DEVELOPMENT OF SOLID PHASE MICROEXTRACTION (SPME) FIBERS FOR VARIOUS ANALYTICAL APPLICATIONS: (I) SELENIUM SPECIATION IN WATERS. (II) SEPARATION AND DETERMINATION OF TRICLOSAN AND TRICLOCARBAN IN WATERS

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ABSTRACT

DEVELOPMENT OF SOLID PHASE MICROEXTRACTION (SPME) FIBERS FOR VARIOUS ANALYTICAL APPLICATIONS: (I) SELENIUM SPECIATION IN WATERS. (II) SEPARATION AND DETERMINATION OF TRICLOSAN AND TRICLOCARBAN IN WATERS

In the first part of this study, four selenium species namely selenite, selenate, selenomethionine and selenocystine in water samples were tried to be separated using single solid phase microextraction (SPME) fiber on the same chromatographic run. Bare silica fibers were immersed into agarose matrix containing nano zerovalent iron (nZVI), ceria (CeO_2) or zirconia (ZrO_2). After characterization of fibers sorption/desorption parameters were optimized and standard reference materials were used to validate the proposed method. Direct mode of SPME method was used to extract the analytes prior to their separation with HPLC and detection with ICPMS. The optimum conditions for the extraction of selenium species with nZVI-agarose fibers are obtained as extraction pH: 4.0, agitation speed: 700 rpm, extraction time: 60 min, desorption matrix: 10.0 mM citrate solution, desorption time: 30 min, solution temperature: 25 °C, ionic strength: no NaCl addition.

In the second part of this study, same fibers were used to separate triclosan (TCS) and triclocarban (TCC) using HPLC-DAD. Among all fibers prepared, nZVI-agarose modified fibers demonstrated the best extraction performance. The optimum conditions for the extraction of TCS and TCC with nZVI-agarose fibers are obtained as extraction pH: 5.0 and 7.0, agitation speed: 400 rpm, extraction time 60 min, desorption matrix: % 90 methanol - %10 water (adjusted to pH 3.0 with acetic acid), desorption time: 30 min, ionic strength: no NaCl addition.

ÖZET

ÇEŞİTLİ ANALİTİK UYGULAMALAR İÇİN KATI FAZ MİKROEKSTRAKSİYON FİBERLERİNİN GELİŞTİRİLMESİ: (I) SULARDA SELENYUM TÜRLEMESİ. (II) SULARDA TRİKLOSAN VE TRİKLOKARBAN AYRIMI VE TAYİNİ

Çalışmanın ilk kısmında, su örneklerinde bulunan dört selenyum türünün (selenit, selenat selenometiyonin ve selenosistin) tek bir SPME fiberi kullanılarak aynı kromatografik uygulamada ayrılmasına çalışılmıştır. Bu amaçla, silika fiberler sıfır değerlikli demir nanoparçacıkları, serya (CeO_2) veya zirkonya (ZrO_2) içeren agaroz matriksine daldırılarak kaplanmıştır. Modifiye edilmiş fiberlerin karakterizasyonu için SEM görüntüleri, EDX ve XRD spektrumları alınmıştır. Fiberlerin karakterizasyon işleminden sonra sorpsiyon/desorpsiyon koşulları optimize edilmiş ve önerilen metotu doğrulamak için standart referans maddeler kullanılmıştır. Direkt SPME metodu kullanılarak ekstrakte edilen analitler HPLC ile ayrılmış ve ICPMS ile tayin edilmiştir. Selenyum türlerinin nZVI-agaroz fiberleriyle yapılan ekstraksiyon işlemi için optimize edilmiş koşulların sonuçları şu şekilde elde edilmiştir; çözelti pH'sı: 4,0, karıştırma hızı: 700 rpm, ekstraksiyon süresi: 60 dk, desorpsiyon matriksi: 10,0 mM sitrat çözeltisi, desorpsiyon süresi: 30 dk, çözelti sıcaklığı: 25 °C, iyonik güç: tuz eklemeden.

Çalışmanın ikinci kısmında, aynı fiberler triklosan HPLC-DAD ile (TCS) ve triklokarban (TCC) ayrımı için kullanılmıştır. Hazırlanan bütün fiberler içerisinde en iyi ekstraksiyon performansını nZVI-agaroz fiberleri göstermiştir. TCS ve TCC maddelerinin nZVI-agaroz fiberleriyle yapılan ekstraksiyon işlemi için optimize edilmiş koşulların sonuçları şu şekilde elde edilmiştir; çözelti pH'sı: 5,0 ve 7,0, karıştırma hızı: 400 rpm, ekstraksiyon süresi: 60 dk, desorpsiyon matriksi: % 90 metanol - %10 su (pH'sı asetik asit ile 3,0'a ayarlanmış), desorpsiyon süresi: 30 dk, iyonik güç: tuz eklemeden.

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CHAPTER 1

INTRODUCTION

1.1. Solid Phase Microextraction (SPME)

Solid Phase Microextraction (SPME) was established as a solvent-free, rapid sample preparation technique for both in the laboratory and on site in 1990 (Arthur and Pawliszyn 1990). The basic principle of SPME is to use a small amount of extracting phase that can be either a high molecular weight polymeric liquid or a high porosity solid sorbent. The most traditional configuration of SPME consists of a small fused silica fiber, generally coated with a polymeric phase. The fiber phase absorbs or adsorbs the analytes until equilibrium is established in the system (Figure 1.1)

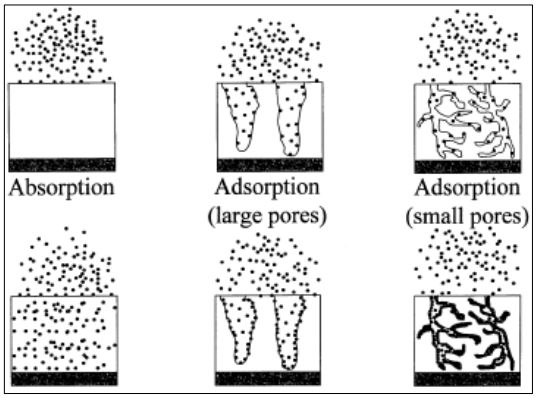


Figure 1.1. Absorptive and adsorptive extraction
(Source: Pawliszyn 2000)

When equilibrium conditions are reached, exposing the fiber for a longer amount of time does not accumulate more analytes. The magnitude of the partition coefficient between the sample matrix and the coating material limits the amount of analyte extracted by the coating at equilibrium (Pawliszyn 1999). To achieve an absorptive extraction the diffusion coefficients of the analytes in the extraction phase must be high.

Conversely, if the diffusion coefficient is low, the analyte is adsorbed on the coating (Pawliszyn 2000).

In SPME, analytes are not extracted exhaustively by the coating. This equilibrium approach is more selective than exhaustive extraction method because of the variance between extracting phase and matrix distribution coefficients that leads to the separation of target analytes and interferences. On the contrary, this approach generally demands calibration for complex matrices. This could be achieved by using standard addition method to determine the amounts of the analytes and to overcome the problem of matrix-to-matrix differences and their influence on distribution constants (Pawliszyn 1999).

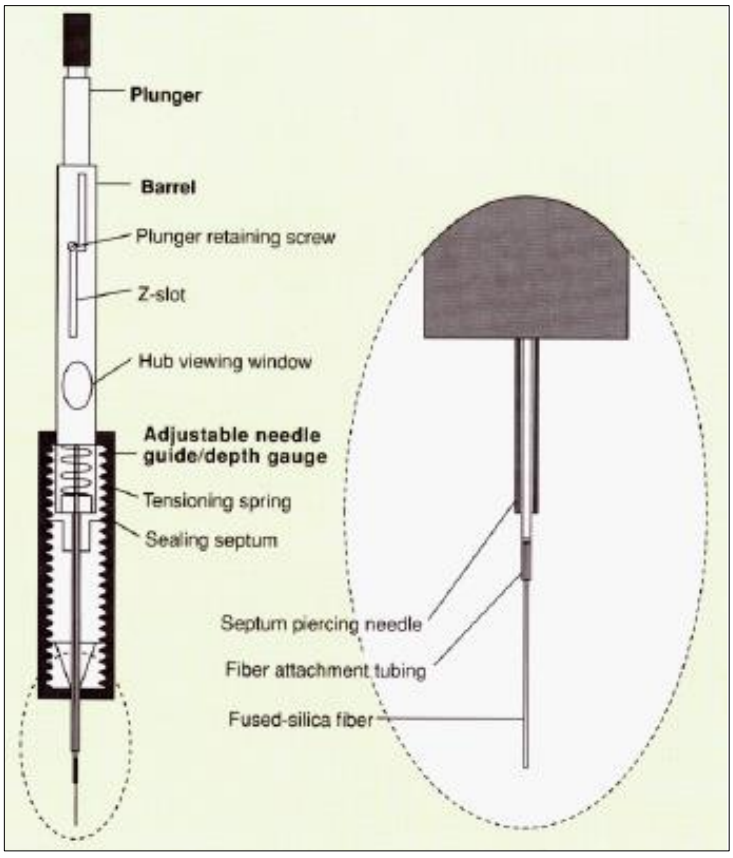


Figure 1.2. Typical SPME device
(Source: Zhang et al. 1994)

Figure 1.2 demonstrates the common view of a commercial solid-phase microextraction fiber and fiber holder. The fiber, attached into a piece of stainless steel tubing, is fixed in a special holder. The holder is equipped with a changeable depth gauge, which makes it possible to control repeatedly how far the needle of the device is allowed to penetrate the sample container or the injector.

SPME can be made by direct extraction or headspace extraction modes (Pawliszyn 2000). Figure 1.3 shows the differences between these modes. In direct mode (Figure 1.3a), the coated fiber is immersed into the sample solution and a direct transformation of nonvolatile analyte occurs. After the equilibrium is completed, the fiber is desorbed by a proper solvent and introduced into an analytical instrument such as high performance liquid chromatograph (HPLC) or inductively coupled plasma mass spectrometer (ICP-MS). For the volatile analytes headspace mode (Figure 1.3b) is principally used. The analytes are in equilibrium in gas phase and extracted from here. As soon as the extraction is finished, the fiber is directly moved into a gas chromatograph injector where analytes are thermally desorbed and quantitatively examined by GC (Pawliszyn 2000).

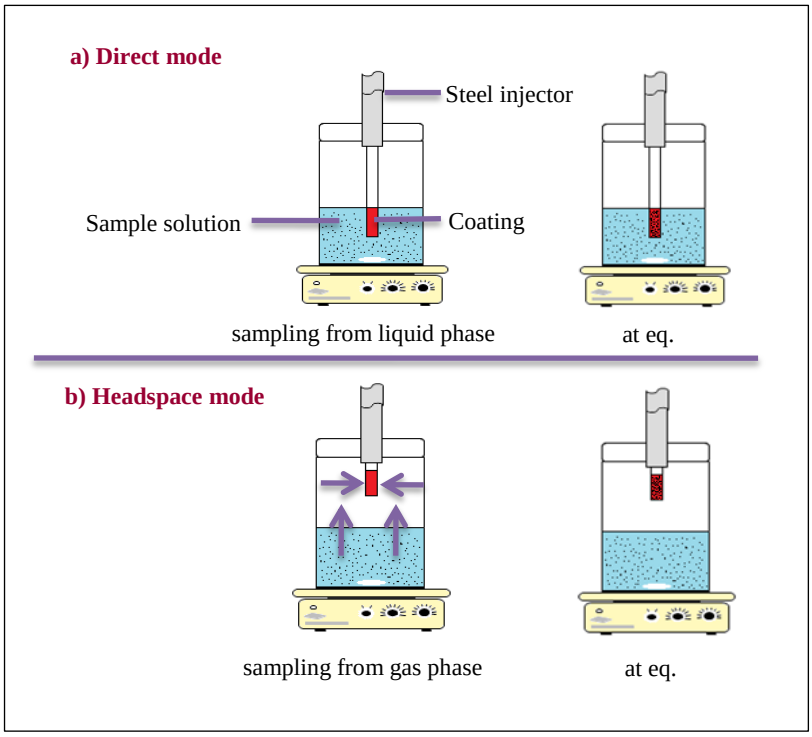


Figure 1.3. Modes of SPME operation: (a) direct extraction, (b) headspace extraction

1.2. Advantages and Disadvantages of SPME

SPME is a solvent free microextraction method. It reduces the amount of organic solvents, and significantly offers shorter analysis times and permits the sample preparation part to be easy. Addition to that it is selective, cost efficient, simple to understand and use, highly sensitive and can be used for the extraction of many kinds of samples of low amounts. It can be applied in any areas such as environmental industry, process monitoring, clinical, forensic, drugs and food analysis (Pawliszyn 2000). Unfortunately, like all the other methods SPME has also some important limitations. One of the most important among them is the reproducibility in analysis and coating quality of the fibers (Dietz et al. 2006).

1.3. Commercially Available Fibers

The coatings of commercial fibers are inspired by commercial gas chromatographic capillary column fillings and there are three main categories. Polydimethylsiloxane (PDMS) is mostly used non polar coating for volatile, semi volatile analytes. Polydimethylsiloxane / Divinylbenzene (PDMS / DVB) is an example for polar volatile analytes. Polyacrylate (PA) and Carbowax / Divinylbenzene (CW / DVB) are used as polar analytes. Finally Polydimethylsiloxane / Carboxen coated fibers are used for trace-level volatiles (Mester et al. 2001). Actually the thickness of the coatings also has an effect on the area of use. Fibers with different polarity provide an increase in recovery of specific analytes with matched-polarity. It reduces the possibility of extracting interferences. Also the homogeneity of coatings is very important (Pawliszyn 1999). PDMS and PA are examples of homogeneous coatings. The constancy of these coatings through organic solvents is improved by cross linking of polymers. Another type of coating is the porous particles embedded in a partially cross-linked polymeric phase. These fibers have lower mechanical stability but they are more selective than homogenous polymer phases. PDMS/DVB, CW/DVB and PDMS/Carboxen are commercially available. Studies showed that increasing the porosity of the fiber coating increases the total capacity and the analyte selectivity of the fiber, moreover analytes are retained more tightly (Pawliszyn 1999).

1.4. Fiber Coatings Developed in Literature

Nowadays the development and characterization of new sorbents for SPME coatings is an important research area regarding to extend the application areas, matrices and analyte types. With the fabrication of new coated fibers, it is aimed to develop solvent-resistive and thermally stable active coatings as well as to increase the mechanical strength of the phase. Table 1.1 summarizes the recent methods applied in the literature for development of new SPME coatings.

Table 1.1. Summary of SPME fiber coatings developed in literature

Coating	Analyte	Detection	Reference
PDMS/DVB/PDMS	pesticides	GC-MS	Naccarato and Pawliszyn 2016
neat crosslinked polymeric ionic liquid-based	polar organic pollutants	HPLC-DAD	Fernandez et al. 2016
magnesium oxinate nanoparticle-modified carbon paste electrode	copper(II)	Cyclic voltammetry	Zhu et al. 2010
sodium dodecylsulfate doped polypyrrole (electrodeposition)	endocrine disruptor pesticides	GC-ECD	Korba et al. 2013
zeolite imidazolate framework-8 coatedmagnetic iron oxide	estrogens	HPLC	Lan et al. 2014
halloysite nanotubes-titanium dioxide (HNTs-TiO_2)	parathion (organophosphorus)	CD-IMS	Saraji et al. 2016
titania sol-gel coated (anodized) alumina fiber (Tetrabutylortotitanate, PEG)	BTEX	GC-FID	Farhadi et al. 2009
NiTi alloy coated with ZrO_2 (electrodeposition)	BTEX, alcohols and trihalomethanes	GC-FID	Budziak et al. 2007
tris(pentafluoroethyl)trifluorophosphate-basd ionic liquids	organophosphate esters	GC-MS	Shi et al. 2016
through-pore sintered titanium disk	polycyclic aromatic hydrocarbons	GC or GC-MS	Zhang et al. 2016
nano-structured lead dioxide (electrochemical deposition)	volatile organoselenium compounds (DMSe and DMDSe)	GC-MS	Ghasemia and Farahani 2012
polymeric ionic liquid-based sorbent	acrylamide	GC-MS	Cagliero et al. 2016

(cont. on next page)

Table 1.1 (cont.).

Coating	Analyte	Detection	Reference
partially sulfonated poly(styrene) (PSP)/3-mercapto propyltrimethoxysilane (MPTS)-N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) immobilized polypropylene hollow fiber	Inorganic and organic forms of arsenic	HPLC-ICP-MS	Chen et al. 2012
chitosan–zinc oxidenanorod composite	Chlorophenol	HPLC	Alizadeh 2016
poly(o-phenylenediamine-co-o-toluidine)/modified carbon nanotubes composite	polycyclic aromatic hydrocarbons	GC-MS	Kazemipour et al. 2016
hydroxylterminated silicone oil-poly(methylhydrosiloxane)	antiestrogens	GC-MS	Liu et al. 2009
carboxylated solid carbon spheres	phenols	GC-MS/MS	Gong et al. 2015
electrospun nanostructured polystyrene	multipesticides	GC-MS	Zali et al. 2015
metal-organic framework MOF-177	polychlorinated biphenyls and polycyclic aromatic hydrocarbons	GC-MS	Wang et al. 2015
zinc–zinc oxide nanosheets	polycyclic aromatic hydrocarbons, phthalates	HPLC-UV	Song et al. 2015
cobalt oxide nanoparticles	benzene, toluene, ethylbenzene and xylene	GC-MS	Gholivand et al. 2014
DNA aptamer	thrombin in plasma	(LC-MS/MS)	Du et al. 2014

1.5. Theoretical Aspects of SPME

SPME is based on multiphase equilibration processes rather than exhaustive extraction. Three phases must be considered for this equilibrium method; fiber coating, gas phase or headspace, and a homogeneous matrix, such as pure water or air. During the sampling period, the analytes transfer between the three phases until equilibrium is reached. The total mass of analyte present during the extraction can be demonstrated with the following mass balance equation (Mester et al. 2001).

$$C_0V_0 = C_cV_c + C_hV_h + C_sV_s \tag{1.1}$$

C_0, C_c, C_h , C_s represent concentrations initially, at equilibrium of analyte in the coating, headspace and sample, respectively, and V_c , V_h , and V_s are the volumes of the coating, headspace and sample. Equation 1.2 shows the relationship between the mass of analyte adsorbed or absorbed on the fiber and headspace-sample distribution coefficient (K_{hs}) and coating-headspace distribution constant (K_{ch}).

$$n = \frac{K_{ch}K_{hs}V_cC_0V_s}{K_{ch}K_{hs}V + K_{hs}V_h + V_s} \tag{1.2}$$

In addition, the distribution coefficient of coating-sample (K_{cs}) can be expressed as in Equation 1.3, which shortens Equation 1.3 to Equation 1.4. For direct extraction mode the relationship would be as in Equation 1.5. There is no terms related to K_{ch} at Eq. (1.4). So, both direct and headspace mode can be applied.

$$K_{cs} = K_{ch}K_{hs} \tag{1.3}$$

$$n = \frac{K_{cs} V_c C_0 V_s}{K_{cs} V_c + K_{hs} V_h + V_s} \tag{1.4}$$

$$n = \frac{K_{cs} V_c C_0 V_s}{K_{cs} V_c + V_s} \tag{1.5}$$

Usually, since the volume of coating is much smaller than the volume of sample (*V*c «*V*s) Eqn. 1.5 turns into Eqn. 1.6. Therefore the sample volume does not play a part. Eqn. 1.6 emphasizes that the extracted amount of analyte on fiber depends on coating volume, initial concentration of the analyte and distribution affinity between the coating and sample matrix.

$$n = K_{cs} V_c C_0 \tag{1.6}$$

1.6. Effect of Various Parameters

1.6.1. Thickness of Coating

In SPME, fiber coating thickness affects the selectivity for analytes, extraction time, sample capacity, desorption time and analyte carryover (Pawliszyn 1999). Increased thickness of the coatings causes longer extraction time, but also enhanced analyte extraction.

1.6.2. Type of Coating

The selection of the type of coating depends on molecular weight, size, boiling point, and vapor pressure of analyte, polarity of both analyte and fiber, functional groups on analyte and fiber and at last concentration range and detector type that will be used. As the general rule “like prefers like”, nonpolar phases will extract nonpolar analytes, whereas polar fibers would be preferred to extract polar analytes (Pawliszyn 1999).

1.6.3. Type of Analyte and Matrix

Headspace extraction mode can be used for volatile analytes because they easily vaporize once they are dissociated from matrix. For semivolatile analytes, the low volatility and relatively large molecular size affect the extraction time by making it longer. This problem can be overcome by increasing the temperature. For nonvolatile analytes direct extraction method can be used if the matrix is not complex. Otherwise headspace extraction is obligatory and another step is applied to turn this nonvolatile analyte into volatile analyte (Zhang et al. 1994).

1.6.4. pH of Solution

Non-ionic polymeric coatings can only extract neutral forms of analytes unless ion-exchange coatings are used. Therefore pH of acidic or basic solutions must be adjusted in order to prevent ionization of analytes. The pH of the aqueous solution should be arranged to at least 2 units lower than the p*K*a of the analyte to ensure that 99% of the acidic compound is in the neutral form. That way the sensitivity will be improved (Pawliszyn 2000).

1.6.5. Extraction Time

Extraction time is related to the mass transfer of analytes between the sample matrix and fiber coatings. There are many parameters affecting the rate of extraction. An increase in temperature speeds up the carrying rate, which results in shorter extraction times. Constantly stirring the analyte solution also may help to decrease the extraction time (Zhang et al. 1994).

1.6.6. Ionic Strength

The interaction between target analytes and the matrix determines the partition coefficients of analytes *(K_{fs})*. By increasing the ionic strength, with addition of a salt (e.g., NaCl or Na_2SO_4) to the aqueous samples, the partitioning of polar organic compounds (but not ions) into the polymer coating can be improved (Zhang et al. 1994).

1.7. Detection with SPME

To date, many detection methods including gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), supercritical fluid chromatography (SFC) and mass spectrometry (ICP-MS, ESI-MS, MALDI-TOF-MS) have been coupled with SPME (Pawliszyn 1999). Specific examples from studies will be mentioned in further sections.

1.8. Selenium

Selenium is a both trace and essential element that has a lot of significant biological functions in human body. Every year, there is a big increase in selenium speciation studies by researchers because of the uncertain nature of selenium. It can be a toxic or an essential element depending on its concentration. New dietary reference

intakes for selenium were calculated as between 15 and 20 µg day-1 for babies and 70 µg day-1 for lactating mothers, whereas the upper limit is 400 µg day-1 (Food & nutrition board & institute of medicine, 2000). The absence of Se can cause many problems such as heart disease, hypothyrodism and a damaged immune system. On the other hand, too much intake of Se results also in gastrointestinal disturbances, hair and nail changes and neurologic manifestations (Colangelo et al., 2014; Zhang et al., 2011).

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.

