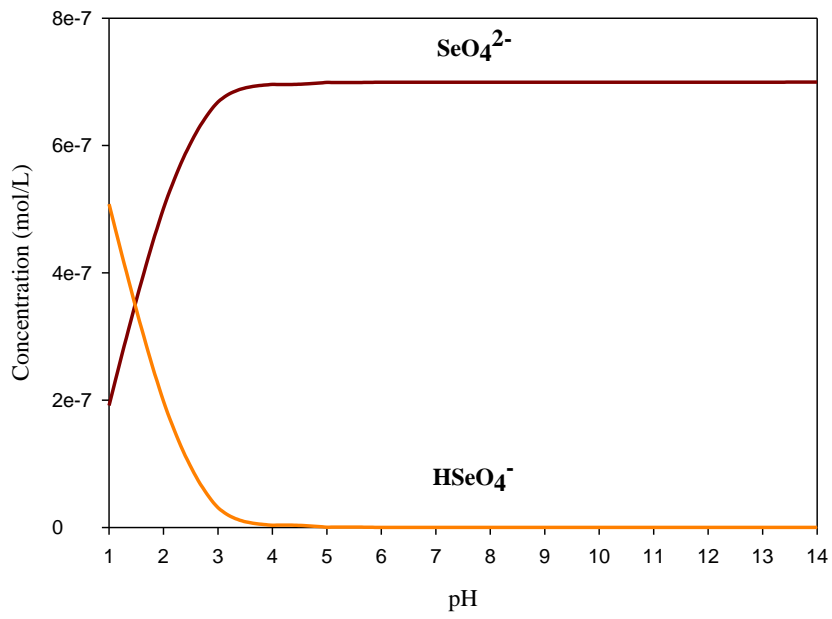
Figure 3.10(b) shows that SH silica is very effective in the sorption of Se(IV) over a wide pH range (>95%), from very acidic regions to pH 4.0, whereas Se(VI) is not retained appreciably at any pH values. Here, the interaction can be attributed to the chelate formation between the mercapto functional groups on the silica surface and the selenium species in the solution, namely, H₂SeO₃ and HSeO₃⁻.

It can be concluded from the previous two paragraphs that, NH₂ silica and SH silica can be used for the separate sorption of Se(VI) and Se(IV), respectively, after a simple pH adjustment step. On the other hand, bifunctional (SN) silica possesses the good features of the two sorbents; it can be used in the separate sorption of Se(IV) or sorption of both species after a pH adjustment step. For example, acidic pHs (<1.0) are convenient for the sorption of only Se(IV) where no sorption is observed for Se(VI). Alternatively, pH can be adjusted to 2.0 or 3.0 and thus Se(IV) and Se(VI) can be retained simultaneously. Although this observation needs to be verified further, it can be speculated that the two functional groups, namely amino- and mercapto-, behave synergistically. This finding also demonstrates the better efficiency of the bifunctional sorbent compared to the mono functionalized silicas.

In order to check whether the superior performance of the bifunctional sorbent is due to the presence of both -NH₂ and -SH functional groups in the mixture at the same time, mono-functionalized silicas were mechanistically mixed at equal portions (50:50) and this new mixture of the sorbents (MIX) was used in a similar manner. The sorption percentages given in Figure 3.10(d) indicate a comparable performance as the bifunctional sorbent. Although the mixed sorbent confirmed the synergistic behaviour of both functional groups, it was not as robust as the bifunctional sorbent and therefore, was not used in further studies. Still, this mixing strategy offers a potential for various applications.

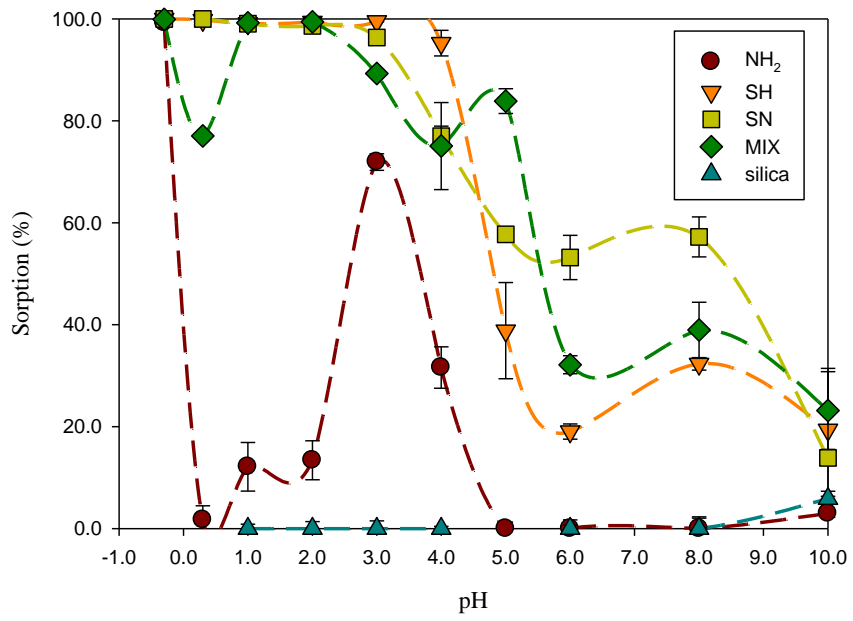


(a)

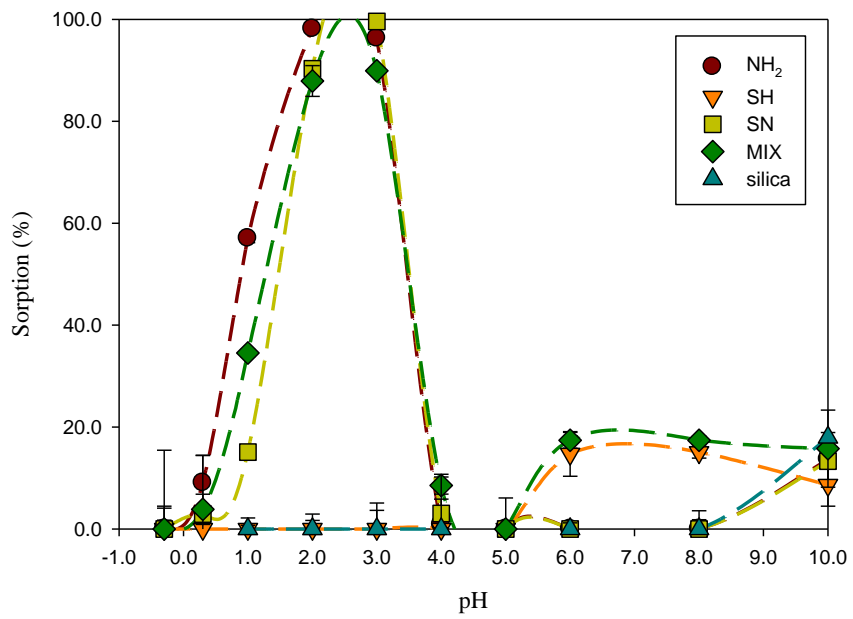


(b)