Organic forms of Se play also an important role in living organisms and their determination is crucial. Selenocystine (SeCys) is found at the active center of glutathione peroxidase and it prevents the oxidative role of peroxides and hydroperoxides and protects immune competent cells and reducing the time for ageing process (Sua et al. 2008). Another organic form of Se is selenomethionine (SeMet) is dominant and has many effects on diseases such as anti-cancer agent against tumor cells, chemo-preventive properties, antioxidant agent in biological systems (Wang et al. 2008, Krause et al. 2009).

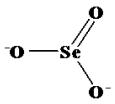
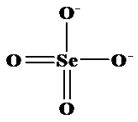
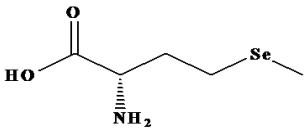
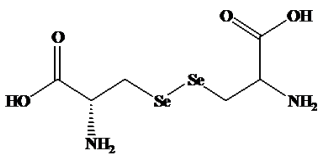
1.8.1. Selenium Species in the Environment and in Biological Systems

The chemical form of an element is very important considering deficiency essentiality and toxicity. Therefore it is necessary to have some knowledge about the existence its forms and their amounts. Several 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, selenocystine, selenocysteine, and dimethylselenide. Methyl group containing species are volatile. 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).

Selenium accumulates in human body usually as selenoamino acids (SeMet, SeCys) and very little amount enters the body as methylated/non-methylated Se through food. Particularly SeMet shows a higher bioavailability than the inorganic species. Selenium exists in cereals, wheat, and most vegetable foods, mainly as SeMet, with a very high bioavailability percentage (85–100%). From dairy products and meats only 10–15% is bioaccumulated. Also Se amount in fish is high, showing a relatively high bioavailability (20–50%). There is chain conversion of Se species into each other as in this order: Se(VI) is reduced to Se(IV); Se(IV) and Se of the selenoamino acids can be transformed to selenides. Selenides are turned into mono-, di-, and trimethylated species. Whereas the trimethyl Se is expelled through urine and the dimethyl form is respired, the monomethylated form is usually released by metabolism of SeMet (Alaejos et al. 2000). Table 1.2 contains the structures of some of the most relevant Se-compounds.

Table 1.2. Structures of Se compounds studied

Se-species	Structure
Selenite	
Selenate	
Selenomethionine	
Selenocystine	

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 1.4 demonstrates that acidic and reducing conditions reduce inorganic selenite to elemental selenium, whereas alkaline and oxidizing conditions favor the formation of selenate. 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).

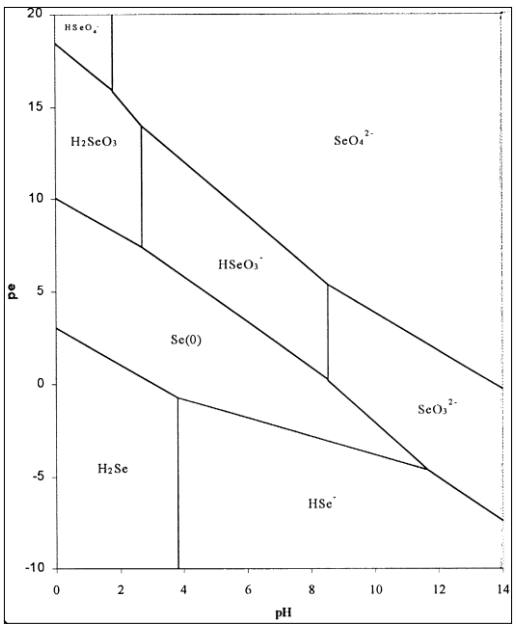


Figure 1.4. Selenium pE-pH diagram of at 25 °C, 1 bar pressure and *I = 0* for a dissolved selenium activity of 10^{-10} mol l^{-1}

Organoselenium compounds play an important role in a number of chemical and biological processes. Similarly, organoselenium species were affected by pH and redox conditions. SeMet has two p*K*a values at pH ~ 2 and the other at pH ~ 9 corresponding to -COOH and $-NH_3^+$ groups and an isoelectric point at 5.75. SeCys has four p*K*a values, two at pH ~ 2 and two at pH ~ 8 (Table 1.3). These compounds are in the zwitterionic form, in the range of pH 2-10. In this form, both NH_3^+ and COO^- groups are

exceptional proton-donor and proton-acceptor groups, respectively, and there is a fast proton transfer from the protonated amino group. Therefore in this pH range, two compounds provide enough concentration of a nonprotonated amino group in the neutral solution.

Table 1.3. pKa values of selenium compounds

Compound	pK_a	Oxidation state of selenium
Selenomethionine	2.2, 9.3	-2
Selenocystine	1.7, 2.3, 7.9, 9.8	-2
H_2SeO_4	1.92	+6
H_2SeO_3	2.46, 7.31	+4

1.8.2. Determination and Speciation of Selenium

So far many studies have been carried out to apply SPME method using commercially available fibers to different application areas, such as different types of water samples, pharmaceutical, clinical, forensic, food, and flavor applications. In addition, researchers have been trying to produce new, thermally stable, with high mechanical strength fiber coatings for element specific analysis. Therefore quite a large number of articles have been published related to the use of SPME method for selenium species.

Amoako et al. (2009) reported the speciation of selenium in yeast- and non-yeast based selenium supplements, with particular focus on the presence and production of S-(methylseleno)cysteine, selenomethionine selenoxide and dimethyldiselenide upon storage and thermal treatment. Commercial PDMS coated fiber was used to extract volatile selenium species for determination by GC–AED and GC–MS. Shahdousti and Alizadeh (2011) applied headspace-solid phase microextraction (HS-SPME) with a polypyrrole (PPy)-coated fiber, as a sample preparation method for determination of Se(IV). It is followed by derivatization with 1,2- diaminobenzene to convert Se(IV) into the piaselenol form and analysis by ion mobility spectrometry (IMS). The method was

applied for determination of selenium in human serum and environmental surface water samples with satisfactory recovery.

Gionfriddo et al. (2012) developed a new analytical method for selenium speciation in selenized and not-enriched selenium potatoes by SPME-GC–triple quadrupole mass spectrometry (SPME-GC–QqQ MS). Five commercial SPME fibers (85 μm CAR/PDMS, 85 μm PA, 50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB, and 100 μm PDMS) and three alkylchloroformates were evaluated and for the exploitation of the GC method, the selected analytes were converted into their *N(O,S)*-alkoxycarbonyl alkyl esters derivatives by direct treatment with alkyl chloroformate in aqueous extracts. The best results were obtained using the DVB/CAR/PDMS fiber and propylchloroformate.

In another study, Campillo et al. (2007) proposed two methods for the simultaneous determination of selenite, dimethylselenide (DMSe) and dimethyldiselenide (DMDSe) using SPME fibers (non-bonded 100 μm PDMS, bonded 65 μm PDMS/DVB, bonded stableFlex 50/30 μm DVB/CAR/PDMS, bonded CW/DVB, bonded 75 μm CAR/PDMS of and bonded 85 μm PA) for sample preconcentration and capillary gas chromatography coupled to atomic emission detection (GC-AED) for separation and detection. The main difference between the methods is the derivatizing agent used to complex the inorganic species: sodium tetraethylborate and 4,5-dichloro-1,2-phenylenediamine. In 2009, Bueno and Pannier reported the quantitative determination of DMSe and DMDSe in normal urine samples without selenium supplementation. This time, headspace SPME (75 μm CAR–PDMS fiber) was coupled with GC-ICPMS.

Tyburska et al. (2011) combined the hydride generation reaction with headspace SPME technique for separation and preconcentration of trace amounts of arsenic and selenium followed by the optical emission spectrometric determination without chromatographic separation. They examined three types of commercial SPME coatings: PDMS/Carboxen Stable Flex 85 μm, PDMS/CAR 75 μm and PDMS 65 μm coating. Dietz et al. (2004) presented the use of a novel and quite simple instrumental approach, multicapillary microwave induced plasma atomic emission spectrometry (MC–MIP-AES) for organoselenium (DMSe, DEtSe and DMDSe) detection in selenium accumulating biological matter. SPME fiber (75 μm CAR/PDMS) was used for preconcentration and sample-matrix separation. Gomes da Silva et al. (2012) introduced

a selenium extraction method using SPME (PDMS/DVB fiber) and graphite furnace (GF). Se(IV) was determined with GC-MS after derivatization.

Apart from the usage of commercial fibers, there are also many studies included the synthesis of fibers with different coating materials. One of them is the study of Vonderheide and her coworkers (2002). Their objective was to illustrate the potential of laboratory prepared SPME fibers based on sol–gel technology for the analysis of selenomethionine (SeMet), selenoethionine (SeEt) and selenocystine (SeCys) using GC separation with ICP-MS detection. Ghasemia and Farahani (2012) developed a novel and efficient speciation method based on the nano-structured lead dioxide as stationary phase of headspace SPME combined with GC–MS, for the determination of volatile organoselenium compounds DMSe and DMDSe in different biological and environmental samples. Mao et al. (2012) prepared an organic-inorganic hybrid SBSE (stirbar sorptive extraction) coating of partially sulfonated polystyrene-titania (PSP-TiO_2) by sol-gel and blending methods to use for the analysis of seleno-amino acids and seleno-oligopeptides in biological samples by HPLC-ICPMS. In another study, Moreno and his coworkers (2013) described a new analytical method for the simultaneous speciation of selenium species in water and human blood plasma using HF-LPME and HPLC-ICPMS. In a new published article, Asiabi et al. (2016) proposed a new nanostructured composite coating consisting of PPy doped with poly ethyleneglycol dimethacrylate (PEGDMA). Coating was electrochemically synthesized on the inner surface of a stainless steel capillary tube and used for on-line electrochemically (EC) controlled in-tube SPME coupled with HG-AAS for determination of total inorganic selenium in water samples.

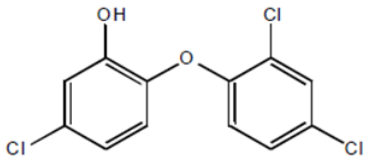
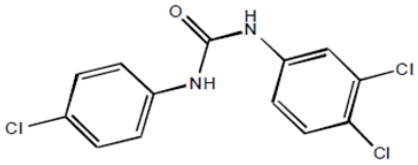
Nyaba et al. (2016) developed a coating alumina nanoparticles functionalized with Aliquat-336A to use with suspended dispersive solid phase microextraction (SDSPME) for the speciation of inorganic selenium prior to their determination with ICPOES. Zhang et al. (2016) used grapheneoxide (GO)–TiO_2 composite material as a SPE sorbent followed by GFAAS determination for speciation of inorganic selenium in environmental waters.

1.9. Triclosan and Triclocarban

Triclosan (5-chloro-2-[2,4-dichloro-phenoxy]-phenol) (TCS) and triclocarban (N-(4-chlorophenyl)-N-(3,4-dichlorophenyl) (TCC) are organic compounds that are present in household and personal care products because of their antibacterial properties (Cha and Cupples, 2009). They are mostly used in shampoos, soaps, creams, mouthwash and tootpaste (Daughton and Ternes, 1999; Chu and Metcalfe, 2007).

Table 1.4 demonstrates the structures of TCS and TCC. TCS is poorly soluble in water (S:0.01 g L^{-1}) because of its high hydrophobicity, at the same time aromatic –OH group provides better solubility above pH 10. pKa value of TCS is 7.9. On the other hand, TCC stays unionized in a wide range of pH values (pKa: 12.7). The solubility of TCC is approxiamately 50 ng mL^{-1} (Delgado et al., 2012).

Table 1.4. Structures of TCS and TCC

Compound	Structure
TCS	
TCC	

1.9.1. Occurrence and Toxicity of TCS and TCC

TCS and TCC are consumed in very high quantities (0.6~10 million kg year $^{-1}$) in household and personal care products (Chu and Metcalfe, 2007). Unfortunately, TCS forms toxic degradation products such as chlorophenols, dioxins, tetra- and pentachlorinated phenoxyphenols, and methyl triclosan and TCC has the potential to act as an endocrine-disrupting compound (Canosa et al., 2007). Therefore their detection in waste and surface waters has attracted great importance globally.

TCS is considered as toxic to aquatic organisms such as algae (Orvos et al., 2002) and was revealed to modulate thyroid function in amphibians at concentrations as low as 0.15 μgL^{-1} (Chu and Metcalfe, 2007). TCC has been added into personal care products for many years and it can also be detected in surface water at concentrations up to 6.75 $\mu g\ L^{-1}$ (Brausch and Rand, 2011). While using these products both antimicrobials are absorbed by the skin and then they can be detected in human plasma, urine, and milk (Venkatesan et al., 2012). Even very low concentrations such as 0.07 and 0.61 mg L^{-1} of TCS have revealed to give harm to metabolic systems, immune function, and hormone production in rats and humans, respectively. The adequate daily intake for TCS is suggested to be as low as 1.9×10^{-4} mg kg^{-1} day^{-1} for humans. Both TCS and TCC could bioaccumulate in aquatic species because of their lipophilic properties (Venkatesan et al., 2012).

1.9.2. Sorption and Determination of TCS and TCC

All these problems caused by TCS and TCC are tried to be overcome with their detection and removal from waters using various methods. Here information will be given regarding these researches.

In 2011, Zhao and his coworkers developed a new method for rapid enrichment and determination of TCS and TCC in water samples. They used ionic liquid/ionic liquid dispersive liquid liquid microextraction (IL/IL-DLLME) and HPLC-ESI-MS-MS. As extraction solvent and disperser solvent they mixed ionic liquid and a hydrophilic ionic liquid. They validated the method by making analysis in tap, river, snow and lake water samples. An injection-port *tert*-butyldimethylsilylated (TBDMS) derivatization

and GC-MS) method was established to determine TCS, and its metabolite: methyltriclosan (MTCS), in wastewater and surface water samples. They used reverse phase C18 SPE cartridges to extract the analytes from water samples (Cheng et al., 2011). In another study, Guo and his coworkers (2009) used dispersive liquid–liquid microextraction (DLLME) combined with ultra-high-pressure liquid chromatography (UHPLC)–tunable ultraviolet detection (TUV) for the preconcentration and determination of TCS, TCC and MTCS in aqueous samples. Baranowska and Wojciechowska (2012) used C18 SPE discs for the extraction of TCS and TCC from water samples and determination of their concentration was done with HPLC-DAD. The detection limits were between 1.9 ng mL^{-1} for TCS and 1.0 ng mL^{-1} for TCC.

Shen et al. (2012) applied a new method regarding to the detection of TCS, TCC and their four related transformation products in water samples using SPME-HPLC-DAD. Commercial PDMS fibers were used throughout the study. Silva and Nogueira attempted to develop a new analytical approach by combining stir bar sorptive extraction and liquid desorption followed by HPLC (SBSE-LD-LC-DAD) for the determination of TCS in commercially available health care products, biological and environmental matrices.

Zhao et al. (2010) used SPE cartridges (Waters Oasis HLB) for the extraction of water and then LC-MS/MS for the detection of TCS and TCC in surface water, sediment and effluents. Behera et al. (2010) investigated the sorption of TCS onto activated carbon, kaolinite and montmorillonite and detected by HPLC. Regiart et al. (2016) performed a sensitive electrochemical sensor with an ordered mesoporous carbon modified screen-printed carbon electrode (SPCE) to detect TCS in river water samples.

1.10. Aim of the Study

The main objective of this thesis study is to develop home-made SPME fiber coatings for analytical applications. These fibers were prepared by embedding with different functional groups using capillary tubes as templates and used for two different groups of analytes. In the first part, it is focused on the production of SPME fiber coatings and their characterization. For this purpose, zerovalent iron (ZVI), ceria (CeO_2)

and zirconia (ZrO_2) were embedded in agarose matrix and attached to the silica fiber. The second part of this study includes the application of these fibers for speciation of selenium species. Therefore selenite, selenate, selenomethionine and selenoscytine were investigated using direct mode SPME coupled with HPLC-ICPMS. In the third part, same fibers were applied for the separation and determination of triclosan and triclocarban using direct mode SPME coupled with HPLC-DAD. For both applications, some important conditions such as pH of the solution, sorption and desorption time, agitation speed, ionic strength of the solution and desorption matrix were optimized. Finally, the method was validated with real samples and standard reference materials.

CHAPTER 2

NEW SPME SORBENTS:

SELENIUM SPECIATION IN WATERS

2.1. Experimental

2.1.1. Chemicals and Reagents

All the chemicals were of analytical reagent grade. Ultrapure water (18.2 MΩ, Millipore) 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 Na_2SeO_3, $5H_2O$ in 1.0% (v/v) HNO_3 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 Na_2SeO_4 in ultrapure water and diluted to 250.0 mL with ultrapure water. Standard Seleno-DL-Methionine stock solution (547.5 mgL^{-1}) was prepared by dissolving 0.068 g of seleno-DL-methionine in 3.0% (v/v) HCl and diluted to 50.0 mL with ultrapure water kept in refrigerator at -20 ^{o}C. Standard Seleno-L-Cystine stock solution (1490 mgL^{-1}) was prepared by dissolving 0.3156 g of seleno-L-cystine in 3.0% (v/v) HCl and diluted to 100.0 mL with ultrapure water kept in refrigerator at 4 ^{o}C. Calibration standards with lower concentrations were prepared daily by appropriate dilution of the stock standard. pH adjustments were done by using 1.0 M, 0.1 M, 0.01 M of HNO_3 and NH_3 solutions. Fiber optic cable was kindly supplied by HES Kablo (Kayseri,Turkey). Acetone was used for removal of polyamide coating of the fibers. 10.0 mM ammonium citrate solutions (pH 5.00) were prepared daily by dissolving proper amount of ACS grade ammonium citric acid diammonium salt in ultra-pure water. Zero valent iron nanoparticles (nZVI) were synthesized from Fe(III) solution which was prepared by dissolving 7.26 g $FeCl_3.6H_2O$ in 30.0 mL solution of absolute ethanol and ultrapure water (4:1 v/v). Sodium borohydride solution ($NaBH_4$, 3.0% (w/v)) was prepared for

reduction of Fe(II) from fine granular product and agarose was used as a immobilization matrix of the nZVI, ZrO_2 and CeO_2.

All solutions were filtered through 0.25 μm cellulose acetate filter and degassed for 15 min in ultrasonic bath before being introduced to HPLC system. Buffer solutions as eluent were prepared daily using dibasic ammonium citrate solution ($C_6H_{14}N_2O_7$). SRM 1643e (NIST) and TMDA 70 were used for the validation of the proposed method.

2.1.2. Instrumentation and Apparatus

Micro images of bare and coated silica fibers were taken using a Philips XL-30S FEG scanning electron microscope (Eindhoven, The Netherlands). Separation of extracted selenium species was achieved in Agilent 1200 Series HPLC system with a 250 x 4.1 mm PRP-X100 (Hamilton) using isocratic elution 10.0 mM ammonium citrate solutions (pH 5.00) as mobile phase at flow rate of 1.4 mL min^{-1}. Detection of each species was realized with inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500ce Series, Tokyo, Japan). Online separation and detection of four selenium species were accomplished by connecting HPLC column outlet directly to ICP-MS concentric nebulizer. To prevent the broadening of the peaks the shortest connection between two instruments was achieved with 67 cm tubing (Figure 2.1). Operating parameters for HPLC and ICP-MS were given in Table 2.1. The pH adjustment of solutions was achieved with Ino Lab Level 1 pH meter (Weilheim, Germany). Extraction of selenium species was performed in multi-position magnetic stirrer RO 10 power IKAMAG.

Figure 2.1. HPLC-ICPMS system

Table 2.1. Operation conditions for HPLC-ICPMS

HPLC		Agilent 1200	
Analytical column		PRP X-100 (250 mm x 4.1 mm, 10 µm)	
Mobile phase		10.0 mM $C_6H_{14}N_2O_7$ solution pH= 5.00	
Flow rate		1.4 mL min^{-1}	
Column temperature		25.0 °C	
Sample volume		100 µL	
ICP-MS		Agilent 7500ce	
Rf power output	1550 W	Interface	Ni sampler cone (1 mm)
Frequency	27 MH		Ni skimmer cone (0.4 mm)
Plasma gas flow rate	15 L/min	Spray chamber temp.	2 °C
Carrier gas flow rate	0.85 L/min	Nebulizer	Concentric
Collision gas flow rate	4.5 mL/min	Dwell time	100 msec
Octopole reaction system		Detected isotopes	^{78}Se, ^{82}Se
Collision gas	He	Integration mode	Peak area

2.1.3. Preparation of SPME Fibers

2.1.3.1. Capillary Template Method for Immobilization of nZVI, ZrO_2, and CeO_2

At first, fibers were cut into 7 cm pieces and for the removal of the polyamide layer they were put into acetone for a while. Bare silica fibers were washed with ultra-pure water. Functionalization of silica fiber was performed sequentially in a two-step procedure. Firstly, nZVI particles were synthesized as described in previous reports (Efecan et al. 2009) by dropwise addition of 100.0 mL of 3.0% (w/v) $NaBH_4$ solution from burette to flask containing 30.0 mL of 1.0 M Fe(III) solution under continuous stirring. After all reducing agent was added the mixture was stirred for additional 20 minutes nZVI particles obtained were filtered and washed with small amount of ultrapure water and many amount of ethanol and further dried overnight in oven at 75 ºC. Secondly, immobilization of the nZVI particles in agarose matrix and coating of the silica fibers with resulting mixture were performed coincidently. Immobilization matrix was prepared by mixing 0.400 g agarose and 20.0 mL of ultra-pure water in a 50 mL beaker. Resulting solution was stirred and boiled on a hot plate until a clear solution was obtained. Solution was cooled to 50 ºC and 0.250 g nZVI was added and stirred for 10 min. Mixture was stored at the specified temperature with continuous stirring during coating of the fibers.

The process of fiber coating was summarized in Figure 2.2. The first step of silica fiber coating process was drawing of the nZVI-agarose mixture into *ca* 3 cm capillary tubing (1 mm i.d.) and immersing a fiber into the capillary tubing. After a few minutes the temperature of the solution in the capillary tubing dropped down to room temperature and the nZVI-agarose matrix was solidified. Removal of the solidified nZVI-agarose coated silica fiber was done easily by pushing the final product from the other open end of the capillary by small a piece of wire. Fiber coating were cut to 2.0 cm from lower end of the silica fiber attachment and allowed to air dry at room temperature overnight. Blank fibers were also prepared in the same manner without addition of nZVI to immobilization matrix. Prepared SPME fibers were characterized by SEM, EDX and XRD.

Commercial CeO_2, ZrO_2 particles were also immobilized by the method described above using the same amounts.