Figure 3.8. Distribution diagram of (a) Se(IV) and (b) Se(VI) in aqueous solutions. (Source: MINTEQ program)



(a)



(b)

Figure 3.9. Effect of solution pH on the sorption of (a) Se(IV) and (b) Se(VI). (20.0 mL 100.0 $\mu\text{g/L}$ Se(IV) or Se(VI) solution, 30 min shaking time, 50.0 mg sorbent, 25.0 $^{\circ}\text{C}$ reaction temperature)

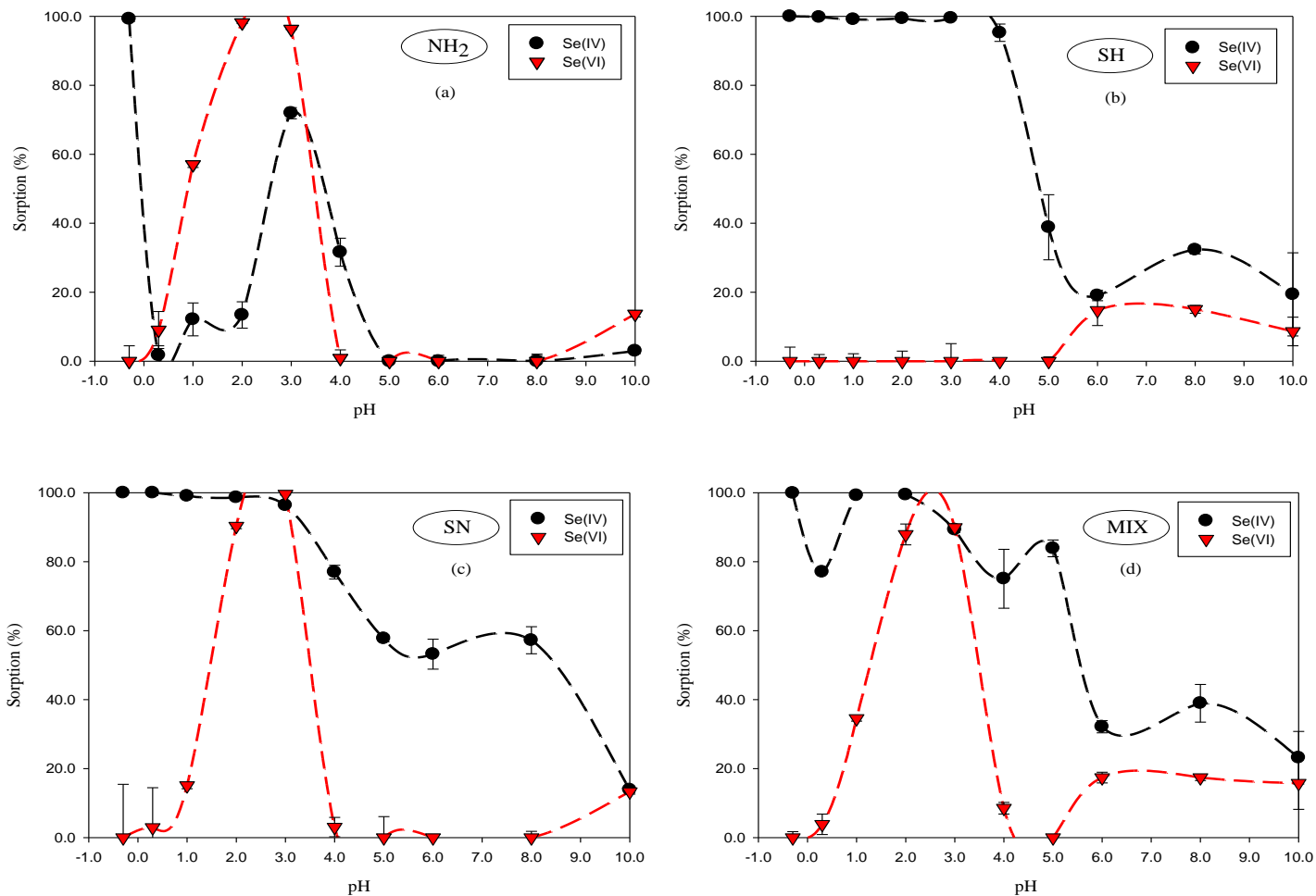


Figure 3.10. Effect of solution pH on the sorption of Se(IV) and Se(VI). The experimental conditions were given under Figure 3.9.

3.3.2. Effect of Shaking Time

Figure 3.11 shows the effect of shaking time on Se(VI) sorption at pH 2.0 for NH₂ silica, SN silica, and MIX silica. As in the case of Se(IV), there is an appreciable sorption (>90%) even in 5 min, but the maximum sorption is obtained in 30 min. In further experiments, a shaking time of 30 min was employed for both selenium forms to make sure obtaining high efficiency in sorption.

The effect of shaking time on Se(IV) sorption was investigated for SH silica, SN silica, and MIX silica, at pHs 2.0 and 3.0. The results are given in Figure 3.11. As a general comment, it can be said that all sorbents retain Se(IV) in a very short time; the percentage sorption was $\geq 95\%$ even in 5 min under the conditions employed at both pHs. As seen from the figures, MIX silica is not robust as the bifunctional sorbent, especially at pH 3.0. In spite of the rapid sorption obtained, small particle size of the sorbents precludes their application in column mode.

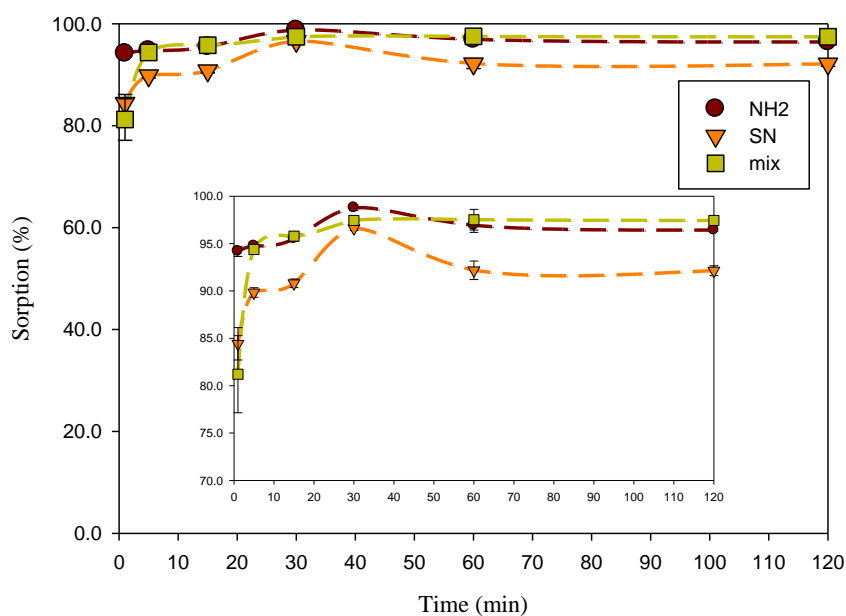
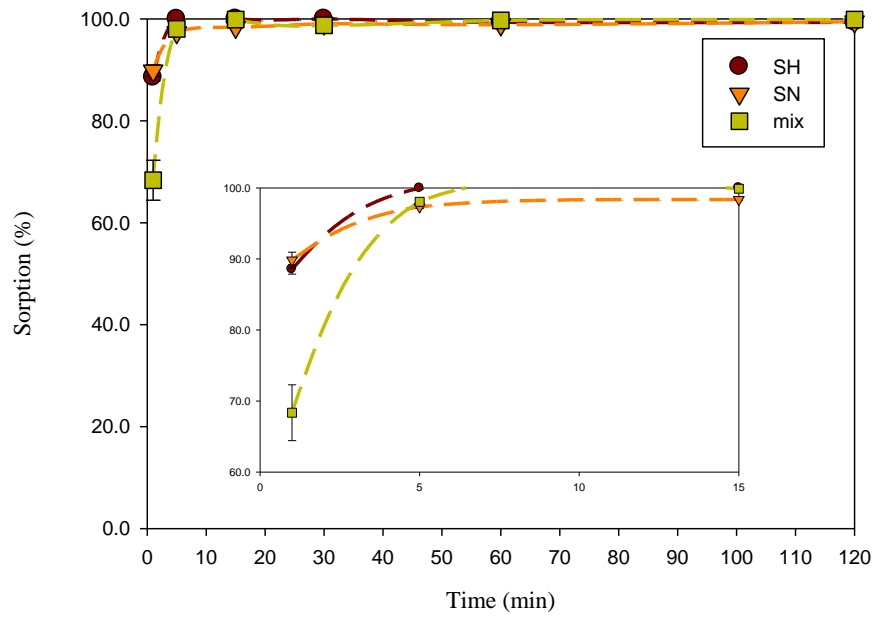
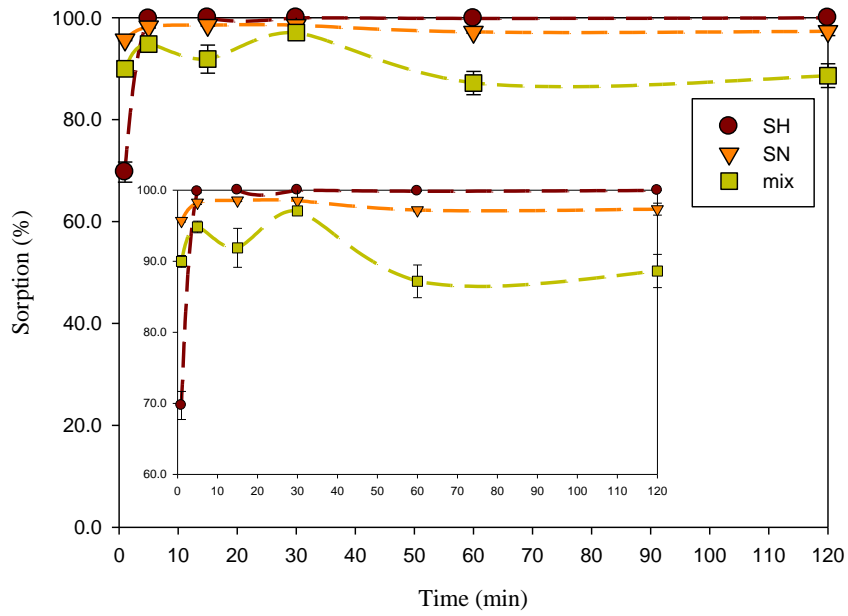


Figure 3.11. Effect of shaking time on Se(VI) sorption at pH 2.0.



(a)



(b)

Figure 3.12. Effect of shaking time on Se(IV) sorption at (a) pH 2.0, (b) pH 3.0.

3.3.3. Effect of Sorbent Amount

Several experiments were made to determine the required amount of the sorbents for the maximum sorption of selenium species. Figure 3.13 demonstrates the results for Se(VI) at pH 2.0. 50.0 mg sorbent amount was decided to the optimum parameter. Figure 3.14 demonstrates the results for Se(IV) at pH 2.0. It can be said that even 5.0 mg of SH silica or SN silica is sufficient for an efficient uptake (>95%) under the experimental conditions applied whereas 10.0 mg was found to give quantitative sorption. For MIX silica, a minimum amount of 10.0 mg was necessary for efficient sorption. At pH 3.0, the sorption performance of MIX silica deteriorated with the increase in the amount of sorbent (Figure 3.14(b)). Although it needs further verification, this finding might have resulted from the more kinetically-favored interaction of Se(IV) with mercapto than with amino groups. In further experiments, 50.0 mg of the sorbents were used to guarantee the quantitative sorption.