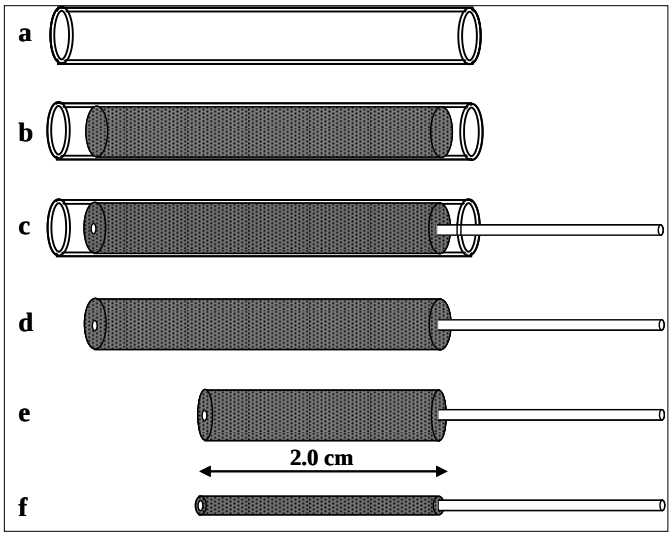


Figure 2.2. Coating procedure of silica fiber; (a) empty capillary tubing ca 3 cm, (b) capillary tubing filled with nZVI-agarose mixture (50 ºC), (c) inserting silica fiber into capillary tubing and cooling for solidification of nZVI-agarose mixture, (d) removal of nZVI-agarose modified silica fiber, (e) cutting the end of the coating of the fiber to obtain 2.0 cm coating on the fiber and (f) air drying of the SPME fiber.

2.1.4. Selenium Speciation

2.1.4.1. Optimization of HPLC-ICPMS

The chromatographic separation of Se(IV), Se(VI), SeCys and SeMet was carried out using an anion exchange column (PRP-X100, 250 mm x 4.1 mm, 10 μm). The mobile phase used was ammonium citrate dibasic. Isocratic program was used throughout the study: 10.0 mM ammonium citrate (pH 5.00). Flow rate were varied from 1.0 to1.5 mL min^{-1}. The separated species of selenium were detected using time resolved analysis mode of ICPMS.

2.1.4.2. Extraction of Selenium Species

SPME was carried out with agarose (blank fibers) and nZVI-agarose, CeO_2-agarose and ZrO_2-agarose fibers for the speciation of Se(IV), Se(VI), SeCys and SeMet. After pH adjustment of the solutions, 15.0 mL aqueous selenium mixture solution containing 10.0 μg/L Se(IV), Se(VI), SeCys and SeMet were added into a 20 mL vial which was stirred with a magnetic bar. The extraction time was 60 min and stirring speed was 700 rpm. All extractions were realized at room temperature (25 °C). Desorption of the analytes was carried out in a home-made container which was prepared from 200-μL pipette. Its tip was blocked at the base by the application of heat (burning). For desorption, the initial conditions were; desorption time: 30 min, desorption volume: 150.0 μL, desorption temperature: 25 °C, eluent: 50.0 mM ammonium citrate solution. HPLC mobile phases and desorption solutions were filtered through 0.20 μm cellulose acetate filter before use. Afterwards, the extraction parameters such as extraction pH, extraction time, agitation (stirring) speed, desorption matrix, desorption time, solution temperature and salt concentration (ionic strength) were optimized.

2.1.5. Optimization of Extraction Parameters

2.1.5.1. Effect of pH on Extraction of Selenium Species

pH of the solution is one of the most important parameters on the extraction of selenium species by fibers. Therefore, preliminary experiments for optimization of extraction parameters started with the investigation of extraction pH. Experiments were carried out at 25 °C and pH of solutions was adjusted to 4.0, 7.0 and 10.0 with dilute HNO_3 or NH_3. The conditions for the extractions were; selenium concentration: 10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 μL, eluent: 10.0 mM citrate solution.

2.1.5.2. Effect of Agitation Time/Speed on Extraction of Selenium Species

Effect of agitation time on the extraction of selenium species by nZVI-agarose fiber was examined for time intervals of 1, 5, 15, 30, 60 and 90 min. The conditions for the extractions were; solution pH: 4.0, selenium concentration: 10.0 μgL^{-1} from in species (Se(IV), Se(VI), SeCys, SeMet), stirring speed: 700 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution. Discretely, the effect of the agitation speed on extraction of the selenium species was studied at 300, 500, 700 and 900 rpm stirring speeds. Extraction and desorption conditions were the same as in the previous trials.

2.1.5.3. Effect of Desorption Matrix/Time on Extraction of Selenium Species

Effect of desorption matrix on the elution of selenium species by nZVI-agarose fiber was investigated for 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 75.0 and 100.0 mM ammonium citrate. The conditions for the extractions were; solution pH: 4.0, selenium concentration: 10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), stirring speed: 700 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution. Separately, the effect of desorption time was studied for 5, 15, 30, 45, 60 min. Extraction and desorption conditions were the same as in the previous trials.

2.1.5.4. Effect of Solution Temperature on Extraction of Selenium Species

To observe the effect of solution temperature on the extraction of selenium species by nZVI-agarose fiber, three different temperatures (25 °C, 40 °C, and 55 °C) were tested. The conditions for the extractions were; solution pH: 4.0, selenium concentration: 10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), stirring

speed: 700 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.

2.1.5.5. Effect of Ionic Strength on Extraction of Selenium Species

Effect of ionic strength on the extraction of selenium species was studied by addition of numerous amount of NaCl into selenium containing solution. The studied concentrations were 0.10 M, 0.010 M and 0.0010 M NaCl. Extraction conditions were; solution pH: 4.0, selenium concentration: 10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), extraction time: 60 min, stirring speed: 700 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.

2.1.5.6. Interference Studies

Interference studies were performed for Sb(III), Sb(V), As(III), As(V), V(IV), V(V), SO_4^{2-} and PO_4^{3-} ions. Each ion was examined by addition into the solution containing 10.0 μgL^{-1} Se species. While the added concentration for Sb(III), Sb(V), As(III), As(V), V(IV), V(V) was 100.0 μgL^{-1}, for SO_4^{2-} and PO_4^{3-} it was 1000.0 μgL^{-1}. Extraction conditions were; solution pH: 4.0, selenium concentration: 10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), extraction time: 60 min, stirring speed: 700 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.

2.1.5.7. Analytical Performance of the Method

Calibration plots were constructed to relate the variation of selenium concentration as a function of the peak area. For this purpose various amounts of selenium species spiked in ultrapure water (0.10 μgL^{-1} to 50.0 μgL^{-1}) and extraction/desorption to HPLC-ICPMS were performed under optimized experimental

conditions. Furthermore, the analytical performance of the developed method was tested via determining relative standard deviations of the peak areas for intra-day and inter-day extractions of the analytes (n=4 and 10, respectively). Extraction conditions were; solution pH: 4.0, selenium concentration: 10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), extraction time: 60 min, stirring speed: 700 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.

2.1.5.8. Method Validation

The applicability of the method vas verified by analyzing two standard reference materials (SRM from NIST, Trace Elements in Water, 1643e and certified reference material TMDA-70 fortified lake water). Extractions were performed in 10-fold diluted samples. Validity of the proposed method was also checked by means of spiked bottled (10.0 μgL^{-1} in each species) and tap water (10.0 μgL^{-1} in each species). Both of the samples were 2-fold diluted. Extraction and desorption conditions were the same as in the previous trials.

2.2. Results and Discussions

2.2.1. Characterization of Prepared SPME Fibers

Scanning electron microscopic images of the bare silica fiber and agarose coated fibers can be seen in Figure 2.3. The diameters of bare silica fiber and agarose coated silica fiber were determined 125 µm and 230 µm, respectively. The surface coating of the fiber can be understood from increased diameter of the silica fiber in addition to surface roughness. Moreover, EDX results of the fibers (Figure 2.4) were used for further identification of the fiber coatings. Carbon peak observed in EDX spectrum of agarose coated fiber and the disappearance of Si peak on the surface were signs of successful coating of agarose (polysaccharide) on fiber.

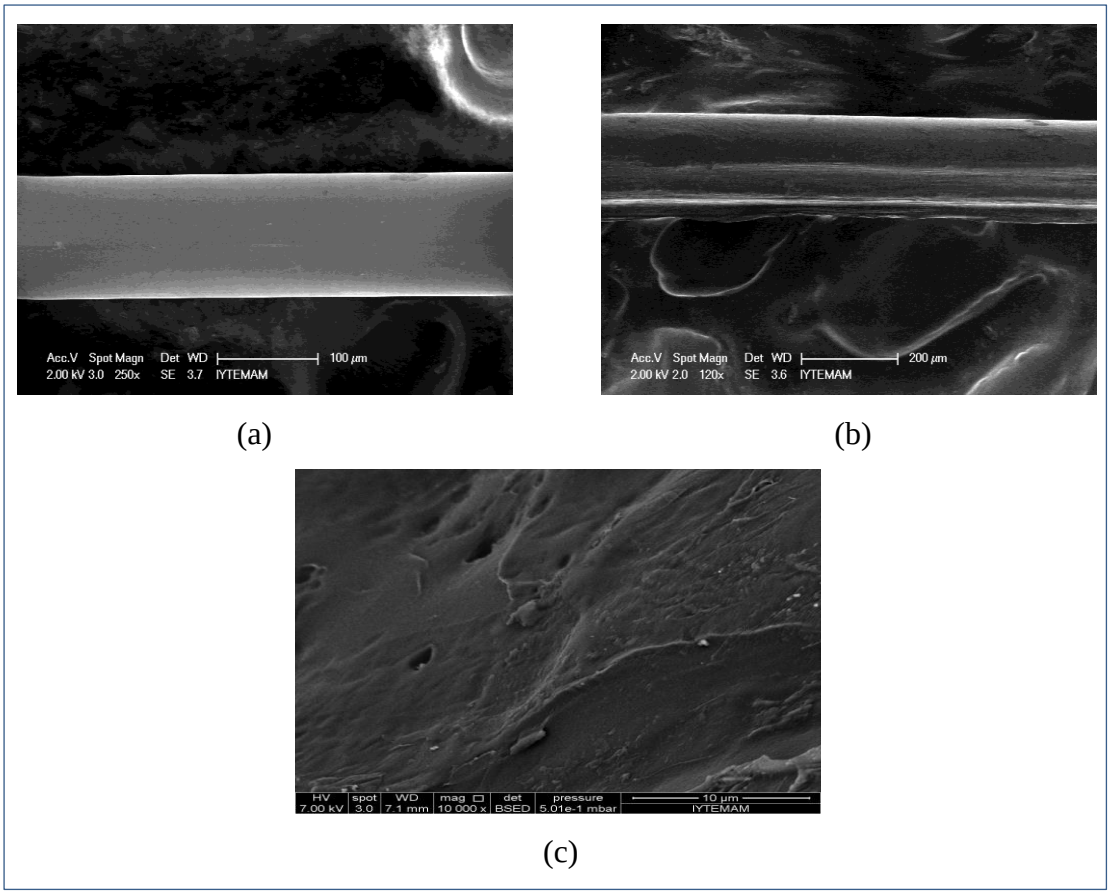


Figure 2.3. SEM images of (a) bare silica fiber (250x) (b) and (c) agarose coated silica fiber at various magnifications

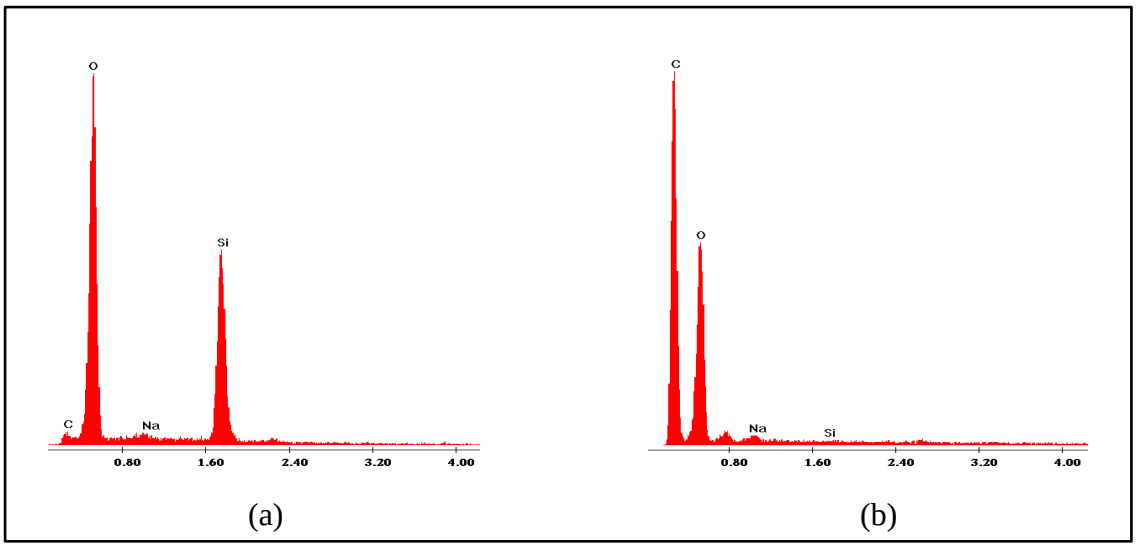


Figure 2.4. EDX spectra of (a) bare silica fiber and (b) agarose coated silica fiber

SEM images of particles (commercial CeO_2 and ZrO_2, and nZVI synthesized by our group) used in capillary template coating method are shown in Figure 2.5. The morphological differences between the particles resulted in different surface areas.

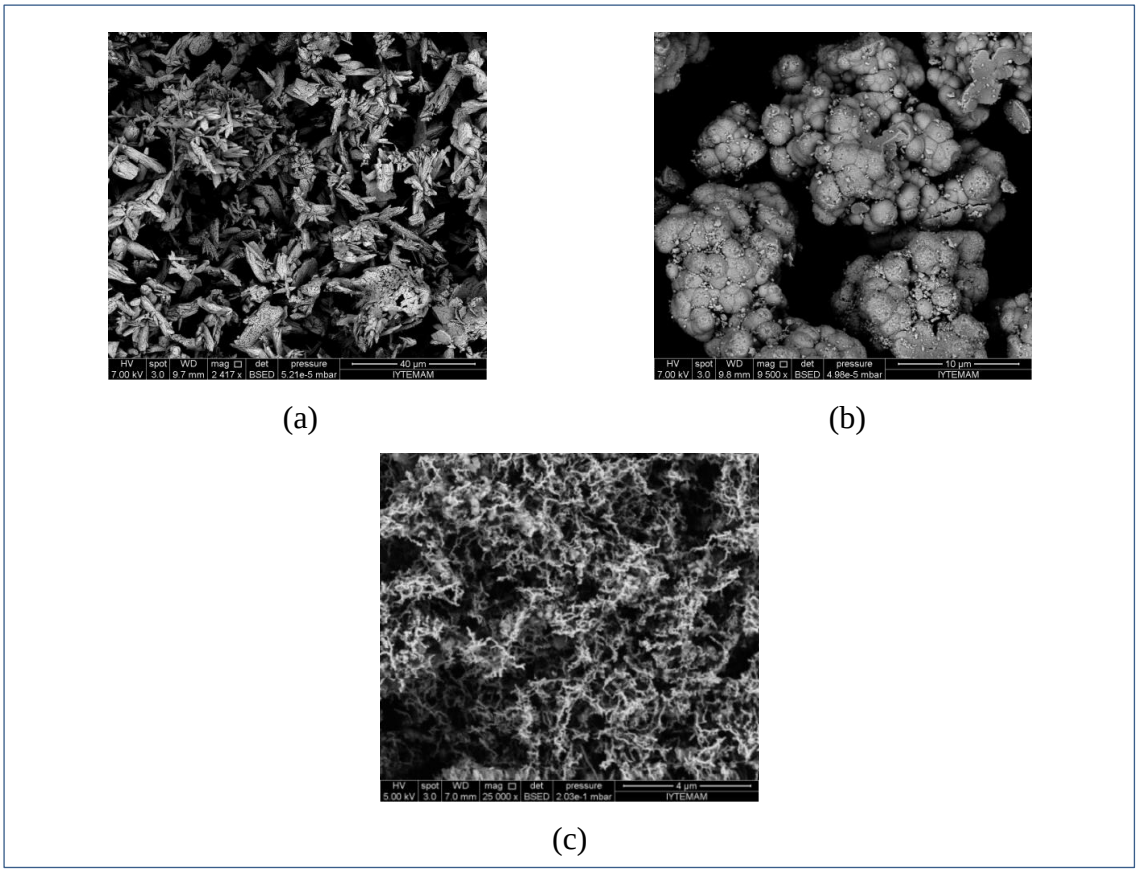
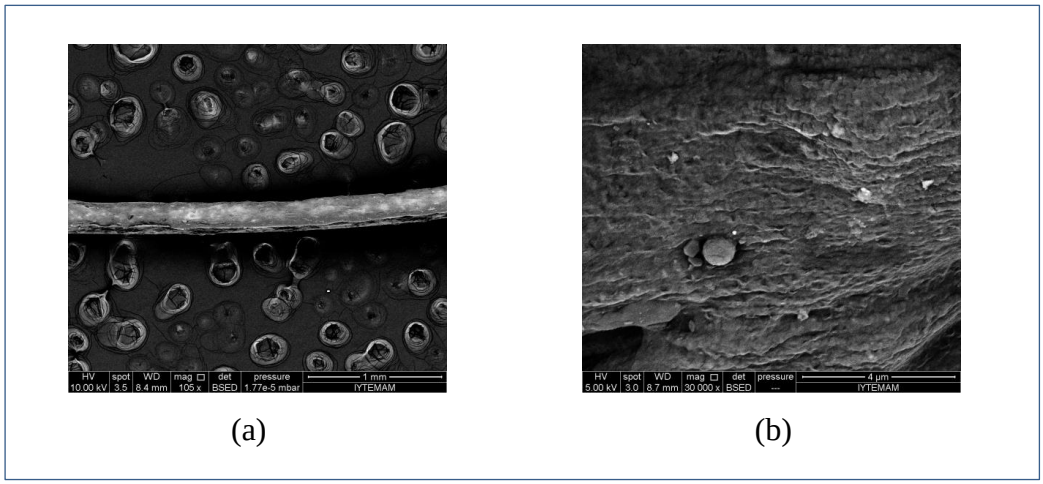


Figure 2.5. SEM images of used particles (a) CeO_2 (2500x), (b) ZrO_2 (9500x) and (c) nZVI (25000x)

SEM images of nZVI-agarose fiber are given in Figure 2.6 at various magnifications. The characteristic chain-like structure of the nZVI particles with particle size ranging within 10 – 60 nm (Efecan et al. 2009) was not observed after immobilization of the nZVI into agarose matrix. The reason of this observation may be the oxidation of nZVI during immobilization process regarding XRD results which will be given below. The nanoparticles were embedded completely inside the matrix. The diameter of the bare fiber and nZVI-agarose fiber was measured as 125 μm and 220 μm, respectively; corresponding to a coating thickness of ca 50 μm. nZVI particles were also validated by EDX analysis by characterization of elemental composition of the coating

from K lines of individual elements Figure 2.9. Results revealed weight percentage of C (29.7 %), O (21.4%) and Na (1.0%) from agarose and Fe (47.3%) from embedded nZVI particles.



(a) (b)

Figure 2.6. SEM images of nZVI-agarose fibers at various magnifications (a) 105x, (b) 30000x

SEM images for ZrO_2-agarose and CeO_2-agarose fibers were demonstrated at Figure 2.7 and 2.8, respectively. ZrO_2-embedded fiber has smoother surface with respect to CeO_2-embedded fiber. The main reason of the differences in roughness results from the morphological difference of the particles. The diameters of the coated fiber were determined as 254 µm and 235 µm for ZrO_2-agarose and CeO_2-agarose fibers, respectively. In addition, EDX analysis during SEM survey demonstrated the high content of iron and zirconium on the fiber surface (Figure 2.9). In contrast, EDX spectrum of ceria functionalized fibers was not indicative of Ce peak. This observation might be explained by the complete coating of the particles with agarose matrix in addition to its small surface area. Therefore XRD pattern of CeO_2-embedded fiber is given in Figure 2.10. Several peaks matching with CeO_2 peaks can be observed.