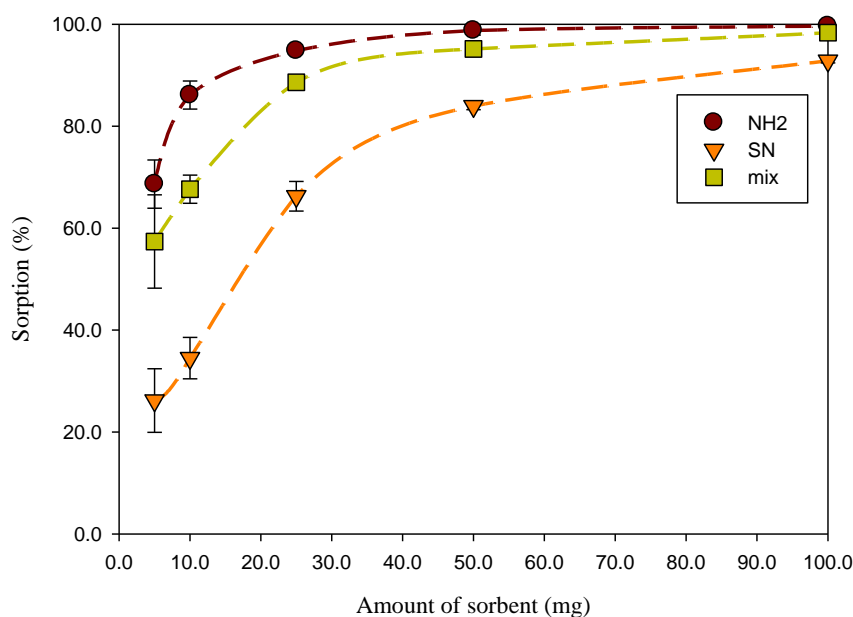
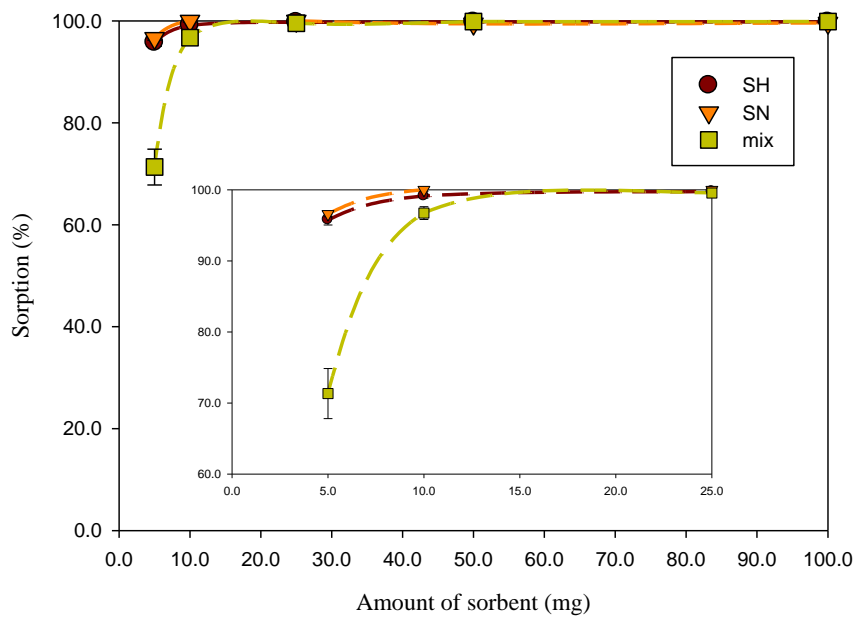
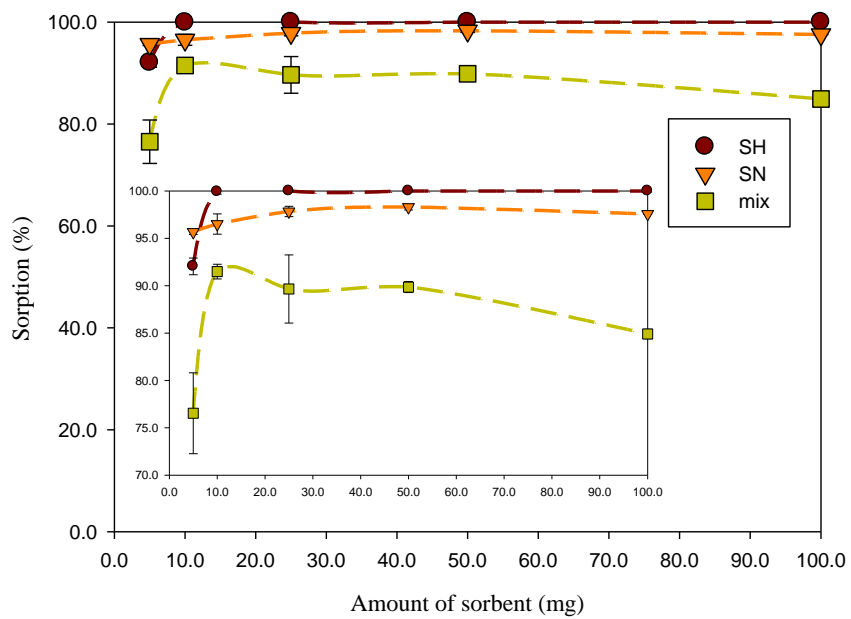


Figure 3.13. Effect of the sorbent amount on Se(VI) sorption at pH 2.0.



(a)



(b)

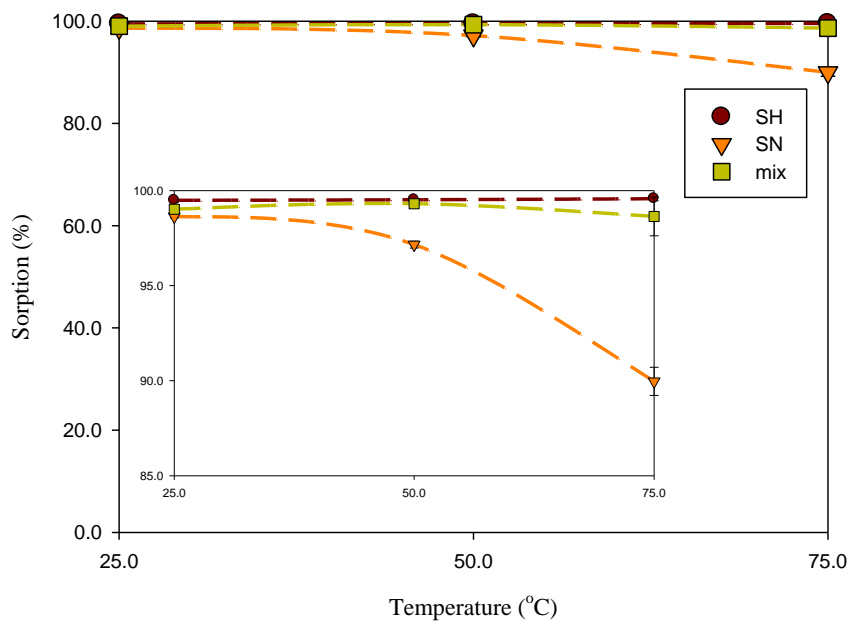
Figure 3.14. Effect of the sorbent amount on Se(IV) sorption at (a) pH 2.0, (b) pH 3.0.

3.3.4. Effect of Sorption Temperature

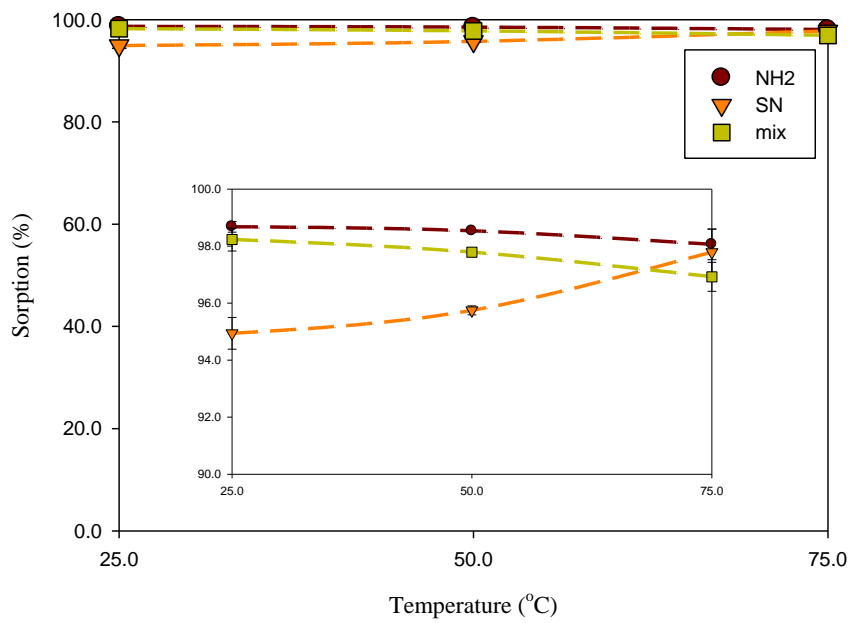
Sorption behavior of the related sorbents towards Se(IV) and Se(VI) was examined at different temperatures. As shown in Figure 3.15(a), SH and MIX sorbents have shown no change with the increase in temperature whereas the sorption of SN silica decreased to 90% demonstrating the exothermic nature of the sorption. The results of Se(VI) study have indicated a very small decrease in the sorption of NH₂ and MIX silicas with increasing temperature whereas SN silica displayed an opposite behavior.(Figure 3.15(b)) These results have shown the necessity of close control of the sorption temperature; for this reason, further experiments were carried out at a constant temperature of 25.0 °C.

3.3.5. Repetitive Loading

As explained in Section 2.6.5, sequential sorption experiments were realized for both forms to check the reusability of the sorbents. As shown in Figure 3.16(a), for Se(IV), all the related sorbents can be used even 10 times sequentially without a significant decrease in the percentage uptake. On the other hand, for Se(VI), the sorption percentage of NH₂ and MIX silicas has decreased slightly whereas the decline for bifunctional silica was noticeable.

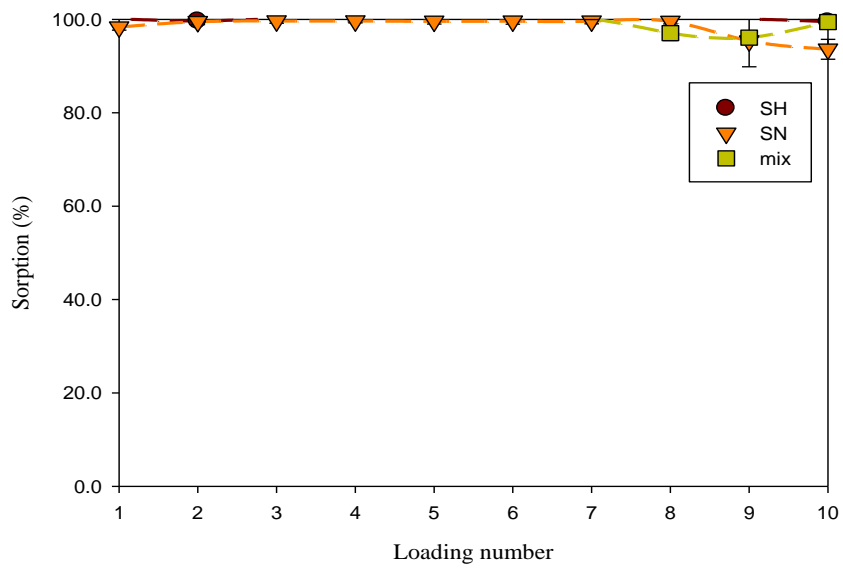


(a)

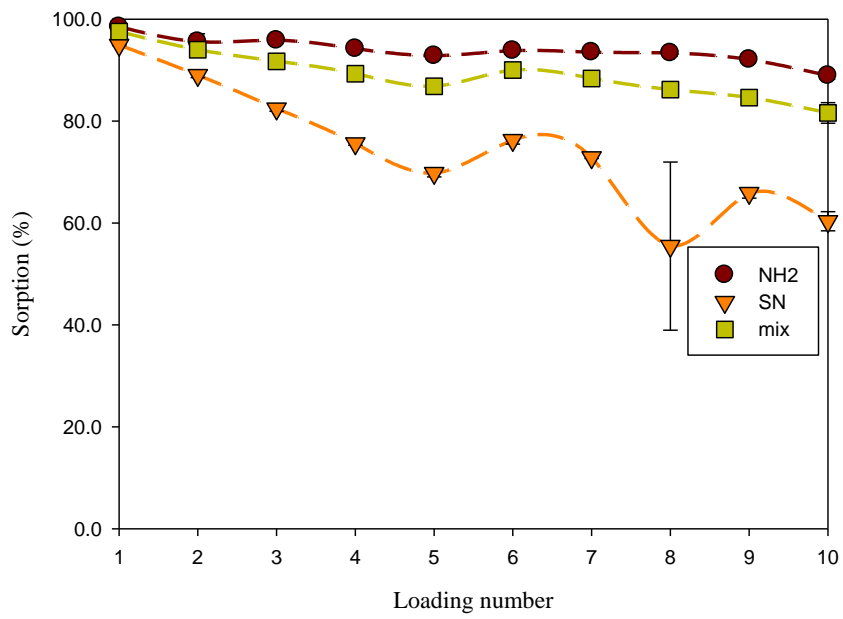


(b)

Figure 3.15. Effect of sorption temperature on (a) Se(IV), (b) Se(VI) sorption at pH 2.0.



(a)



(b)

Figure 3.16. Effect of repetitive loading on (a) Se(IV), (b) Se(VI) sorption at pH 2.0.