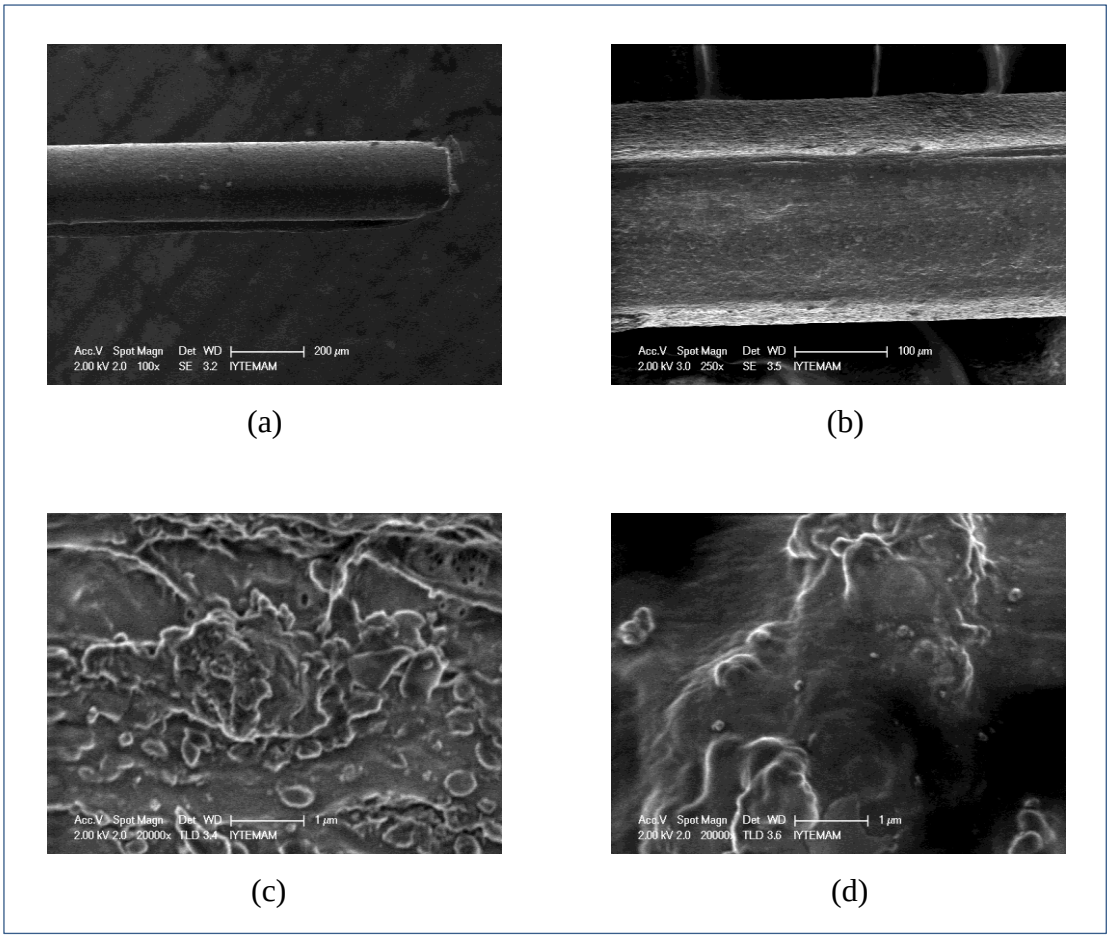


Figure 2.7. SEM images of ZrO_2-agarose fibers at various magnifications (a) 100x, (b) 250x, (c) and (d) 20000x

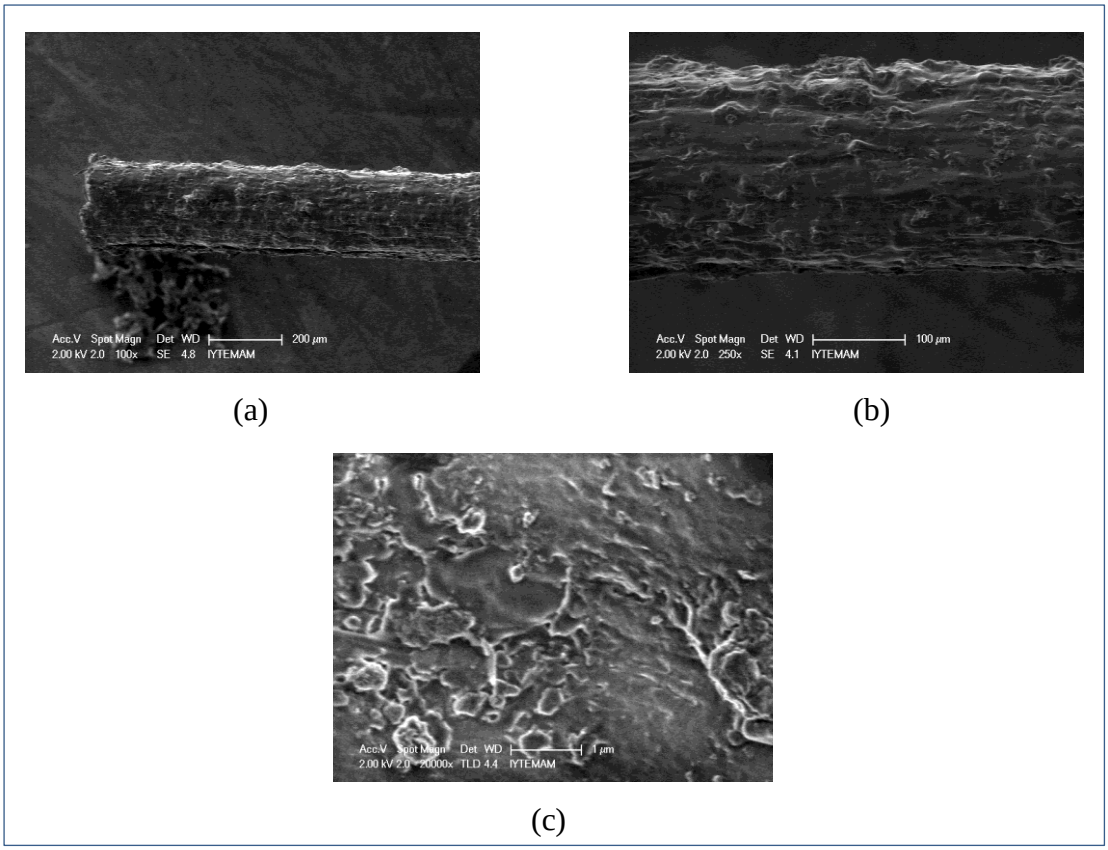


Figure 2.8. SEM images of CeO_2-agarose fibers at various magnifications (a) 100x, (b) 250x, and (c) 20000x

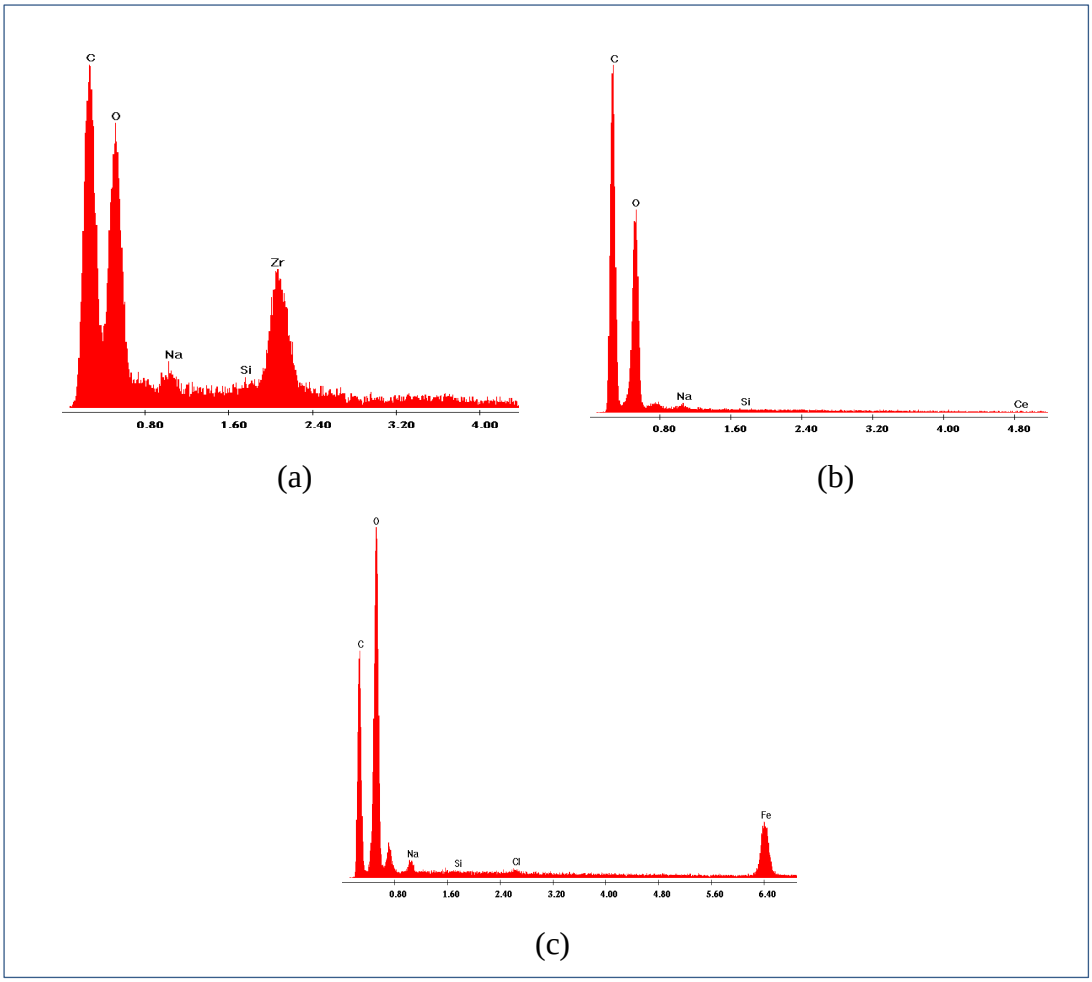


Figure 2.9. EDX spectra of (a) ZrO_2-agarose fiber, (b) CeO_2-agarose fiber and (c) nZVI-agarose fiber

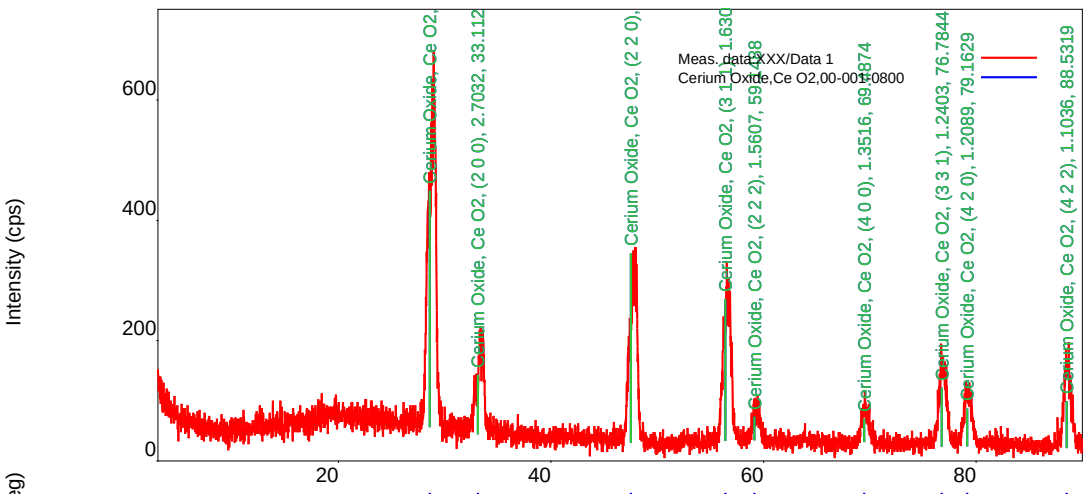


Figure 2.10. XRD pattern of ground CeO_2-agarose fiber

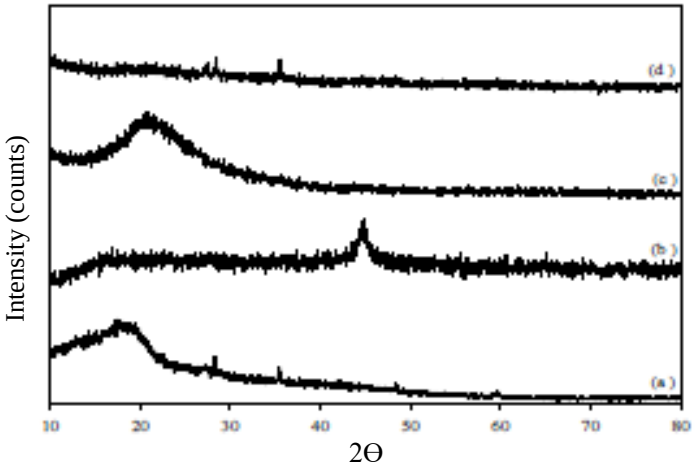


Figure 2.11. XRD pattern of the (a) agarose powder, (b) nZVI, (c) ground silica fiber and (d) ground nZVI-agarose fiber

Figure 2.11 demonstrates the XRD results of agarose powder, synthesized nZVI, ground bare silica fiber and ground nZVI-agarose coated SPME fiber. On the XRD pattern of agarose there are four reflections. These are found at 2Θ degree of 28, 35, 48, and 59. nZVI particles indicated a strong reflection at 2Θ degree of 45 that shows the existence of Fe^0. The XRD pattern of nZVI-agarose fiber clearly demonstrates the disappearance of the reflection of zero valent state and reflections of iron oxide particles at 2Θ degree of 36 were detected.

Previous works of our study group had shown that zero valent iron, ceria and zirconia can be used as solid phase extraction (SPE) sorbents for the speciation and removal of inorganic selenium. Additionally, nZVI-agarose coated silica fibers were used for the speciation of different arsenicals such as As(III), As(V), dimethyl arsinic acid (DMA), monomethyl arsonic acid (MMA), arsenobetaine (AsB) For the adaptation of this knowledge into a developing method like SPME, agarose was used like a glue to attach these functional groups onto silica fibers. This time the purpose was to speciate also the organic forms of selenium. According to the results of preliminary studies it has been decided to do the rest of the study only with nZVI-agarose fiber. To the best of our knowledge, there has been no report established for speciation of organoseleniums with nZVI-agarose coated silica fibers.

2.3. Selenium Speciation with nZVI-agarose SPME Fibers

2.3.1. Optimization of Chromatographic Parameters

The chromatographic separation of selenium species was carried out using an anion exchange column (PRP-X100, 250 mm x 4.1 mm, 10 μm). Selenium species that had been separated using an anion exchange column were sent directly into ICP-MS and detected in 'time resolved analysis' mode. ^{82}Se isotope was selected as it is the most preferable isotope of selenium. Signals of the other isotopes, namely ^{78}Se and ^{80}Se were also measured in previous studies. While ^{78}Se isotope gave similar results (counts/second) to ^{82}Se isotope, ^{80}Se resulted in very high intensity values because of the interference from ^{40}Ar - ^{40}Ar dimer. Most of the buffers that are capable of separating selenium species result in salt deposition onto sample and skimmer cones in the ICP-MS. Therefore, ammonium citrate dibasic buffer was chosen as the mobile phase. At first, all species were eluted from the column less than 9 min when they were injected into the column with 1 mL/min flow rate using 10 mM ammonium citrate (pH: 5.00) as the mobile phase. To lower the amount of time gradient elution was applied by changing the concentration of eluent from 10.0 mM to 30.0 mM. Since it did not work, flow rate of the solution was increased from 1.0 mL/min to 1.5 mL/min. At that time eluent was prepared also in 2% methanol. Methanol didn't show good results causing a shoulder in SeCys peak Figure 2.12. The best chromatogram was obtained using 1.4 mL/min flow rate and 10.0 mM citrate buffer (at pH: 5.0) leading an analysis time of 420 sec. The acquired chromatogram can be seen in Figure 2.13.

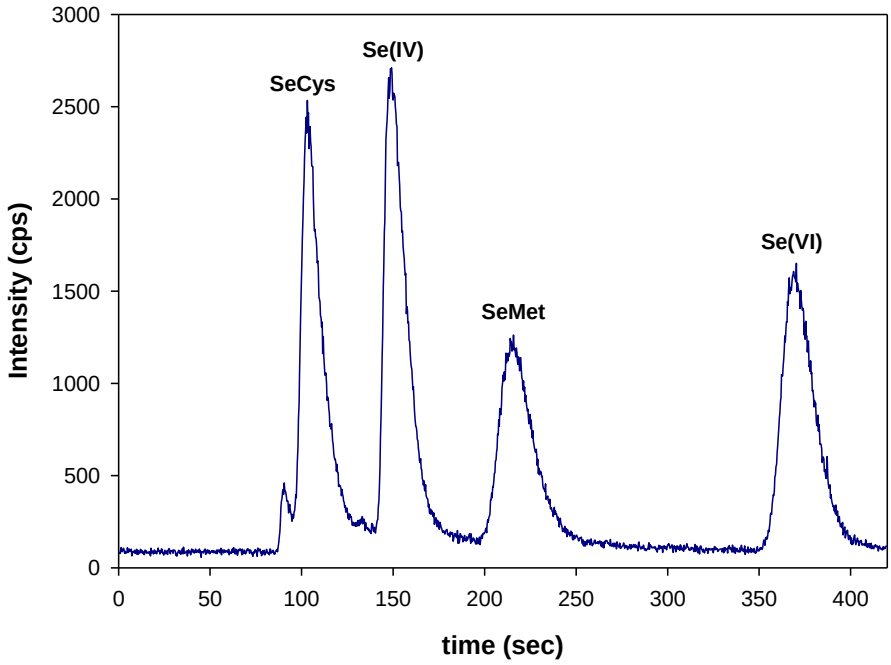


Figure 2.12. Chromatographic separation of selenium species (flow rate: 1.4 mL/min, mobile phase: 10.0 mM citrate buffer in 2% methanol

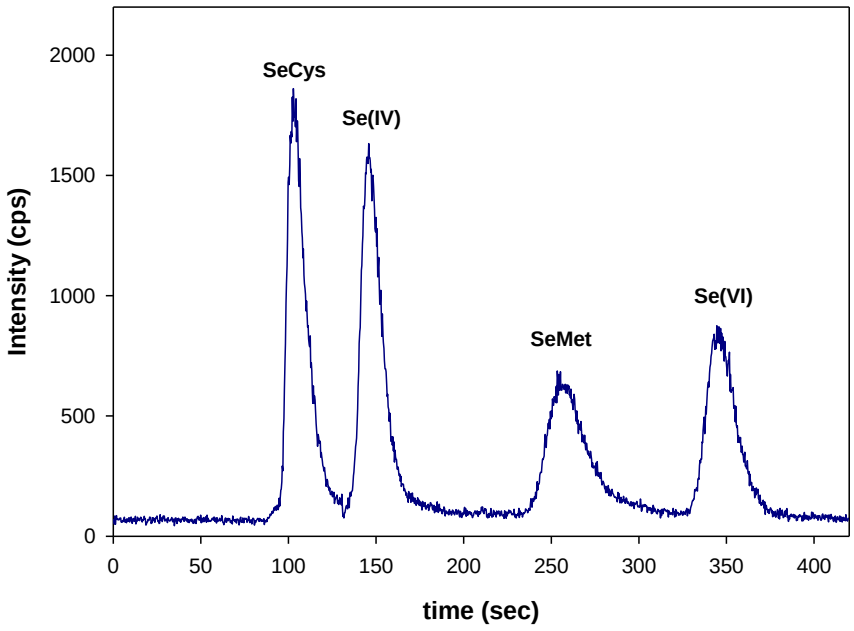


Figure 2.13. Chromatographic separation of selenium species (flow rate: 1.4 mL/min, mobile phase: 10.0 mM citrate buffer

2.3.2. Desorption of Selenium Species

At first, proper mobile phase solution has been determined by considering the chemical properties of the analytical column used. The suggested eluent was ammonium citrate buffer at pH 5.00, therefore experiments were carried out with 10.0 mM citrate buffer solution. Secondly, the retention times were determined by injecting each species separately. They were found to be at around 110 sec, 150 sec, 260 sec and 350 sec for SeCys, Se(IV), SeMet and Se(VI), respectively. The sample volume was 100 μL as recommended.

2.3.3. Optimization of Extraction Parameters

2.3.3.1. Effect of pH on Extraction of Selenium Species

One of the most important parameters to be optimized is the extraction pH. Firstly, agarose coated fibers were tested to understand whether sorption occurs between Se species and agarose without embedding a functional group in it. Figure 2.14 demonstrated clearly that there is no sorption at all at any pH. Effect of pH on extracted amount of each selenium species by nZVI-agarose were investigated at pH 4.0, 7.0, and 10.0 (Figure 2.15).

According to a previous article Iso-Electric-Point (IEP) for nZVI particles was determined to be 8.1-8.2 (Efecan et. al. 2009). While solution pH was below IEP, surface of the fiber is positively charged; if it is above IEP, then the surface of the fiber is negatively charged. Selenium species used and their pKa values were given in Table 2.2. It can be clearly seen that these fibers are not able to extract the selenium species at pH 10.0, whereas for the effective extraction of Se(IV), Se(VI) and SeMet the solution pH must be 4.0, for SeCys it must be 7.0.

All species of selenium are negatively charged at pH 10.0 as Se(IV) and Se(VI) (-2), SeCys (-). Because the surface of the fiber is also negatively charged, there is no interaction due to electrostatic attraction. With the decrease of pH under 8.0 (at pH 4.0 and 7.0) the surface of the fiber is positively charged. Thus there is an electrostatic

attraction between nZVI and negatively charged selenium species. Se(IV) and Se(VI) have the same charges at pH 4.0 and 7.0. On the other hand, the positive charges on the surface of fiber were increased with the drop of pH from 7.0 to 4.0. Thus the interaction between analytes and nZVI particles were also increased. SeCys is -2 charged at pH 4.0, at pH 7.0, -3 charged form exists also partially causing higher attraction at this pH.

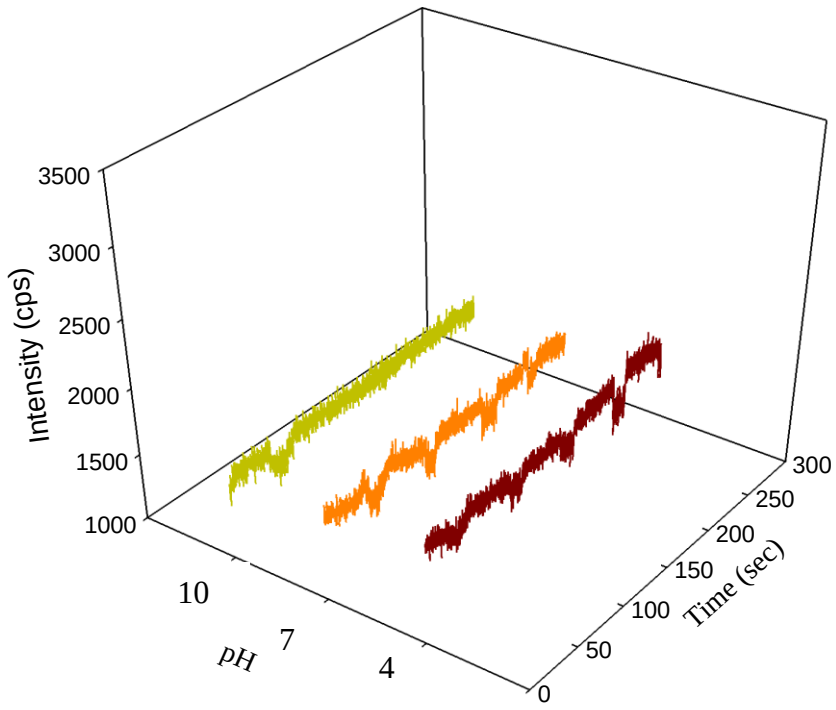


Figure 2.14. Selenium extraction with agarose fibers (10.0 µgL-1 in each species (Se(IV), Se(VI), SeMet, SeCys), stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution)

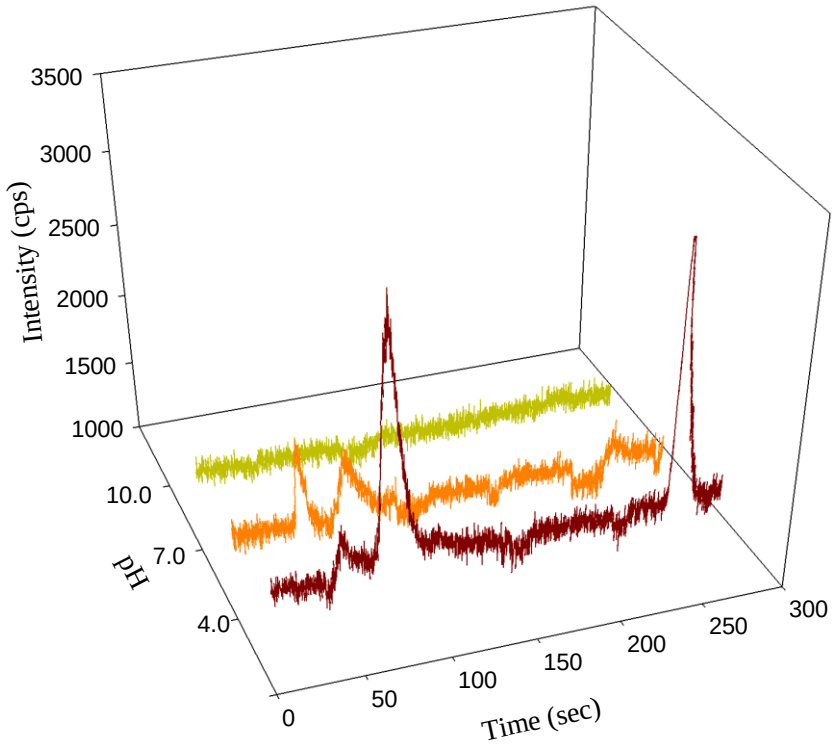
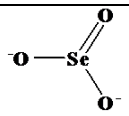
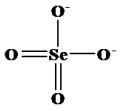
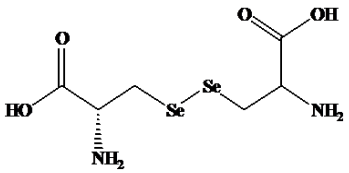
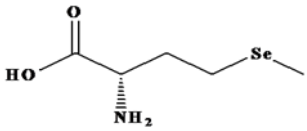


Figure 2.15. Selenium extraction with nZVI-agarose fibers (10.0 μgL^{-1} in each species (Se(IV), Se(VI), Se(Met), SeCys), stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution)

Table 2.2. pKa values of selenium species

Se species	Structure	pKa
Selenite, Se(IV), SeO_3^{2-}		2.5 7.3
Selenate, Se(VI), SeO_4^{2-}		1.9
Selenocystine, SeCys		1.7 2.3 7.9 9.8
Selenomethionine, SeMet		2.2 9.3

However ZrO_2-agarose embedded fibers did not work at pH 7.0 and 10.0 but at pH 4.0 only Se(IV) signal can be seen (Figure 2.16). According to point of zero charge value (2.8) of ZrO_2, fiber is negatively charged above pH 2.8. Therefore it was expected that there is no interaction between negatively charged Se species and ZrO_2-agarose embedded fibers. Similar results are obtained with CeO_2-agarose (pzc: 2.4) embedded fibers (Figure 2.17). At pH 4.0, Se(IV) and Se(IV) peaks can be noticed. This interaction may be explained by the result of a complex formation between CeO_2 and Se compounds forming an insoluble precipitate (Shi et al., 2009). Organoselenium species did not retain on the fibers at all.

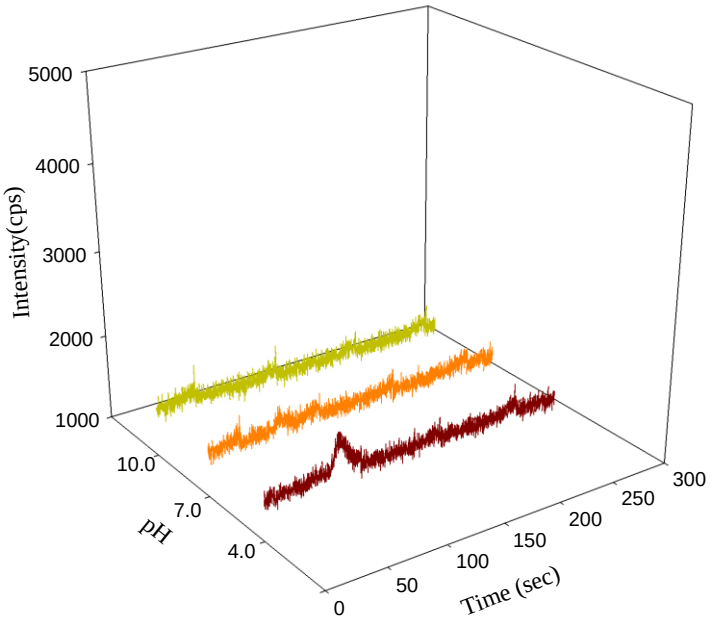


Figure 2.16. Selenium extraction with ZrO_2-agarose fibers (10.0 µgL-1 in each species (Se(IV), Se(VI), Se(Met), SeCys), stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution)

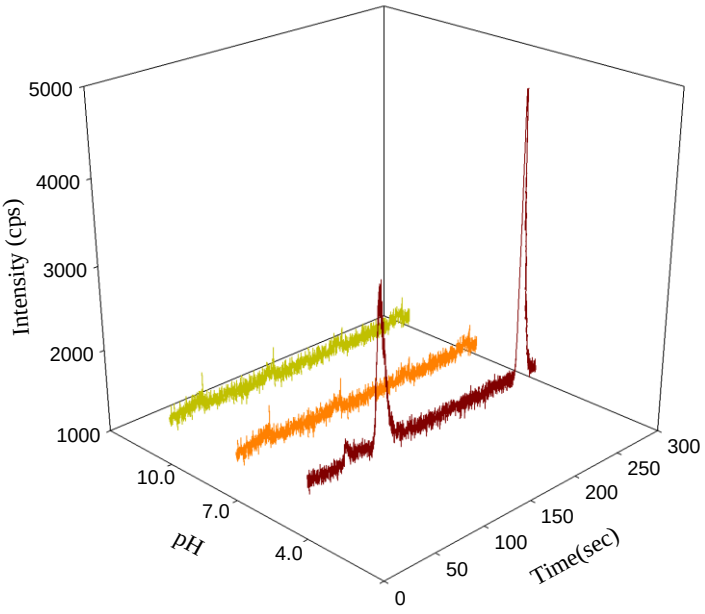


Figure 2.17. Selenium extraction with CeO_2-agarose fibers (10.0 µgL-1 in each species (Se(IV), Se(VI), Se(Met), SeCys), stirring speed: 700 rpm, extraction time:60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution)

2.3.3.2. Effect of Agitation Time/Speed on Extraction of Selenium Species

Contact time of the solution with fibers on extraction of selenium species was seen to be highly important. To enlighten this, 1, 5, 15, 30, 60, and 90 min. of extraction times were tried and the best efficiency was obtained in 90 min (Figure 2.18). But since this time is very long, 60 min was chosen to be optimum time. Actually it seems like there is not a great difference between 30 min and 60 min for Se(IV) and Se(VI) but the signals of organoselenium species are decreasing appreciably at 30 min. The reason of this long equilibrium period can be explained with the fact that the interaction between selenium species and functional particles embedded in agarose matrix was limited with diffusion.

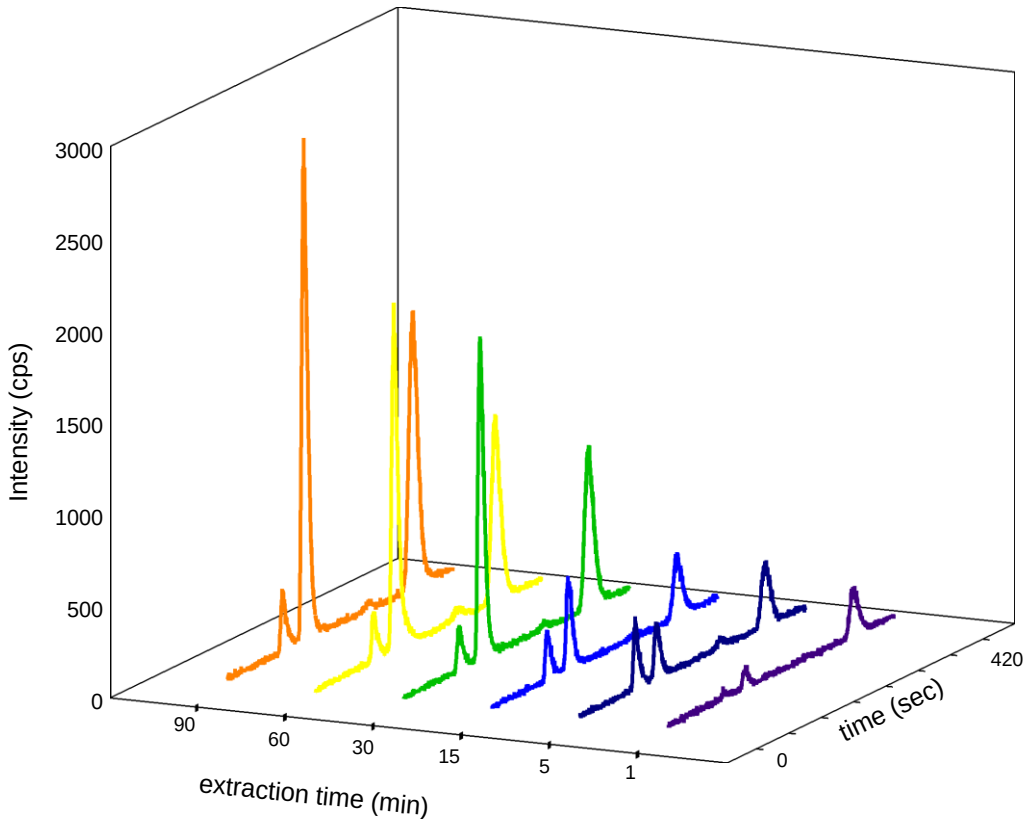


Figure 2.18. The effect of extraction time on selenium extraction (10.0 µgL-1 in each species (Se(IV), Se(VI), SeCys, SeMet), solution pH: 4.0, stirring speed: 700 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution)

The effect of agitation speed on extraction of selenium species was tested at 300, 500, 700, 900 rpm using nZVI-agarose fibers (Figure 2.19). Extracted amounts of each selenium species were increased at higher rates. At 700 rpm the optimum results for all species were obtained. In the case of 900 rpm a vortex occurred, so the sorption of species got lower. In fact better results were obtained at 500 rpm except for SeCys. Since the area of SeCys peak is already smaller than peaks of Se(IV) and Se(VI), 700 rpm was chosen to be used in further studies.

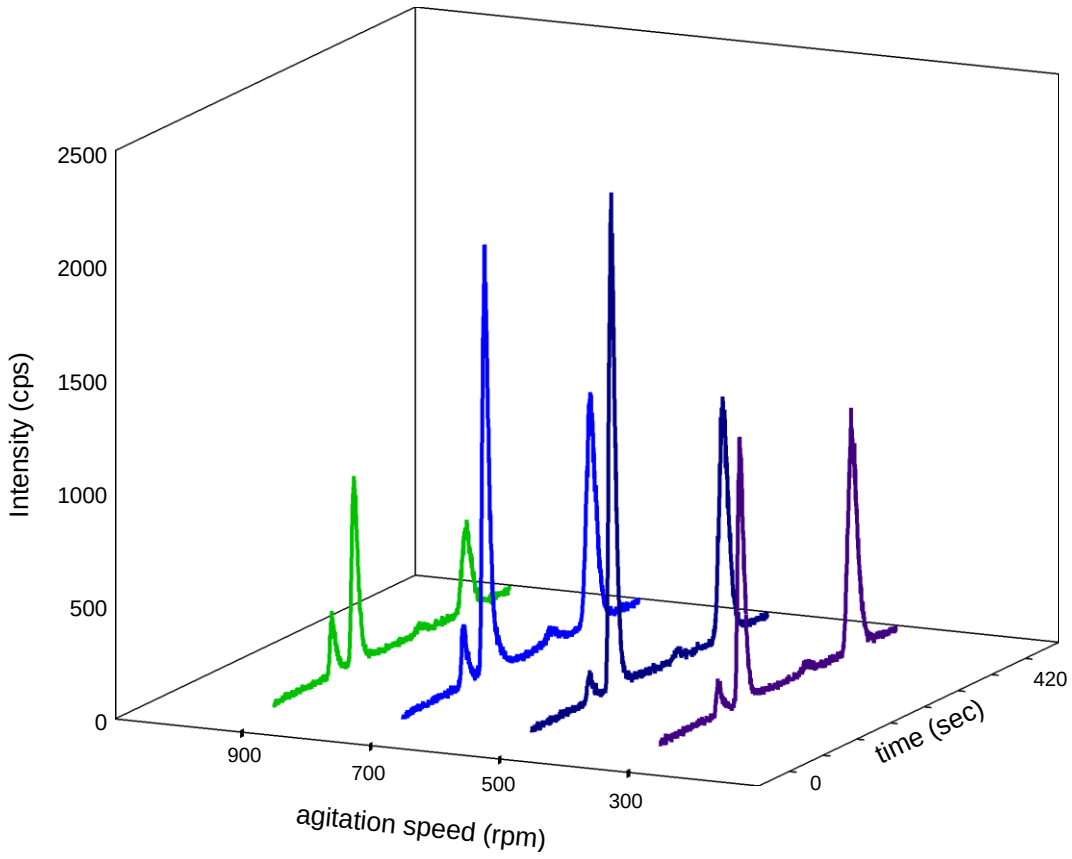


Figure 2.19. The effect of agitation speed on selenium extraction (10.0 µgL-1 in each species (Se(IV), Se(VI), SeCys, SeMet), solution pH: 4.0, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution)

2.3.3.3. Effect of Desorption Matrix/Time on Extraction of Selenium Species

Effect of desorption matrix on the elution of selenium species by nZVI-agarose fiber was investigated for 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 75.0 and 100.0 mM ammonium citrate (Figure 2.20) Even using 5.0 mM citrate solution desorbs all species. With 10.0 mM of eluent the peak areas were increased. For higher concentrations the results did not change much. Starting with 50.0 mM solution, SeCys and Se(IV) peaks were not well separated. Therefore 10.0 mM citrate (same as the mobile phase) was chosen for further experiments.

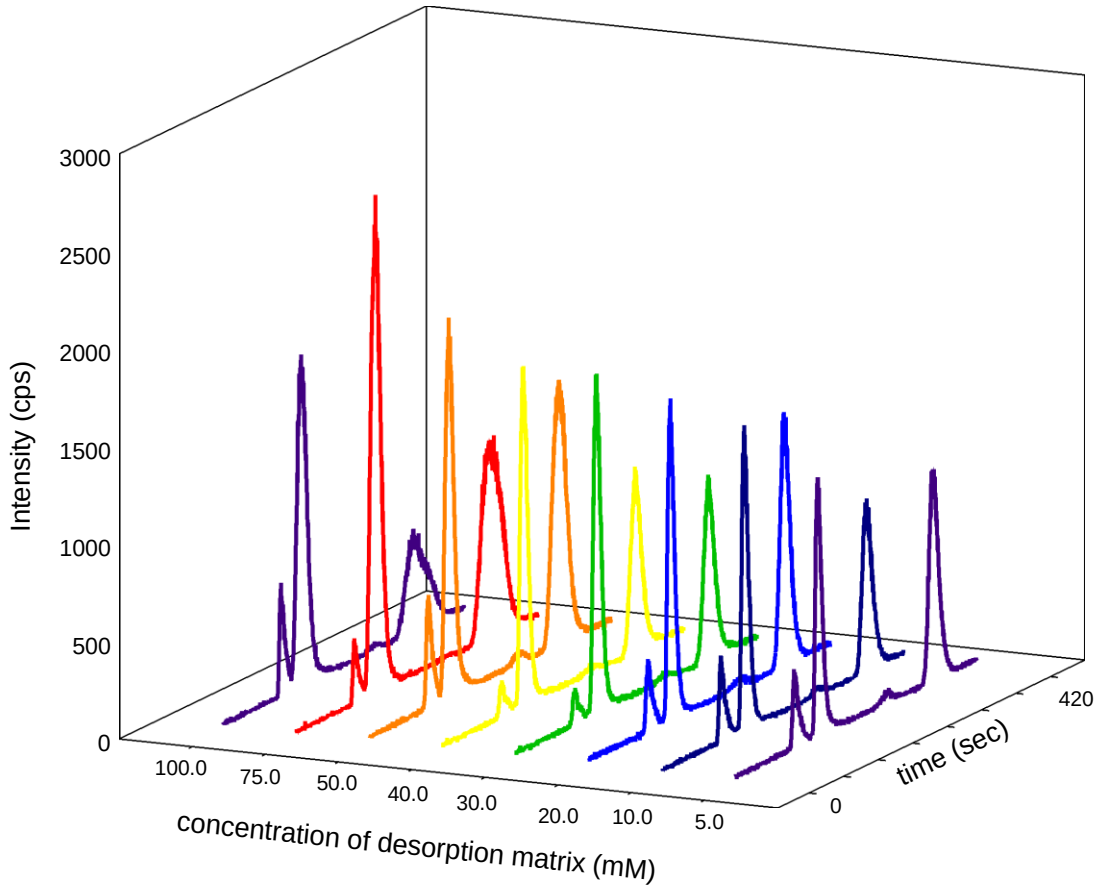


Figure 2.20. The effect of desorption matrix on selenium extraction (10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), solution pH: 4.0, stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL.)

Effect of desorption time was studied for 5, 15, 30, 45, 60 min (Figure 2.21). There appears to be an increase in intensity with longer times, but in fact after 30 minutes, peak areas were lowered although peaks were sharper having. Therefore, 30 min was chosen as the optimum time since peak area was used throughout the study.

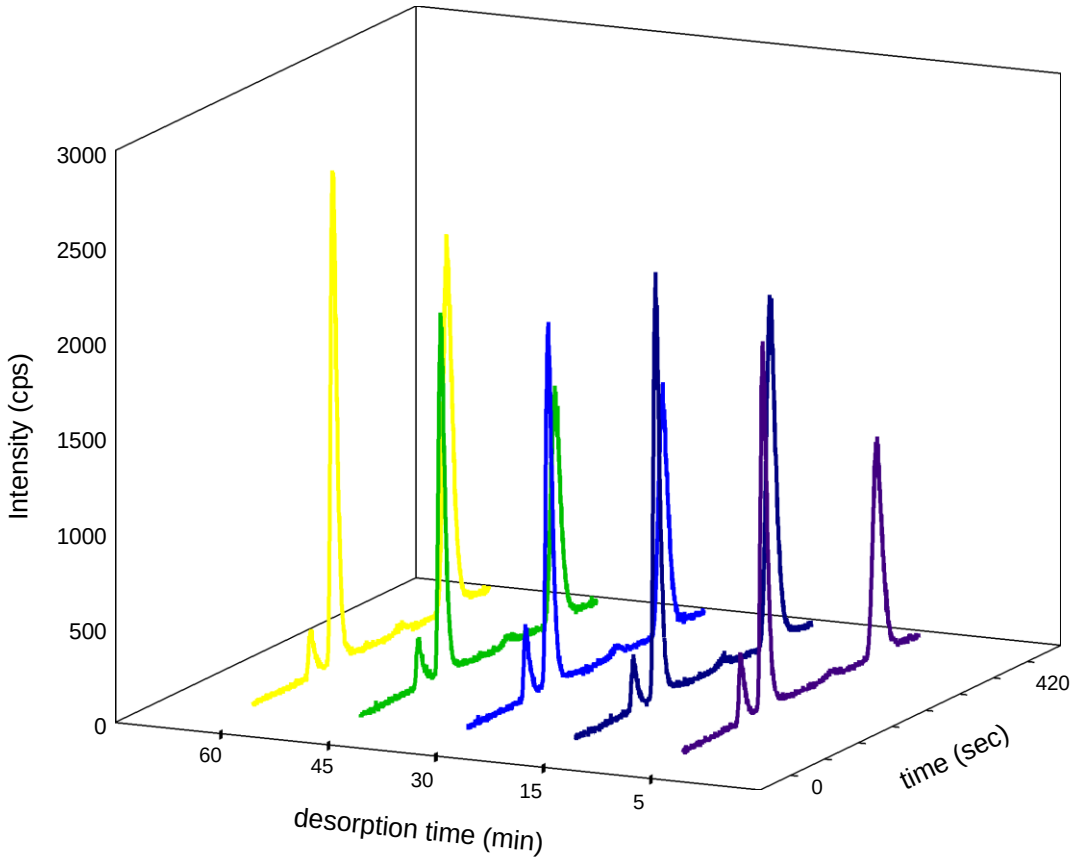


Figure 2.21. The effect of desorption time on selenium extraction (10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), solution pH: 4.0, stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption volume: 150 µL, eluent: 10.0 mM citrate solution.)

2.3.3.4. Effect of Extraction Temperature on Extraction of Selenium Species

To observe the effect of solution temperature on the extraction of selenium species by nZVI-agarose fiber, three different temperatures (25 °C, 40 °C, 55 °C) were tested (Figure 2.22). The temperature change did not show any big difference in peak areas, but if the peak heights were considered 25 °C demonstrated much better results. Thus further experiments were carried out at 25 °C. In the headspace microextractions of volatile compounds increasing the solution temperature increases the amounts of the extracted analytes (Pawliszyn 1999) by altering the equilibrium concentrations of analyte in the headspace and solution. In case of direct mode microextraction a prediction of the effect of the temperature on the extraction of analyte is not easy. It depends on the exothermic or endothermic nature of the extraction and as well as on the volatility of the analytes. Increasing the extraction temperature decreases the amount of extracted analytes. This finding shows the exothermic nature of the extraction of the analytes by the fibers.

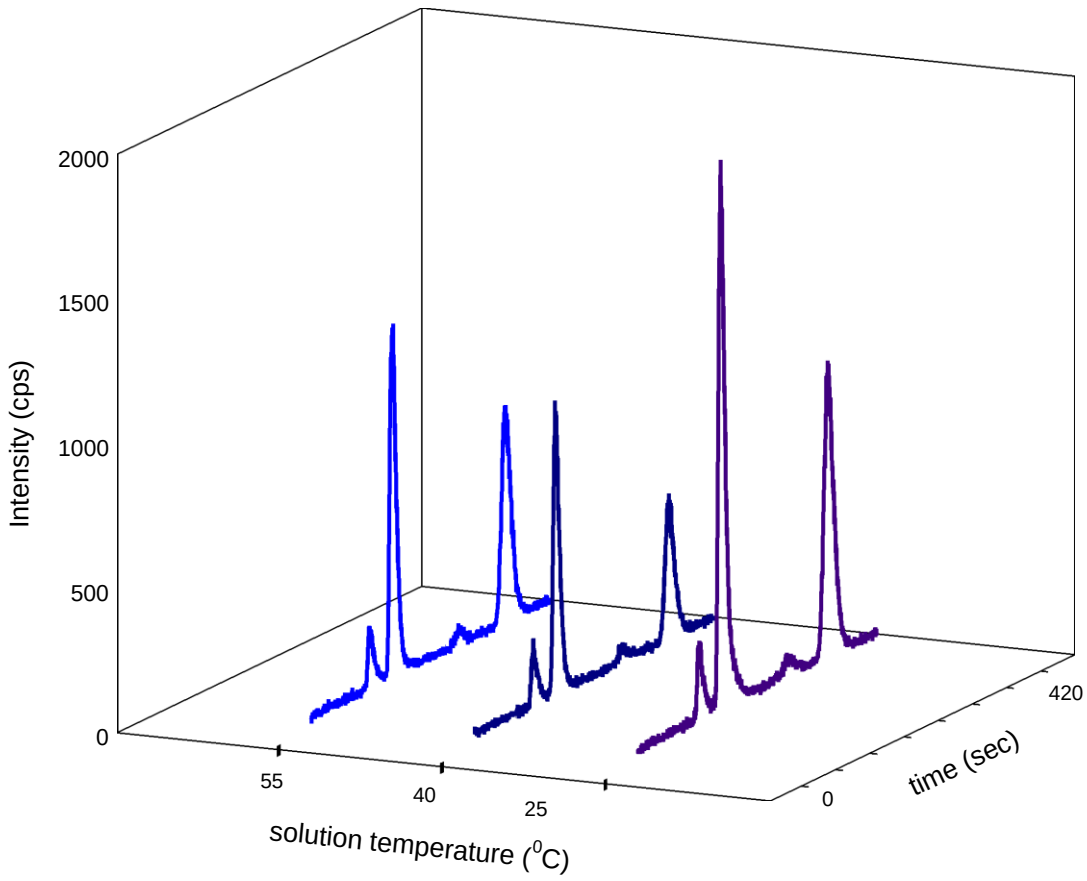


Figure 2.22. The effect of solution temperature on selenium extraction (10.0 µgL-1 in each species (Se(IV), Se(VI), SeCys, SeMet), solution pH: 4.0, stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.)