3.3.6. Effect of Initial Concentration

Sorption efficiency of SH, NH₂ and SN silicas was tested as a function of initial concentration of Se(IV) alone and Se(IV)+Se(VI) together at the related pHs. As shown in Table 3.4, with the increase in the initial concentration of Se(IV), a slight decrease in percentage sorption has first occurred for 50.0 mg L⁻¹, and then for 100 mg L⁻¹ the capacity of the SH silica has decreased almost to 50%. Table 3.5 demonstrates the results of the sorption percentages of Se(IV) using SN and NH₂ silicas in 2.0 M HCl. Bifunctional silica has shown almost quantitative sorption at an initial concentration of 10.0 mg L⁻¹ while the percentage sorption of NH₂ silica started to decrease even for an initial concentration of 1.0 mg L⁻¹ to 25.6%. These findings are also consistent with the previous results given in Section 3.2.1 in a way that the silica sorbent functionalized only with NH₂ groups is not sufficient even for such a low concentration as 1.0 mg L⁻¹. In addition, this feature demonstrates the better efficiency of the bifunctional sorbent over mono-functionalized silicas. Table 3.6 indicates the sorption percentages of Se(IV)+Se(VI) at pH 2.0 and 3.0 using SN silica. As seen, an initial concentration of 10.0 mg L⁻¹ for both selenium species can efficiently be retained by the bifunctional sorbent after which the sorption percentages start to decrease gradually.

Table 3.4. Effect of initial Se(IV) concentration on the sorption by SH silica at pH 2.0.

Concentration (mg L⁻¹)	% sorption
0.10	99.4 ± 0.1
1.0	98.5 ± 0.1
10.0	99.7 ± 0.2
50.0	88.2 ± 1.7
100.0	51.4 ± 5.5

Table 3.5. Effect of initial Se(IV) concentration on the sorption by SN and NH₂ silica in 2.0 M HCl.

Concentration (mg L ⁻¹)	SN	NH ₂
0.10	99.9 ± 0.1	99.3 ± 0.8
1.0	99.7 ± 0.1	25.6 ± 0.1
10.0	97.9 ± 0.1	~ 0
50.0	~ 0	~ 0
100.0	~ 0	~ 0

Table 3.6. Effect of initial Se(IV) + Se(VI) concentration on the sorption by SN silica at pH 2.0 and 3.0.

Concentration (mg L ⁻¹)		% sorption	
Se(IV)	Se(VI)	pH2	pH3
0.10	0.10	94.3 ± 0.3	98.2 ± 0.2
1.0	1.0	95.8 ± 0.2	99.3 ± 0.1
10.0	10.0	85.8 ± 0.5	97.9 ± 0.1
50.0	50.0	59.3 ± 2.7	34.9 ± 0.6
100.0	100.0	56.5 ± 1.9	20.7 ± 1.5

3.4. Desorption Studies

For Se(IV), a strong oxidant, potassium iodate (KIO₃); and for Se(VI), 2.0 M HCl was used for desorption. In the case of Se(IV), following the sorption step with the optimized conditions, the sorbent was shaken for an hour in a solution of 0.2% (m/v) KIO₃ in 1 M HCl and then was removed from the mixture by filtration. The selenium concentration in the eluate was determined by SFI-HGAAS. In the case of Se(VI), the desorption step was realized with the use of 2.0 M HCl in a similar procedure. The sorption parameters were as follows; 100.0 µg/L concentration of Se(IV) or Se(VI), shaking time of 30 min, solution volume of 20.0 mL, reaction temperature of 25.0°C, solution pH of 2.0. The sorbents used were SH silica, SN silica and MIX for Se(IV) and NH₂ silica, SN silica and MIX for Se(VI).

Results of the desorption study performed as explained in Section 2.6 are given in Table 3.7. The elemental analysis results obtained before and after the desorption step have shown that at least N and S of the functional groups were still present on the surface of silica. Therefore, it can be concluded that the use of KIO_3 results in the oxidation of Se(IV) to Se(VI) and thus the retained selenium was released from the sorbents where the sorbents displays no affinity for Se(VI) . In the case of Se(VI) , on the other hand, 2.0 M HCl was used as the eluent. The reason for this was the inability of the sorbents to retain Se(VI) in very acidic regions. The results show the success of the eluents used for desorption.

Table 3.7. Percentage desorption results of Se(IV) and Se(VI) .

	Sorbent	Eluent	% Recovery
Se(IV)	SH silica	0.2% (m/v) KIO_3 in 1 M HCl	90.4(\pm 4.6)
Se(VI)	NH_2 silica	2.0 M HCl	83.8(\pm 5.7)
Se(IV)	SN silica	0.2% (m/v) KIO_3 in 1 M HCl	84.5(\pm 1.2)
Se(VI)	SN silica	2.0 M HCl	83.4(\pm 4.2)
Se(IV)	MIX	0.2% (m/v) KIO_3 in 1 M HCl	95.9(\pm 5.5)
Se(VI)	MIX	2.0 M HCl	86.4(\pm 2.5)

3.5. Method Validation

As mentioned in Section 2.8, the proposed sorbents were applied to spiked samples of bottled drinking and tap water. Firstly, the sorption performance of the sorbents was checked. The results are summarized in Table 3.8 and 3.9 for Se(IV) and Se(VI) , respectively. As can be seen from Table 3.8, the related sorbents displayed very efficient performances towards Se(IV) at the indicated pHs; the sorption percentages were above 97.7% in all cases. In contrast to Se(IV) , the situation is more complicated for Se(VI) . According to Table 3.9, the choice of the sorbent must be based on the pH of the sample since pH 2.0 is more convenient for NH_2 silica whereas pH 3.0 provides better results for the bifunctional sorbent for both water types. MIX silica, on the other hand, exhibits a superior performance at both pHs for both water types.

In line with the sorption results mentioned above, the spike recoveries after sorption/elution steps were acceptable for Se(IV) for the three sorbents investigated, namely, SH, SN, and MIX silicas. As can be seen from Table 3.10, the percentage recoveries changed between 82.2 ± 7.1 and 109.4 ± 3.6 . For Se(VI), MIX sorbent has shown better spike recoveries for Se(IV) and Se(VI) species as given in Table 3.11. For spiked bottled drinking and tap water samples, the respective percent recoveries were 87.1 ± 3.6 and 74.5 ± 6.6 . The relatively lower results for the bifunctional sorbent might be attributed to the possible interfering species present in the water samples.

Table 3.8. Percent sorption of spiked selenite in bottled drinking and tap water.

% Sorption of Se (IV)						
pH	Bottled Drinking Water			Tap Water		
	SN silica	MIX	SH silica	SN silica	MIX	SH silica
1.0	98.6±0.1	97.7±0.1	97.8±0.3	101.6±0.1	99.9±0.8	100.9±0.1
2.0	98.7±0.1	97.9±0.3	98.4±0.1	101.5±0.1	100.9±0.4	101.4±0.1
3.0	98.9±0.1	92.3±1.0	99.2±2.0	100.9 ±0.1	100.2±0.3	101.6±0.1

Table 3.9. Percent sorption of spiked selenate in bottled drinking and tap water.