2.3.3.5. Effect of Ionic Strength on Extraction of Selenium Species

The effect of the ionic strength on the extraction of selenium species was investigated by addition of 0.10 M, 0.010 M and 0.0010 M of NaCl into selenium containing solution (Figure 2.23). Addition of 0.0010 M NaCl has provided slightly better extraction for SeCys but at the same time a dramatic decrease in Se(IV) and Se(VI) peak areas were observed. Addition of 0.010 M NaCl caused an increase in the signals of inorganic species (Se(IV) and Se(VI)) but also caused decreased peak areas for organic species (SeCys and SeMet). In case of 0.10 M NaCl addition, there appears to be a decrease for all peak areas. Therefore no salt was added in further experiments.

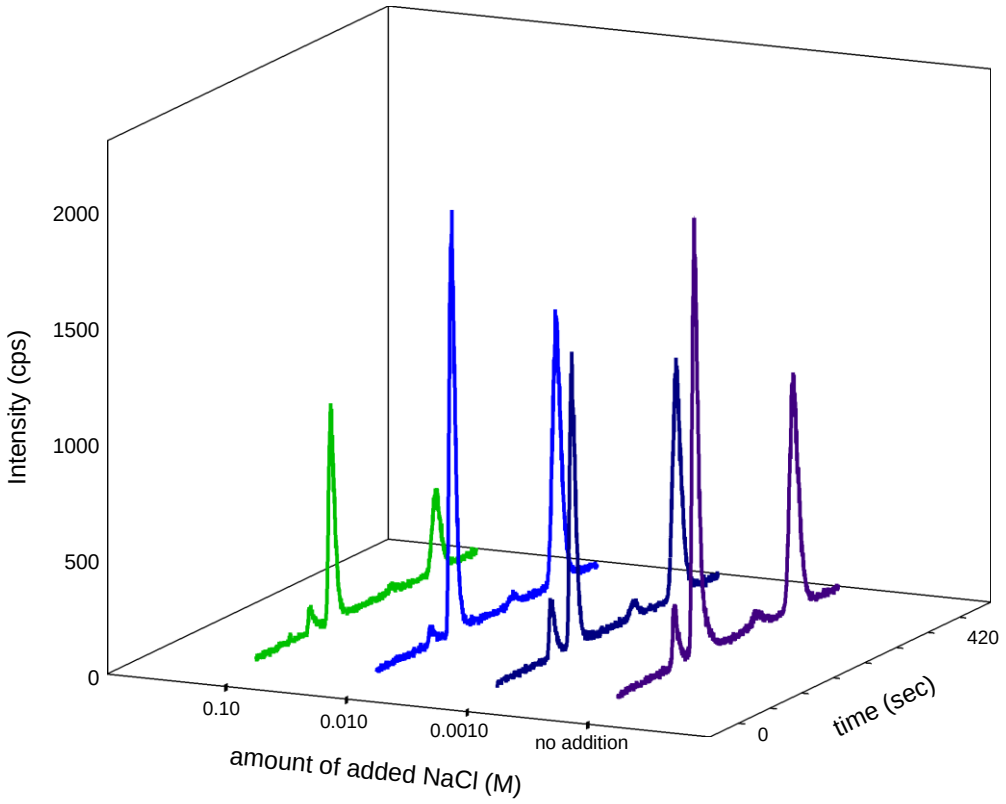
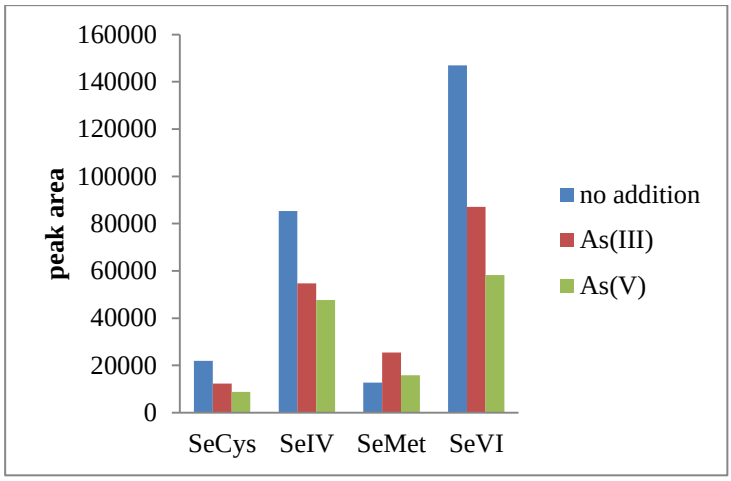


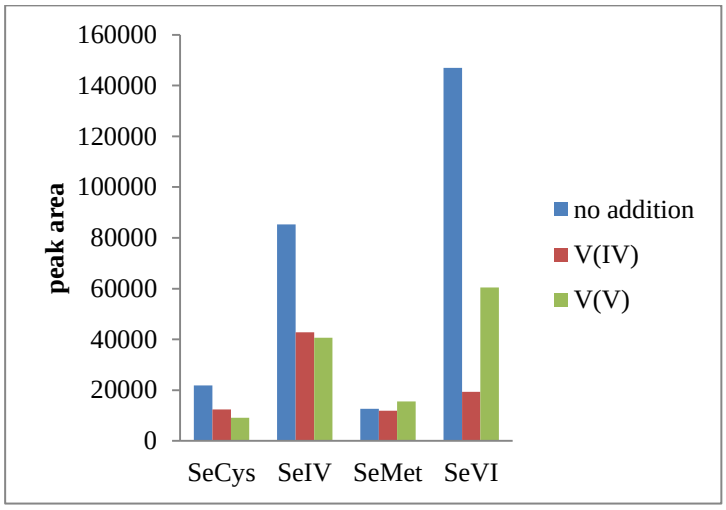
Figure 2.23. The effect of ionic strength on selenium extraction (10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet), solution pH: 4.0, stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.)

2.3.3.6. Interference Studies

Interference studies were performed for Sb(III), Sb(V), As(III), As(V), V(IV), V(V), SO_4^{2-} and PO_4^{3-}. Figure 2.24 demonstrated the effect of each species on extraction of Se(IV), SeCys, SeMet and Se(VI). As can be understood from Figure 2.24, As(III), As(V), V(IV), and V(V) showed interference in extraction of all selenium species except for SeMet. SO_4^{2-} and PO_4^{3-} interfere with all species. On the other hand, Sb(III) and Sb(V) interfered only with SeCys and did not affect the other species.



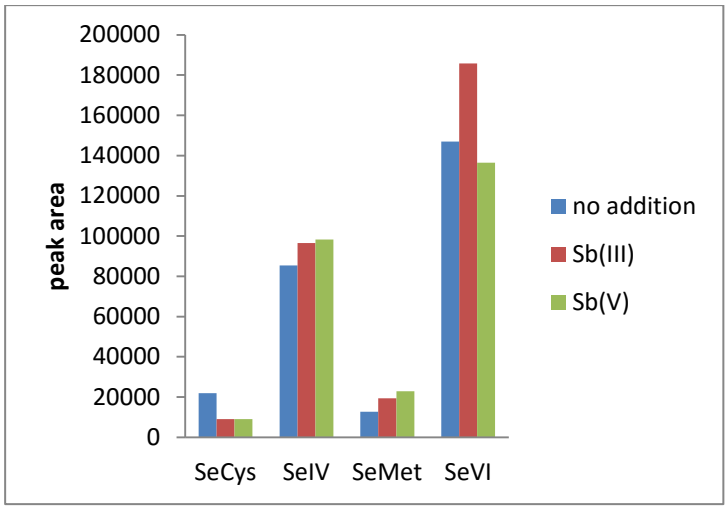
(a)



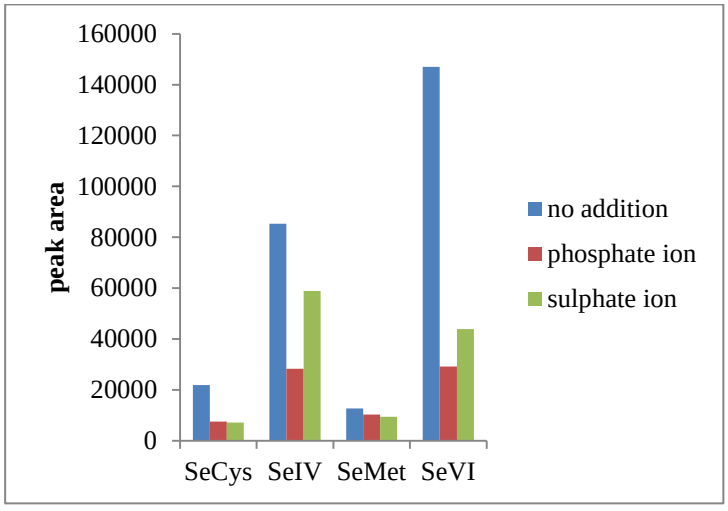
(b)

(cont. on next page)

Figure 2.24. Summary of the interference study; Extraction conditions; extraction time: 60 min, Se concentration: 10.0 µgL-1, solution pH: 4.0, stirring speed: 700 rpm, solution volume: 15 mL, Desorption conditions; desorption time: 30 min, 150 µL 10.0 mM citrate solution, the concentration of the species was 100.0 µgL-1 for (a), (b) and (c), the concentration of the species was 1000.0 µgL-1 for (d).



(c)



(d)

Figure 2.24 (cont.)

2.3.3.7. Analytical Performance of the Method

Calibration plots were constructed to relate the peak area as a function of selenium concentration (Figure 2.25). For this purpose various amounts of selenium species were prepared in ultrapure water (0.1 μgL^{-1} to 50.0 μgL^{-1}) and extraction/desorption to HPLC-ICPMS were performed under optimized experimental conditions. Regression coefficients for each species demonstrated good correlations.

While for SeCys and SeMet the lowest concentration was 1 μgL^{-1}, for Se(IV) and Se(VI) it was 0.10 μgL^{-1}.

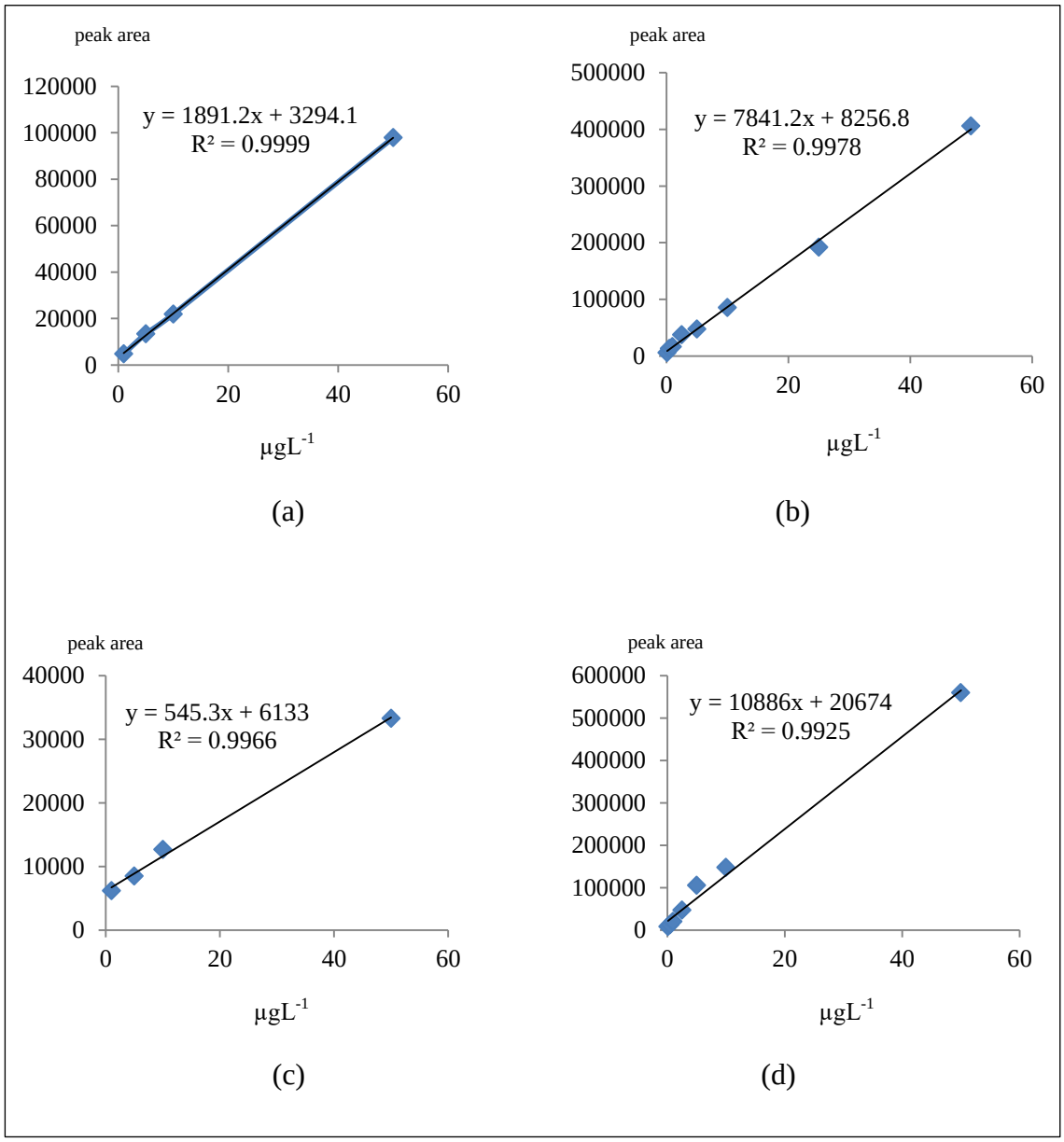


Figure 2.25. Calibration plots of (a) SeCys, (b) Se(IV), (c) SeMet, (d) Se(VI)

In addition, the limit of detection (LOD), and the limit of quantification (LOQ) values for each Se species were also calculated (Table 2.3). Furthermore, the analytical performance of the developed method was tested via determining relative standard

deviations of the peak areas for intra-day and inter-day extractions of the analytes with 4 and 10 fibers, respectively (Table 2.4).

Table 2.3. LOD (3s) and LOQ (10s) obtained with SPME fibers (Extraction conditions; extraction time: 60 min, stirring speed: 700 rpm, solution volume: 15 mL, Desorption conditions; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution

Se Species	LOD μgL^{-1}	LOQ μgL^{-1}
Se(IV)	0.021	0.070
SeCys	0.056	0.187
SeMet	0.043	0.143
Se(VI)	0.024	0.081

Table 2.4. Relative standard deviations obtained for inter-day and intra-day extractions (n= 4 and 10 for inter-day and intra-day extractions, respectively.)

Se Species	Inter-day RSD (%)	Intra-day RSD (%)
Se(IV)	10.5	9.4
SeCys	3.5	15.3
SeMet	6.8	12.3
Se(VI)	5.8	21.3

In order to confirm the nature of the extraction as being exhaustive or microextraction, a solution containing each Se species (10.0 μgL^{-1}) was exposed to SPME procedure with nZVI-agarose fibers. The calibration plots of each species were constructed by injection of several concentrations of Se species into HPLC-ICPMS without extraction with SPME fiber. Then the concentration of Se species in the original solution was determined by HPLC-ICPMS with these calibration curves. The extraction efficiency of each analyte was found as 7.4%, 5.8%, 5.5% and 6.5% SeCys, Se(IV),

SeMet and Se(VI), respectively. The non-exhaustive nature of the extraction indicates that the proposed method is microextractive in nature.

2.3.3.8. Method Validation

The method was applied to tap and bottled water samples. 10.0 μgL^{-1} in each species (Se(IV), Se(VI), SeCys, SeMet) were added and the optimum parameters were applied. Both samples were 2 fold diluted because of their complex matrix. For bottled water samples, Figure 2.26 illustrates the chromatograms of both spiked and non-spiked samples. It seems to be that there is no selenium species in bottled water or their concentrations are below LOD. For the spiked bottled water sample, the concentrations of spiked analytes were Se(IV) 8.32 ± 0.53 μgL^{-1}, Se(VI) 7.40 ± 0.68 μgL^{-1}. SeMet and SeCys could not be extracted. In respect of tap water samples, Se(IV) and Se(VI) concentrations were found to be as 0.78 ± 0.08 μgL^{-1} and 0.71 ± 0.08 μgL^{-1}, respectively (Figure 2.27). For the spiked tap water sample, the concentrations of spiked analytes were SeCys 9.02 ± 0.36 μgL^{-1}, Se(IV) 9.42 ± 0.67 μgL^{-1}, Se(VI) 4.84 ± 1.01 μgL^{-1}. SeMet could not be extracted. Two standard reference materials (SRM from NIST, Natural Water – Trace Elements, Cat. No. 1643e and certified reference material TMDA-70 fortified lake water) were used to validate the method. According to the results that are shown in Table 2.5, an acceptable correspondence was found between the certified and determined values.

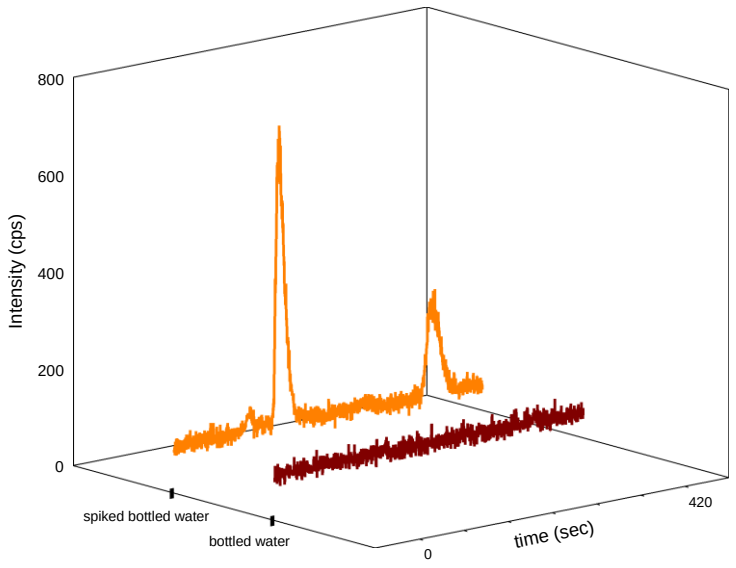


Figure 2.26. Bottled water extraction spiked with 10.0 µg/L in each selenium species (solution pH: 4.0, stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.)

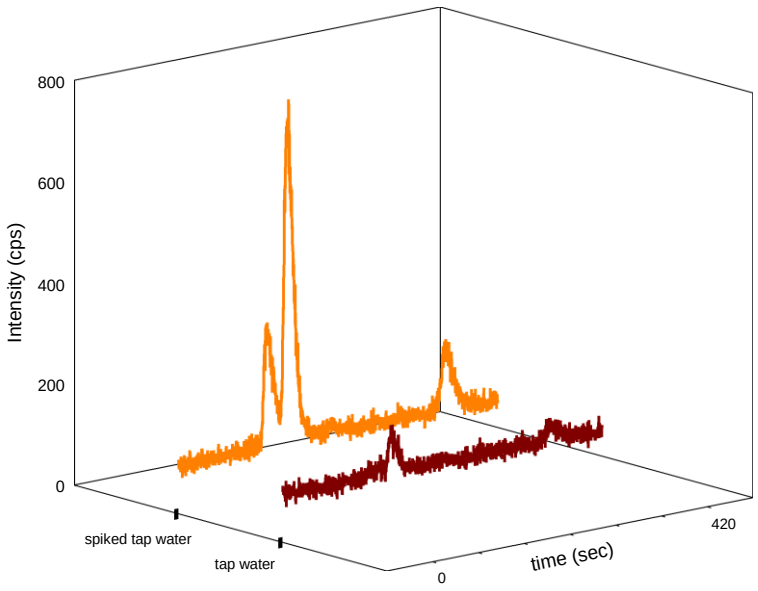


Figure 2.27. Tap water extraction spiked with 10.0 µg/L in each selenium species (solution pH: 4.0, stirring speed: 700 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 10.0 mM citrate solution.)

Table 2.5. Method validation with SPME fibers. (Extraction conditions; extraction time: 60 min, solution pH: 4.0, stirring speed 700 rpm, solution volume: 15 mL, Desorption conditions; desorption time: 30 min, 150 µL 10.0 mM citrate solution.)

Se species	SRM 1643e [a]		TMDA-70 [a]	
	Determined μgL^{-1}	Certified μgL^{-1}	Determined μgL^{-1}	Certified μgL^{-1}
SeCys	ND*		ND	
Se(IV)	10.53		15.9	
SeMet	ND		ND	
Se(VI)	0.40		5.19	
Total Se	10.93	11.97 ± 0.14**	21.09	25.8 ± 1.98**

a: extractions were performed in 1/10 diluted samples

***** Not detected

****** Speciation of selenium in SRM 1643e and in TMDA-70 was not indicated in the certificate.

CHAPTER 3

NEW SPME SORBENTS:

SEPARATION AND DETERMINATION OF TRICLOSAN AND TRICLOCARBAN IN WATERS

3.1. Experimental

3.1.1. Chemicals and Reagents

All the chemicals were of analytical reagent grade. Ultrapure water (18.2 MΩ, Millipore) was used throughout the study. Glassware and plastic containers were washed with detergent and then acetone.

Standard TCS and TCC stock solutions (100.0 mg/L) were prepared monthly in methanol in dark glass bottles and stored at –20 °C in refrigerator. Triclocarban (3,4,4'-trichlorocarbanilide) (99%) was obtained from Sigma-Aldrich and triclosan (5-Chloro-2-(2,4-dichlorophenoxy)phenol), (99%) from Alfa Aesar. Methanol was HPLC grade (Sigma–Aldrich, St. Louis, MO, USA). Working solutions were prepared daily by diluting the individual stock solution with the same solvent. pH adjustments were done by using 1.0 M, 0.1 M, 0.01 M of HCl and NaOH solutions. All solutions were filtered from 0.25 μm cellulose acetate filter paper (Sartorius) and degassed for 15 min in ultrasonic bath before being introduced into HPLC system.

3.1.2. Instrumentation and Apparatus

Separation of extracted selenium species was achieved in Agilent 1200 Series HPLC system with a C30 (YMC, 250 mm x 4.6 mm) column using isocratic elution with 90% methanol – 10% ultrapure water (adjusted to pH 3.0 with acetic acid) as mobile phase at a flow rate of 0.8 $mLmin^{-1}$. The pH adjustments of solutions were

achieved with Ino Lab Level 1 pH meter (Weilheim, Germany). IKA yellow line OS 5 basic orbital shaker (Staufen, Germany) was used for efficient mixing.

Table 3.1. Operation conditions for HPLC

HPLC	Agilent 1200
Analytical column	C30 (250 mm x 4.6 mm, 5 μm)
Mobile phase	90% methanol, 10% UPW (adjusted to pH: 3.0 with acetic acid)
Flow rate	0.8 mL/min
Column temperature	30 ºC
Sample volume	20 μL
Selected λ's	220, 230, 265, 280

3.1.3. Separation and Determination of TCS and TCC

3.1.3.1. Optimization of HPLC

The chromatographic separation of TCS and TCC was carried out using C30 bonded silica based reversed-phase column. Mixture of methanol-ultrapure water (adjusted to pH 3.0 with acetic acid) was chosen as the mobile phase. TCS and TCC were injected into the column with 0.8 mL/min flow rate at 30 °C column temperature and the total separation was lasted almost 8 min. DAD detector was used to detect the analytes at four different wavelengths (220, 230, 265, 280).

3.1.3.2. Extraction of TCS and TCC

SPME was carried out with agarose (blank fibers), nZVI-agarose, CeO_2-agarose, ZrO_2-agarose fibers for the extraction of TCS and TCC. After pH adjustment of the solutions, 15.0 mL aqueous mixture containing 100.0 μg/L TCS and TCC were stirred

in 20 mL amber vial using IKA yellow line OS 5 basic orbital shaker. All extractions were realized at room temperature (25 °C). All solutions were prepared in amber glassware to prevent possible photodegradation of TCS and TCC during SPME process. The extraction time was 120 min and stirring rate was 240 rpm. Desorption of the analytes was carried out in a container which was prepared from 200-µL pipette. Its tip was blocked by the application of heat (burning). For desorption, the initial conditions were; desorption time: 30 min, desorption volume: 150.0 µL, desorption temperature: 25 °C, eluent: 90 % methanol – 10 % ultrapure water (adjusted to pH 3.0 with acetic acid). HPLC mobile phases and desorption solutions were filtered through 0.20 µm cellulose acetate filter paper before use. Afterwards, the extractions parameters such as extraction pH, extraction time, agitation (stirring) speed, desorption matrix, desorption time, salt concentration (ionic strength) and organic modifier were optimized.

3.1.4. Optimization of Extraction Parameters

3.1.4.1. Effect of pH on Extraction of TCS and TCC

pH of the solution is one of the most important parameters on the extraction of TCS and TCC by the prepared fibers. Therefore, preliminary experiments for optimization of extraction parameters started with the investigation of extraction pH. Extraction studies were carried out at 25 °C after the initial pH of solutions was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 10.0 with dilute HCl or NaOH. The conditions for the extractions were; TCS and TCC concentration: 100.0 $\mu g L^{-1}$ in each species, stirring speed: 240 rpm, extraction time: 120 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 90% MeOH – 10 % UPW (adjusted to pH 3.0 with acetic acid).

3.1.4.2. Effect of Agitation Time/Speed on Extraction of TCS and TCC

Effect of agitation time on the extraction of TCS and TCC by nZVI-agarose was investigated for time intervals of 5, 15, 30, 60, 90 and 120 min. The conditions for the extractions were; solution pH: 5.0, 7.0, analyte concentration: 100.0 μgL^{-1} in each species (TCS and TCC), stirring speed: 240 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 90% MeOH – 10 % UPW (adjusted to pH 3.0 with acetic acid). Separately, the effect of the agitation speed on extraction of the TCS and TCC was studied at 80, 160, 240, 320 and 400 rpm stirring speeds. Extraction and desorption conditions were the same as in the previous trials.

3.1.4.3. Effect of Desorption Matrix/Time on Extraction of TCS and TCC

Effect of desorption matrix on the elution of TCS and TCC by nZVI-agarose was investigated for 100% MeOH and 90% MeOH – 10 % UPW. The conditions for the extractions were; solution pH: 5.0, 7.0, extraction time: 60 min, analyte concentration: 100.0 μgL^{-1} in each species (TCS and TCC), stirring speed: 240 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 90% MeOH – 10 % UPW (adjusted to pH 3.0 with acetic acid). Separately, the effect of desorption time was studied for 5, 15, 30, 45, 60 min. Extraction and desorption conditions were the same as in the previous trials.

3.1.4.4. Effect of Ionic Strength on Extraction of TCS and TCC

Effect of ionic strength on the extraction of TCS and TCC was investigated by addition of several amount of NaCl into TCS and TCC containing solution. The investigated concentrations were 0.50 M, 0.10 M, 0.010 M and 0.0010 M NaCl. The conditions for the extractions were; solution pH: 5.0, extraction time: 60 min, analyte concentration: 100.0 μgL^{-1} in each species (TCS and TCC), stirring speed: 400 rpm,

solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 μL, eluent: 90% MeOH – 10 % UPW (adjusted to pH 3.0 with acetic acid).

3.1.4.5. Interference Studies

Interference studies were performed for bisphenol A (BPA) and β-estradiol. Each compound was examined by addition into the solution containing 100.0 μgL^{-1} TCS and TCC. The added concentration for BPA and β-estradiol was 100.0 μgL^{-1}. The conditions for the extractions were; solution pH: 5.0, extraction time: 60 min, analyte concentration: 100.0 μgL-1 in each species (TCS and TCC), stirring speed: 400 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 μL, eluent: 90% MeOH – 10 % UPW (adjusted to pH 3.0 with acetic acid).

3.2. Results and Discussions

3.2.1. Optimization of Chromatographic Parameters

At first, for the chromatographic separation of TCS and TCC, a C18 column was tested. Although mobile phases with different strengths, various flow rates and gradient programs were tried peaks of TCS and TCC could not be separated with a high resolution. Therefore a more specific column namely C30 bonded silica based reversed-phase column was used throughout the study with very good resolution. An isocratic program has lead TCS and TCC to be eluted at retention times approximately 5.1 min and 7.2 min, respectively. The mobile phase used was the mixture of 90 % methanol – 10 % ultrapure water (adjusted to pH 3.0 with acetic acid). Acetic acid is used to improve the chromatographic peak shape and to provide a source of protons in reverse phase HPLC. Different percentages of methanol-UPW mixtures and various flow rates were tried to obtain best resolution in shortest time. Finally, it was decided to use a 0.8 mL/min flow rate at 30 °C column temperature and the total separation was lasted

almost 8 min. DAD detector was used to detect the analytes at four different wavelengths (220, 230, 265, and 280). Results were calculated based on 280 ʎ.

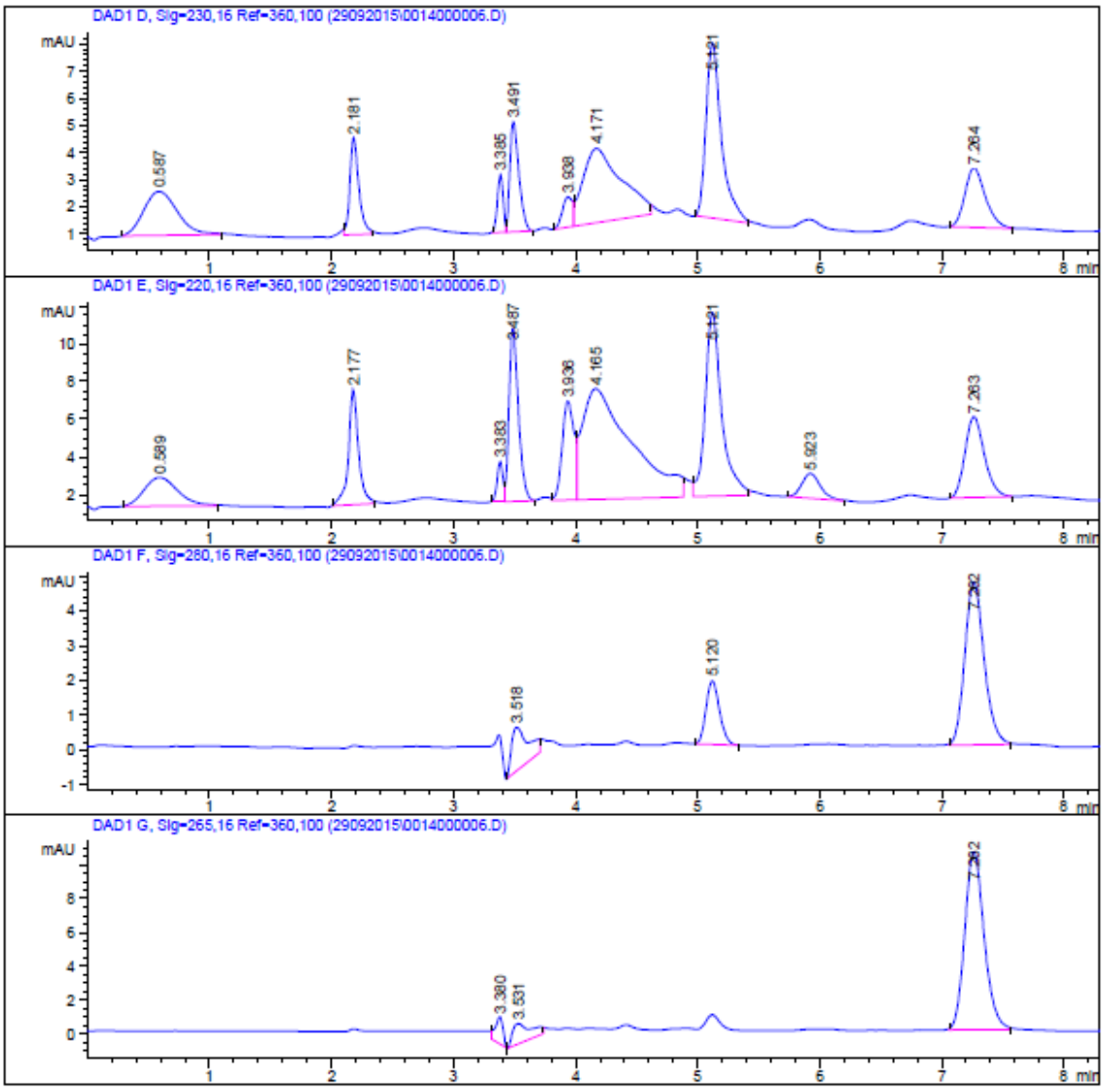


Figure 3.1. Chromatographic separation of TCS and TCC (flow rate: 0.8 mL/min, mobile phase: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid)

3.2.2. Desorption of TCS and TCC

At first, proper mobile phase solution has been determined by considering the chemical properties of the analytical column used. The suggested eluent was methanol or acetonitrile, since the standard stock solutions are prepared in methanol, this eluent was chosen for initial experiments. Different proportions of methanol-water mixtures were tested to obtain best results. At last it is decided to use 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid). Secondly, the retention times were determined by injecting each species separately. They were found to be at around 5.1 min and 7.2 min for TCS and TCC, respectively. The sample volume was 20 μL as recommended.

3.2.3. Optimization of Extraction Parameters

3.2.3.1. Effect of pH on Extraction of TCS and TCC

One of the most important parameters to be optimized is the extraction pH. Firstly, agarose coated fibers were tested to understand whether sorption occurs between TCS and TCC and agarose without embedding a functional group on it at pH 4.0, 5.0, 6.0, 7.0, 8.0 and 10.0. Figure 3.2 indicates that both TCS and TCC are retained on agarose fibers to some extent. Embedding nZVI particles onto agarose caused an increase in peak areas of TCS and TCC (Figure 3.3). In case of CeO_2-agarose fibers, TCS signal did not result in high peak areas whereas standard deviation values of TCC signals were high enough to end the experiments with this coating. ZrO_2-agarose fibers have revealed no sorption capacity for TCS at all, therefore this fiber was abandoned too.

According to the dissociation constant of TCS (pK_a = 8.14), more than 50 % of TCS is estimated to be deprotonated at pH > pK_a. When the pH is above 9.0, the anionic form of TCS is dominant in solution and the overall surface charge on the nZVI-agarose fiber is partially negative (pzc of nZVI = 8.1-8.2). In this case, the sorption capacity is reduced due to electrostatic repulsion between the deprotonated TCS and negatively charged nZVI. Even at pH 8.0 this result can be seen. At acidic pH, TCS is mainly non-

dissociated and the surface charge of nZVI-agarose fiber is positive. Therefore repulsive electrostatic interactions are lowered and sorption is improved. In case of TCC (pK_a = 12.7) there is an increase in sorption capacity until a sharp drop at pH 10.0. According to this result, pH 5.0 and pH 7.0 were chosen as the extraction pH for further optimization experiments.

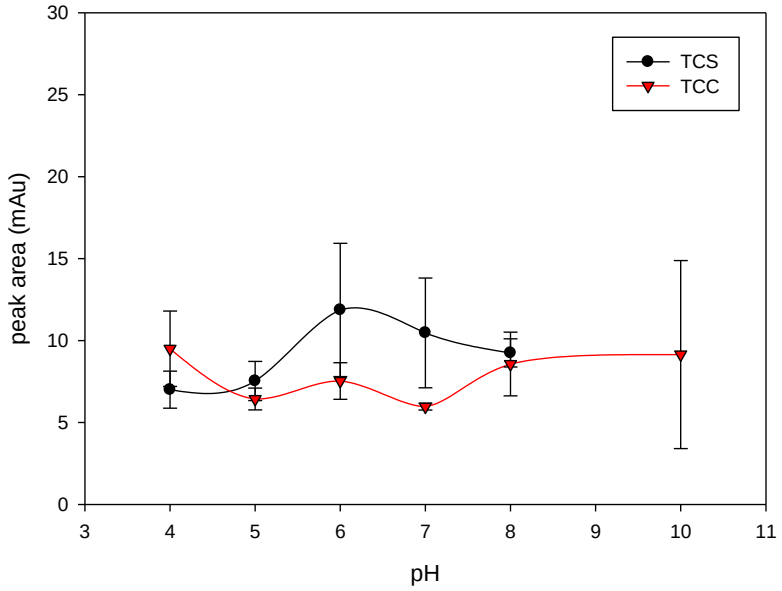
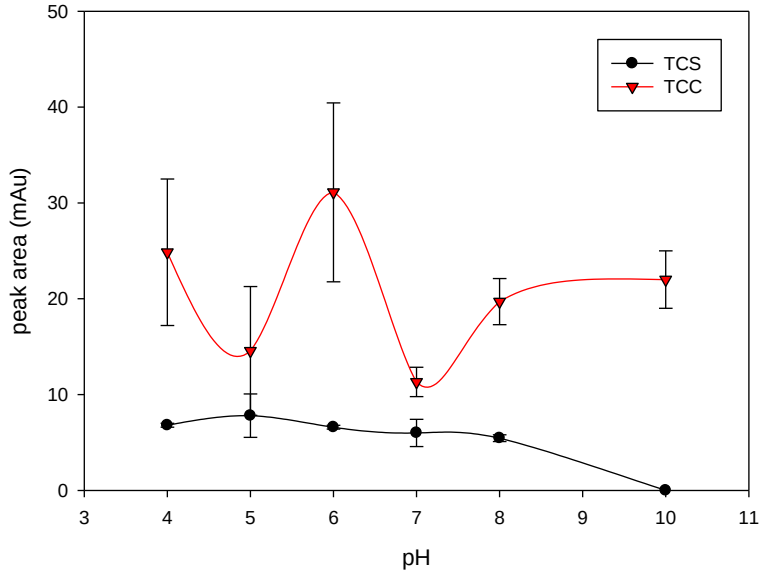
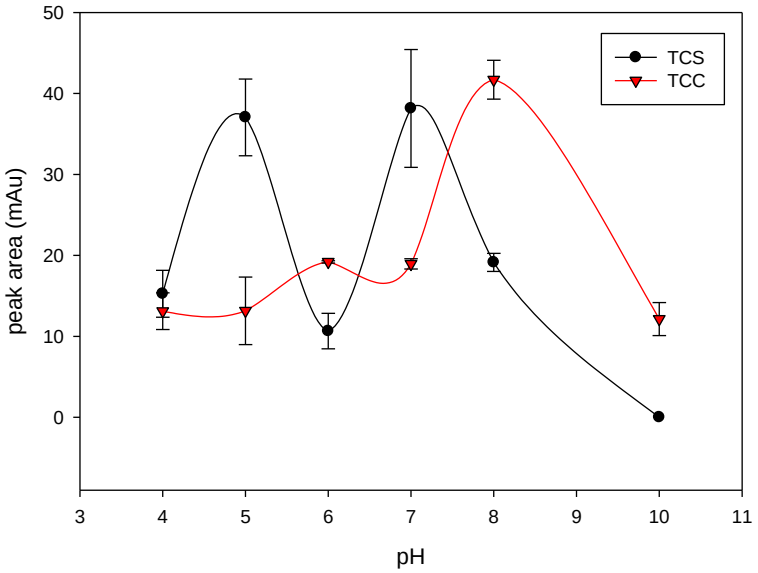


Figure 3.2. TCS and TCC extraction with agarose fibers (100.0 µgL-1 in each species (TCS and TCC), stirring speed: 400 rpm, extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid))



(a)



(b)

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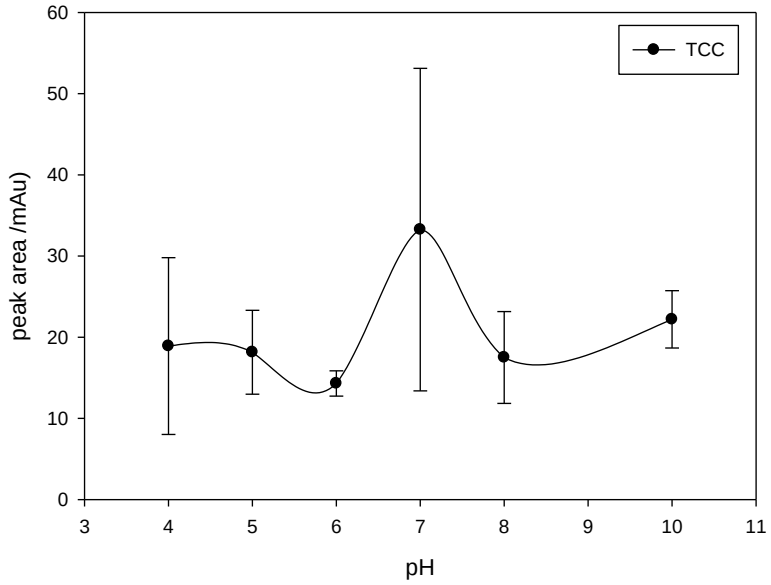
Figure 3.3. TCS and TCC extraction with (a) CeO_2-agarose fibers, (b) nZVI-agarose fibers and (c) ZrO_2-agarose fibers. (100.0 µgL-1 in each species (TCS and TCC), stirring speed: 240 rpm, extraction time: 120 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid)



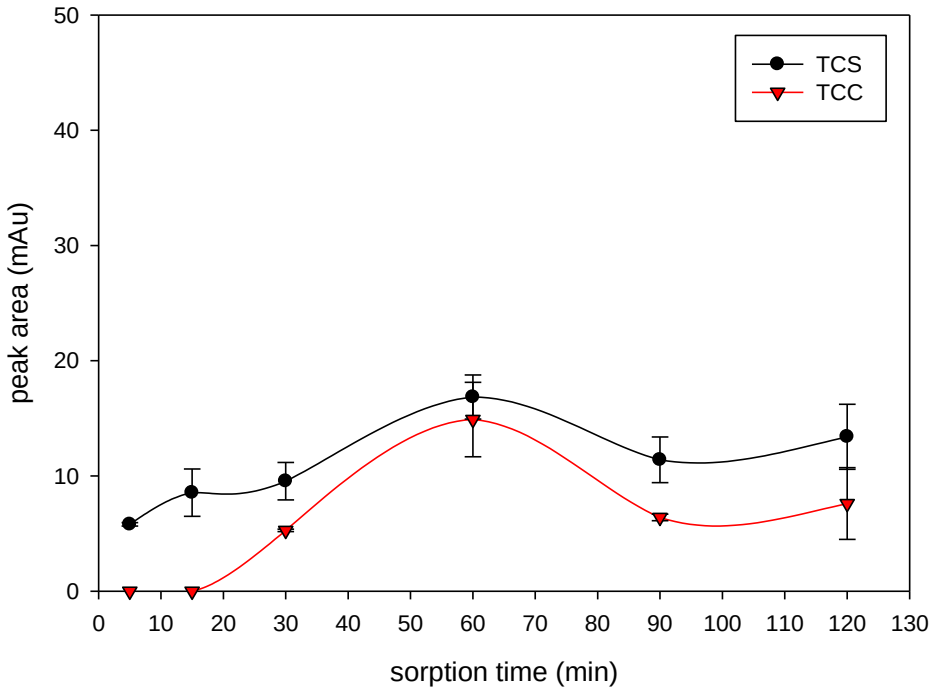
(c)

Figure 3.3 (cont.)

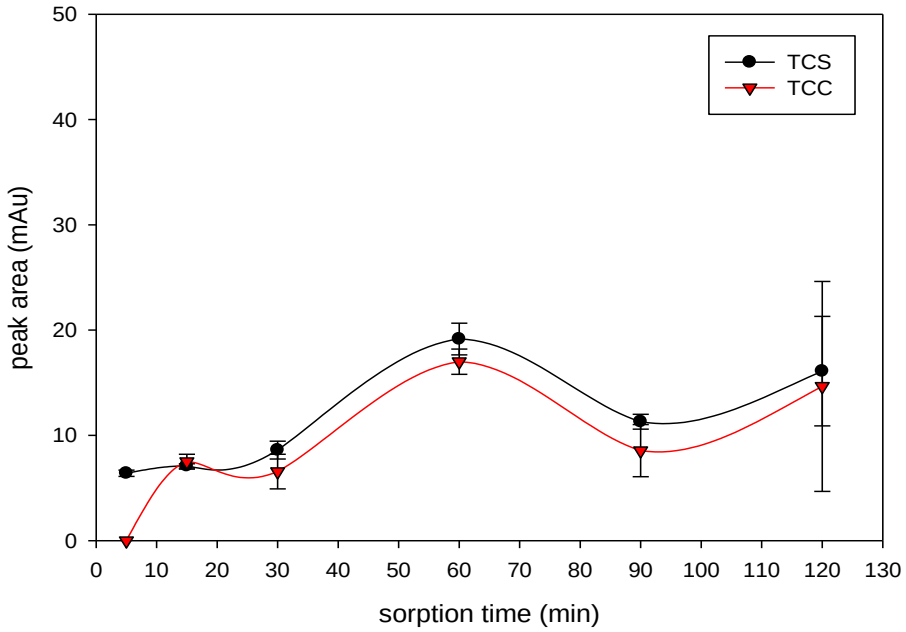
3.2.3.2. Effect of Agitation Time/Speed on Extraction of TCS and TCC

Since the sorption time is very important in SPME (equilibrium method), various extraction times were investigated for nZVI-agarose fibers at pH 5.0 and pH 7.0. Extraction times of 5, 15, 30, 60, 90, 120 min were tried and for both pH it is clearly observed that in 60 min equilibrium is reached (Figure 3.4)

The effect of agitation speed on extraction of TCS and TCC was tested at 80, 160, 240, 320 and 400 rpm. Extracted amounts of each analyte were increased at higher rates. At 400 rpm the best results were obtained for nZVI-agarose fiber (Figure 6a and 6b).