% Sorption of Se (VI)						
pH	Bottled Drinking Water			Tap Water		
	NH ₂ silica	SN silica	MIX	NH ₂ silica	SN silica	MIX
2.0	95.5±0.2	69.6±0.3	92.4±0.3	91.3±0.2	21.5±4.2	83.4±0.5
3.0	65.0±8.1	99.1±0.1	98.2±0.4	58.4±0.9	81.4±0.3	82.2±1.3

Table 3.10. Percent recoveries of spiked selenite in bottled drinking and tap water.

% Recovery of Se(IV)						
pH	Bottled Drinking Water			Tap Water		
	SN silica	MIX	SH silica	SN silica	MIX	SH silica
2.0	86.8±2.4	109.4±3.6	93.2±5.8	90.5±1.3	82.2±7.1	95.5±2.8

Table 3.11. Percent recoveries of spiked selenate in bottled drinking and tap water.

% Recovery of Se(VI)						
pH	Bottled Drinking Water			Tap Water		
	SN silica	MIX	NH ₂ silica	SN silica	MIX	NH ₂ silica
2.0	57.3±20.4	87.1±3.6	75.8±3.9	52.6±9.1	74.5±6.6	73.5±2.6

CHAPTER 4

CONCLUSION

In this study, three novel sorbents have been synthesized and used for the speciation of selenite and selenate in waters. Characterization of the sorbents was realized using SSNMR, SEM, TGA, BET surface area analysis and elemental analysis and the successful attachment of the functional groups namely, amine (NH₂), mercapto (SH), or both, on the surface of silica has been demonstrated.

For each sorbent, sorption parameters were optimized and performance data were obtained by using atomic spectrometric techniques. It has been shown that either Se(IV) or Se(VI) can be retained and determined with mono-functionalized silicas after a simple pH adjustment step. Bifunctional silica, on the other hand, has been shown to be applicable for the sorption of Se(IV) alone or Se(IV) and Se(VI) together. The better performance of the bifunctional sorbent and mechanically-mixed silica (NH₂-silica and SH-silica in 1:1 ratio) acquired for the spiked water samples is promising and may offer a new strategy in the speciation studies.

REFERENCES

- Ari U., Volkan M., Aras N.K. 1991. Determination of selenium in diet by Zeeman effect graphite furnace atomic absorption spectrometry for calculation of daily dietary intake. *J. Agric. Food Chem.* 39: 2180-2183.
- Bedwall R.S., Nair N., Sharma M.P., Mathur R.S. 1993. Selenium its biological perspectives. *Med. Perspect.* 41: 150-159.
- Blitz I.P., Blitz J.P. Gun'ko V.M., Sheeran D.J. 2007. Functionalized silicas: Structural characteristics and adsorption of Cu(II) and Pb(II). *Colloids and Surfaces A* 307: 83-92.
- Burke A.M., Hanrahan J.P., Healy D.A., Sodeau J.R., Holmes J.D., Morris M.A. 2009. Large pore bi-functionalised mesoporous silica for metal ion pollution treatment. *J. Hazard. Mat.* 164: 229-234.
- Cabañero A.I., Madrid Y., Camara C. 2007. Mercury–selenium species ratio in representative fish samples and their bioaccessibility by an in vitro digestion method. *Biol. Trace Elem. Res.* 119: 195–211.
- Chen M., Yang T., Wang J. 2009. Precipitate coating on cellulose fibre as sorption medium for selenium preconcentration and speciation with hydride generation atomic fluorescence spectrometry. *Anal. Chim. Acta* 631: 74-79.
- Chand V., Prasad S. 2009. Trace determination and chemical speciation of selenium in environmental water samples using catalytic kinetic spectrophotometric method. *J. Hazard. Mat.* 165: 780-788.
- Chappuis P., Poupon J. 1991. Le rôle du sélénium dans la défense du stress oxydatif. *Cah. Nutr. Diet.* 26: 295-297.
- Cuvín-Aralar M.L., Furness R.W. 1991. Mercury and selenium interaction: a review. *Ecotoxicol. Environ. Saf.* 21: 348-364.
- Díaz J.P., Navarro M., López H., López M.C. 1997. Determination of selenium levels in dairy products and drinks by hydride generation atomic absorption spectrometry: correlation with daily dietary intake. *Food Addit. Contam.* 14: 109-114.
- Díaz-Alarcon J.P., Navarro-Alarcon M., Lopez-Ga de la Serrana H., Lopez-Martinez M.C. 1994. Determination of selenium in fresh fish from southeastern Spain for calculation of daily dietary intake. *J. Agric. Food Chem.* 42: 334-337.
- Dumont E., Vanhaecke F., Cornelis R. 2006. Selenium speciation from food source to metabolites: a critical review. *Anal. Bioanal. Chem.* 385: 1304–23.

- Ellingsen D.G., Holland R.I., Thomassen Y., Landro-Olstad M., French W., Kjuus H. 1993. Mercury and selenium in workers previously exposed to mercury vapour at a chloroalkali plant. *Br. J. Ind. Med.* 50: 745-752.
- Frost D.V. 1983. What do losses in selenium and arsenic bioavailability signify for health? *Sci. Total Environ.* 28: 455-466.
- Gondi F., Dauto G., Feher J., Bogy G., Alfthan G. 1992. Selenium in Hungary. The rock-soil-human system. *Biol. Trace Elem. Res.* 35: 299-306.
- Harwood J.J., Su W. 1997. Analysis of organic and inorganic selenium anions by ion chromatography-inductively coupled plasma atomic emission spectroscopy. *J. Chromatogr. A.* 788: 105-111.
- Hershey W., Oostdyk T.S., Keliher E.N. 1988. Determination of arsenic and selenium in environmental and agricultural samples by hydride generation atomic absorption spectrometry. *J. Assoc. Anal Chem.* 71: 1090-1093.
- Izgi B., Gucer S., Jacimovic R. 2005. Determination of selenium in garlic (*Allium sativum*) and onion (*Allium cepa*) by electro thermal atomic absorption spectrometry. *Food chem.* 99: 630-637.
- Kápolna E., Shah M., Caruso J.A., Fodor P. 2007. Selenium speciation studies in Se-enriched chives (*Allium schoenoprasum*) by HPLC-ICP-MS. *Food chem.* 101: 1398-1406.
- Keshan Disease Research Group. 1979. Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. *Chi. Med.* 92: 477-482.
- Levander O.A., Burk R.F. 1994. *Selenium. In: Modern Nutrition in Health and Disease.* ed. Shils M.E., Olson J.A., Shike M. Philadelphia: Lea and Febiger : 242-251.
- Li Y., Hu B., He M., Xiang G. 2008. Simultaneous speciation of inorganic selenium and antimony in water samples by electrothermal vaporization inductively coupled plasma mass spectrometry following selective cloud point extraction. *Water Research* 42: 1195-1203.
- Liang P., Chen X.G. 2005. Preconcentration of rare earth elements on silica gel loaded with 1-phenyl-3-methyl-4-benzoylpyrazol-5-one prior to their determination by ICP-AES. *Anal. Sci.* 21: 1185-1188.
- Lin T. 2007. Inorganic selenium speciation in groundwaters by solid phase extraction on Dowex 1X2. *J. Hazard. Mat.* 149: 80-85.
- Liu Q. 2009. Determination of ultra-trace amounts of inorganic selenium species in natural water by ion chromatography-inductively coupled plasma-mass spectrometry coupled with nano-Al₂O₃ solid phase extraction. *Cent. Eur. J. Chem.* 8(2): 326-330.

- Lorena C.L., Peralta-Zamora P., Bueno M.I. 1998. Pre-concentration of rare earths using silica gel loaded with 1-(2-pyridylazo)-2-naphthol (PAN) and determination by energy dispersive X-ray fluorescence. *Talanta* 46: 1371–1378.
- Maleki N., Safavi A., Doroodmand M.M. 2005. Determination of selenium in water and soil by hydride generation atomic absorption spectrometry using solid reagents. *Talanta* 66: 858-862.
- Miekeley N., Pereira R.C., Casartelli E.A., Almedia A.C., Carvalho M.F.B. 2005. Inorganic speciation analysis of selenium by ion chromatography-inductively coupled plasma-mass spectrometry and its application to effluents from a petroleum refinery. *Spectrochimica Acta Part B*. 60: 633-641.
- Najafi N.M., Seidi S., Alizadeh R., Tavakoli H. 2010. Inorganic selenium speciation in environmental samples using selective electrodeposition coupled with electrothermal atomic absorption spectrometry. *Spectrochimica Acta Part B* 65: 334-339.
- Navarro-Alarcon M., Lopez-Martinez M.C., 2000. Essentiality of selenium in human body: relationship with different diseases. *Sci. Total Environ.* 249: 347-371.
- Neve J., Henry M., Peretz A., Mareschi J.P. 1987. L'importance nutritionnelle du selenium. *Cah. Nut.r Diet.* 22: 145-162.
- Ortuno J., Ros G., Periago M., Martinez C., Lopez G. 1996. Selenium bioavailability and methods of evaluation. *Food Sci. Technol. Int.* 2: 135-150.
- Panigati M., Falcicola L., Mussini P., Beretta G., Facino R.M. 2007. Determination of selenium in Italian rices by differential pulse cathodic stripping voltammetry. *Food Chem.* 105: 1091-1098.
- Pappa E., Pappas A., Surai P. 2006. Selenium content in selected foods from the Greek market and estimation of the daily intake. *Sci. Total Environ.* 372: 100–8.
- Polatajko A., Jakubowski N., Szpunar J. 2006. State of the art report of selenium speciation in biological samples. *J. Anal. Atom Spectrom.* 21: 639-654.
- Sahin F., Volkan M., Howard A.G., Ataman Y.O. 2003. Selective pre-concentration of selenite from aqueous samples using mercapto-silica. *Talanta* 60: 1003-1009.
- Sarkar A.P., Datta P.K., Sarkar M. 1996. Sorption recovery of metal ions using silica gel modified with salicylaldehyde. *Talanta* 43: 1857-1862.
- Saygi K.O., Melek E., Tüzen M., Soylak M. 2007. Speciation of selenium(IV) and selenium(VI) in environmental samples by the combination of graphite furnace atomic absorption spectrometric determination and solid phase extraction on Diaion HP-2MG. *Talanta* 71: 1375-1381.
- Simonoff M., Simonoff G., ed. 1991. Le s'elenium et la vie. Paris: Masson.

- Skoog D.A., Holler F.J. and. Nieman T.A 1998. Principles of Instrumental Analysis, Philadelphia: Saunders College Publishing.
- Stripeikis J., Tudino M., Troccoli O., Wuilloudb R., Olsina R., Martinez L. 2001. On-line copper and iron removal and selenium(VI) pre-reduction for the determination of total selenium by flow-injection hydride generation-inductively coupled plasma optical emission spectrometry. *Spectrochimica Acta Part B*. 56: 93-100.
- Tarafdar A., Pramanik P. 2006. Synthesis of amino-functionalized mesoporous silica–zirconia mixed oxide using sodium silicate and zirconium carbonate complex. *Micropor. Mesopor. Mat.* 91: 221–224.
- Tuzen M., Saygi K.O., Soylak M. 2007. Separation and speciation of selenium in food and water samples by the combination of magnesium hydroxide coprecipitation graphite furnace atomic absorption spectrometric determination. *Talanta* 71:424-429
- Valencia M.C., Nicolas E.A., Valley L.F. 1999. Speciation of selenium (IV) in natural waters by solid phase spectrophotometry. *Talanta* 49: 915-921.
- Visual MINTEQ program ver. 2.61
- Wada O., Kurihara N., Yamazaki N., Jpn. J. 1993. Essentiality and toxicity of trace elements. *J. Nutr. Assess.* 10: 199-210.
- Yersel M., Erdem A., Eroğlu A.E., Shahwan T. 2005. Separation of trace antimony and arsenic prior to hydride generation atomic absorption spectrometric determination. *Analytica Chimica Acta* 534: 293–300.
- Zheng F., Hu B. 2009. Dual silica monolithic capillary microextraction (CME) on-line coupled with ICP-MS for sequential determination of inorganic arsenic and selenium species in natural waters. *J. Anal. Atom Spectrom.* 24: 1051-1061.
- Zhao Q.X., Chen Y.W., Belzile N., Wang M. 2010. Low volume microwave digestion and direct determination of selenium in biological samples by hydride generation atomic fluorescence spectrometry. *Analytica Chimica Acta*. 665: 123–128.
- Zhang L., Liu N., Yang L., Lin Q. 2009. Sorption behavior of nano-TiO₂ for the removal of selenium ions from aqueous solution. *J. Hazard. Mat.*, doi:10.1016/j.jhazmat.2009.05.098.
- Zhang X., Shi B., Spallholz J.E. 1993. The selenium content of selected meats, seafoods, and vegetables from Lubbock, Texas. *Biol. Trace Elem. Res.* 39: 161-169.
- Zhang Y., William T. Frankenberger Jr. 2001. Speciation of selenium in plant water extracts by ion exchange chromatography-hydride generation atomic absorption spectrometry. *Sci. Total Environ.* 269: 39-47.