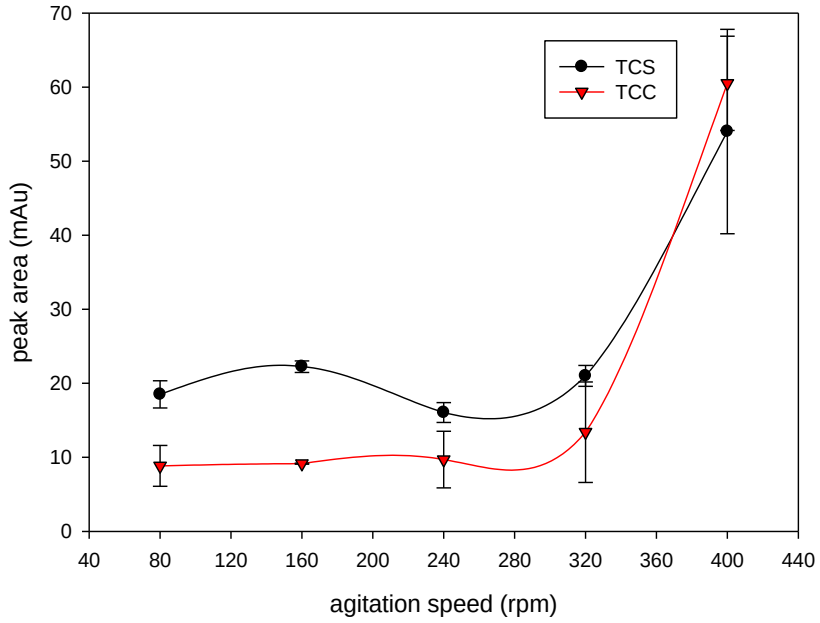


(a)

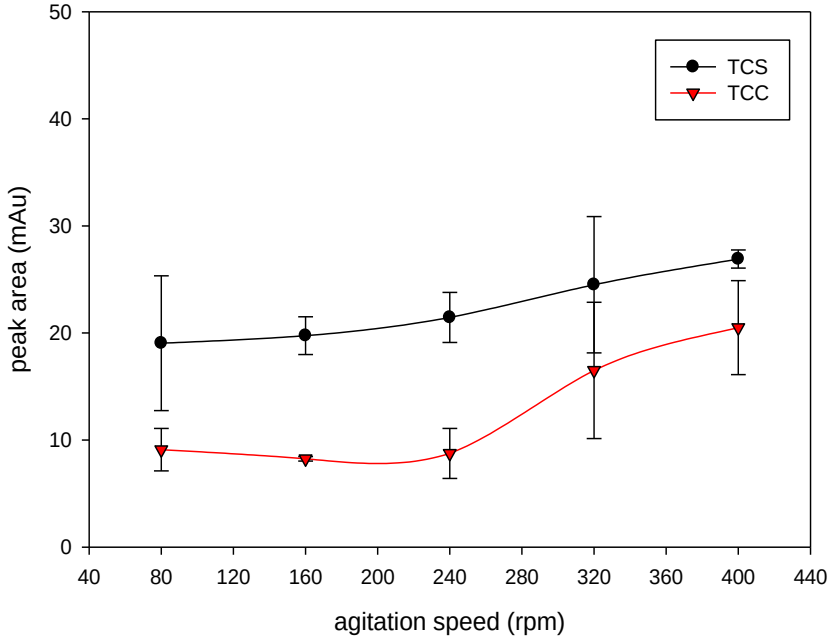


(b)

Figure 3.4. The effect of extraction time on TCS and TCC extraction. (a) at pH 5.0, (b) at pH 7.0 (100.0 µgL-1 in each species (TCS and TCC), stirring speed: 240 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid)



(a)



(b)

Figure 3.5. The effect of agitation speed on TCS and TCC extraction. (a) at pH 5.0, (b) at pH 7.0 (100.0 μgL^{-1} in each species (TCS and TCC), extraction time: 60 min, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL, eluent: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid)

3.2.3.3. Effect of Desorption Matrix/Time on Extraction of TCS and TCC

Desorption matrix of analytes was chosen as the mobile phase at first. % 100 MeOH was also used as desorption matrix to figure out if there will be an increase in peak areas (Figure 3.6). At pH 5.0, a decrease has occurred for both TCS and TCC. At pH 7.0, in case of TCS the signal was also reduced with the use of % 100 MeOH, whereas TCC has demonstrated better desorption from nZVI-agarose fibers. Since both analytes must be considered at the same time, desorption matrix was selected as the mixture of %90 methanol with % 10 water (adjusted to pH 3.0 with acetic acid).

Effect of desorption time was studied for 5, 15, 30, 45, 60 min. Figure 3.6a indicates clearly that five minutes is enough for the desorption of analytes at pH 5.0 and even higher peak areas are obtained. But the standard deviations are also increased enormously. Therefore, 30 min was chosen as the optimum desorption time with smallest deviations among 15, 45 and 60 min. In case of pH 7.0, while there is a stable trend for TCS, a fluctuation has occurred for TCC. Then again 30 min seems to be the optimum time for desorption.

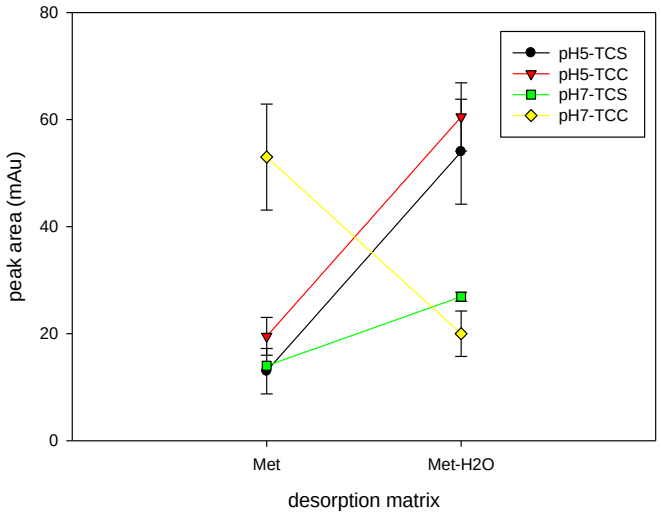
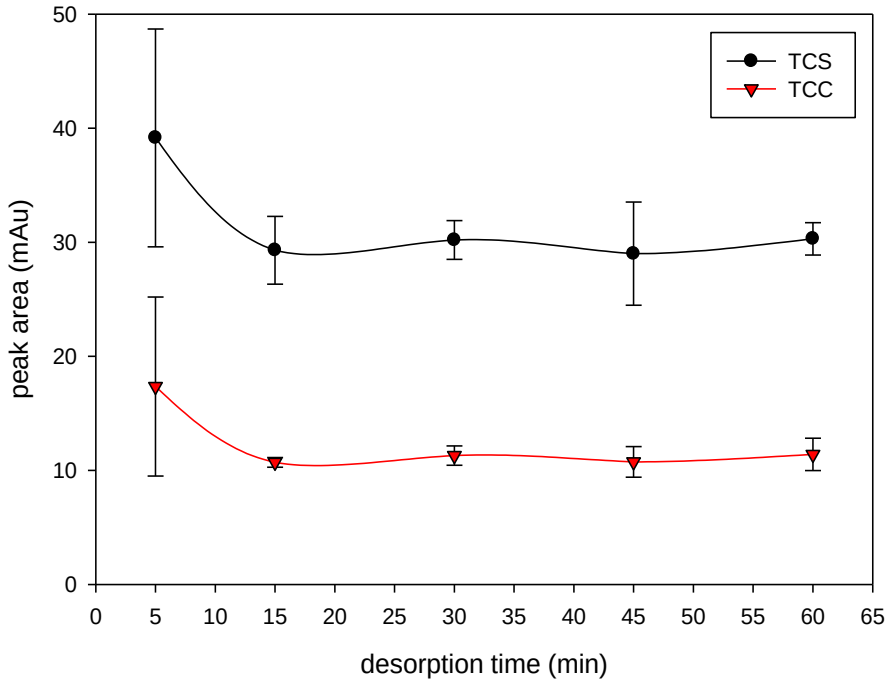
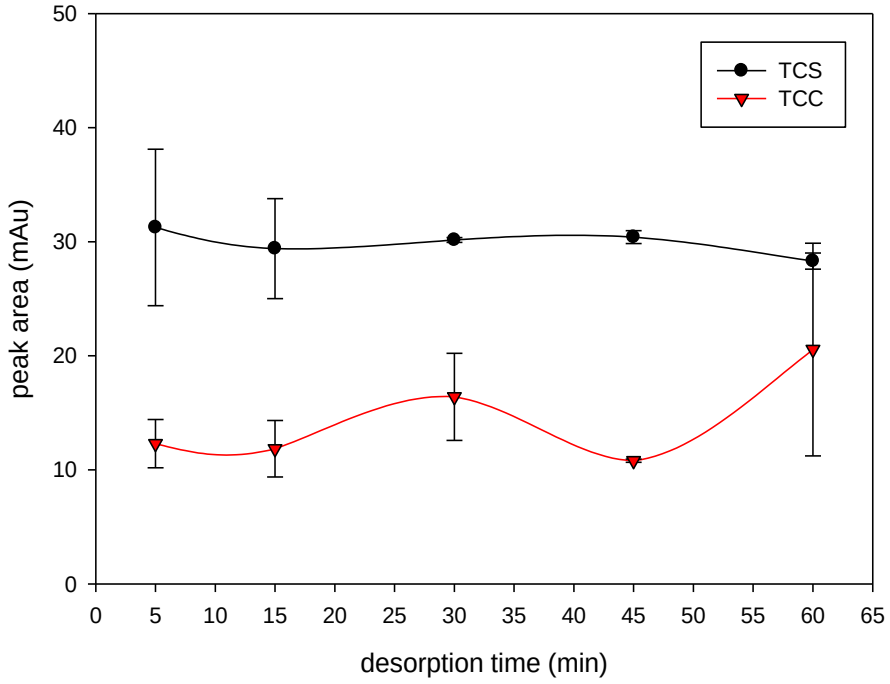


Figure 3.5. The effect of desorption matrix on TCS and TCC extraction. (at pH 5.0 and pH 7.0, 100.0 µgL-1 in each species (TCS and TCC), extraction time: 60 min, stirring speed: 400 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption time: 30 min, desorption volume: 150 µL)



(a)

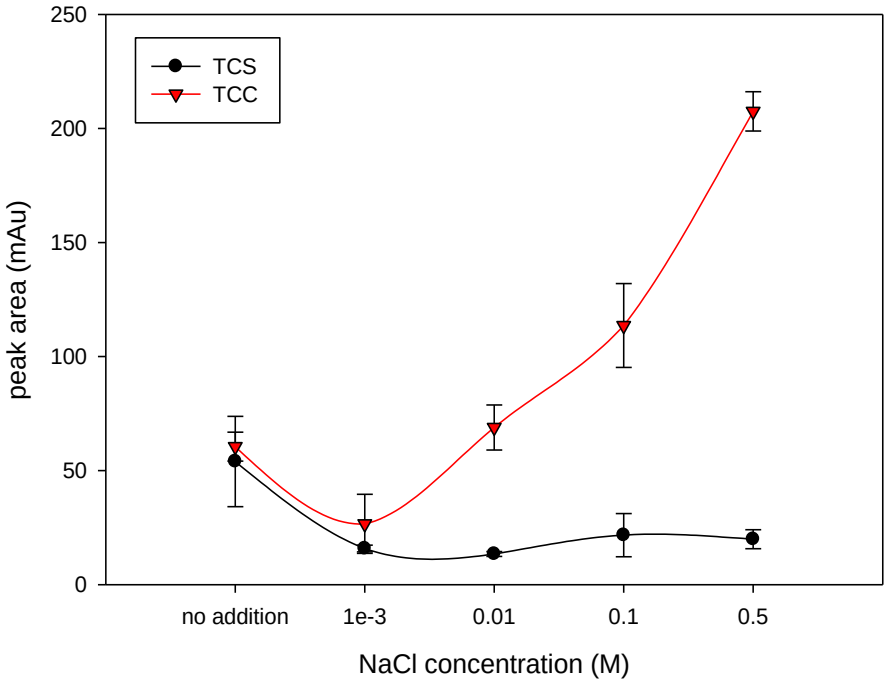


(b)

Figure 3.6. The effect of desorption time on TCS and TCC extraction. (a) at pH 5.0, (b) at pH 7.0 (100.0 µgL-1 in each species (TCS and TCC), extraction time: 60 min, stirring speed: 240 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption volume: 150 µL, eluent: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid)

3.2.3.4. Effect of Ionic Strength on Extraction of TCS and TCC

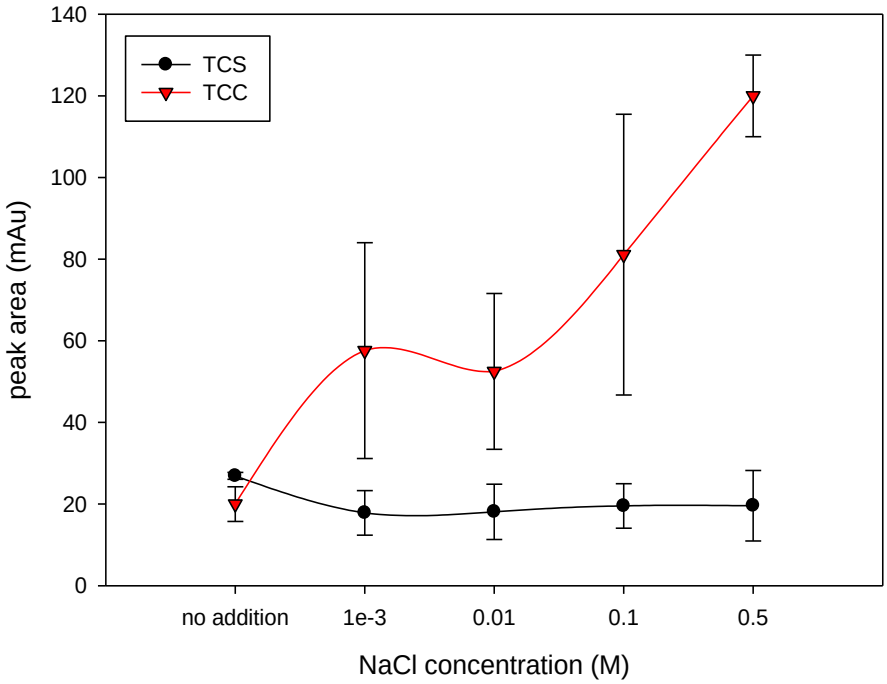
The investigated concentrations were 0.50 M, 0.10 M, 0.010 M and 0.0010 M NaCl. Increasing the ionic strength of the solution usually yields in a decrease in solubility of analytes. Therefore, it is usually expected that analytes are more easily retained on the fiber. Figure 3.7 has presented that for TCC at both pH 5.0 and pH 7.0 the expectation has been met resulting in very high peak areas. But also it can be clearly seen that standard deviation got bigger too. On the contrary, results of TCS demonstrated a decrease in peak areas with the increase of concentration of added salt into the aqueous solution. Probably the increase in sorption of TCC caused this decrease in sorption of TCS if their sorption process can be assumed competitive. Overall, for further experiments it has been decided not to put any salt into the solutions.



(a)

(cont. on next page)

Figure 3.7. The effect of ionic strength on TCS and TCC extraction. (a) at pH 5.0, (b) at pH 7.0 (100.0 µgL-1 in each species (TCS and TCC), extraction time: 60 min, stirring speed: 400 rpm, solution volume: 15.0 mL. Desorption conditions were; desorption volume: 150 µL, desorption time: 30 min, eluent: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid)



(b)

Figure 3.7 (cont.)

3.2.3.5. Interference Studies

Interference studies were performed for bisphenol A (BPA) and β-estradiol. Each compound was examined by addition into the solution containing 100.0 μgL^{-1} TCS and TCC. The added concentration for BPA and β-estradiol was 100.0 μgL^{-1}. The experiments were conducted at the optimum conditions for pH 5.0. Figure 3.8 shows that both bisphenol A and β-estradiol interfere with TCS and TCC.

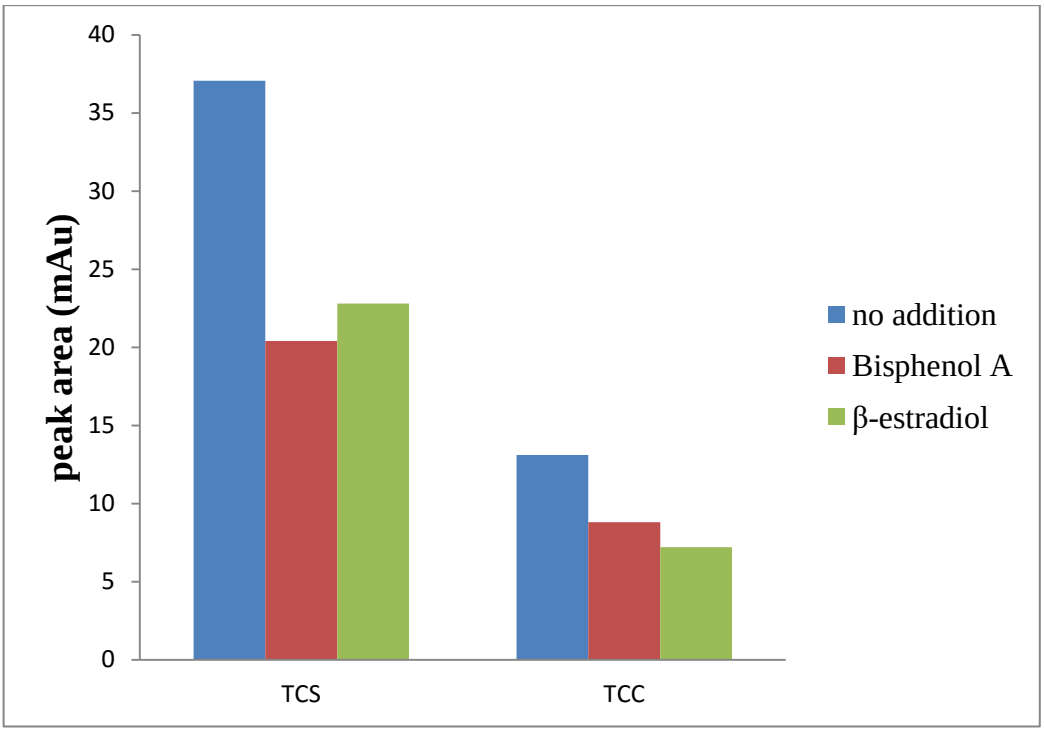


Figure 3.8. Summary of the interference study; Extraction conditions; extraction time: 60 min, TCS and TCC concentration: 100.0 µgL-1, solution pH: 5.0, stirring speed: 400 rpm, solution volume: 15 mL, Desorption conditions; desorption time: 30 min, desorption volume: 150 µL,eluent: 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid, the added concentration of BPA and β-estradiol was 100.0 µgL-1)

3.2.3.6. Reproducibility of the Fiber

The limit of detection (LOD), and the limit of quantification (LOQ) values for TCS and TCC were calculated as (Table 3.2). Furthermore, the analytical performance of the developed method was tested via determining relative standard deviations of the peak areas for intra-day and inter-day extractions of the analytes with 4 and 10 fibers, respectively (Table 3.3).

Table 3.2. LOD (3s) and LOQ (10s) obtained with SPME fibers (Extraction conditions; extraction pH: 5.0, extraction time: 60 min, stirring speed: 400 rpm, solution volume: 15 mL, Desorption conditions; desorption time: 30 min, desorption volume: 150 μL, eluent: 90 % MeOH – 10 % H_2O (adjusted to pH 3.0 with acetic acid)

	LOD μgL^{-1}	LOQ μgL^{-1}
TCS	7.47	24.9
TCC	4.2	14.1

Table 3.3. Relative standard deviations obtained for inter-day and intra-day extractions (n= 4 and 10 for inter-day and intra-day extractions, respectively.)

	Inter-day RSD (%)	Intra-day RSD (%)
TCS	12.5	8.4
TCC	13.1	7.3

3.2.3.7. Method Validation

The method was applied to tap, bottled and sea water samples. 100.0 μgL^{-1} TCS and TCC were added and the optimum parameters were applied at pH 5.0. While bottle and tap water samples were 2 fold diluted, sea water sample was 10 fold diluted because of their complex matrix. For bottled and tap water samples, Table 3.4 illustrates the determined values of both spiked and non-spiked samples. TCS and TCC could not be detected in non-spiked samples. For the spiked bottled water sample, the concentrations of spiked analytes were TCS 98.8 ± 1.2 μgL^{-1}, TCC 105 ± 3 μgL^{-1}. In respect of spiked tap water samples, TCS and TCC concentrations were found to be as 83.2 ± 0.84 μgL^{-1} and 112 ± 2 μgL^{-1}, respectively. In sea water no TCS was detected but the concentration of TCC was found to be as 73.3 ± 1.8 μgL^{-1}. For the spiked sea water sample, the concentrations of spiked analytes were TCS 86.3 ± 4.2 μgL^{-1}, TCC 161 ± 6.7 μgL^{-1}.

Table 3.4. Method validation with SPME fibers (Extraction conditions; extraction pH: 5.0, extraction time: 60 min, stirring speed: 400 rpm, solution volume: 15 mL, Desorption conditions; desorption time: 30 min, desorption volume: 150 µL, eluent: 90 % MeOH – 10 % H_2O (adjusted to pH 3.0 with acetic acid)

	Bottled water (μgL^{-1})	Spiked bottled water (μgL^{-1})	Tap water (μgL^{-1})	Spiked tap water (μgL^{-1})	Sea water (μgL^{-1})	Spiked sea water (μgL^{-1})
TCS	ND*	98.8 ± 1.2	ND	83.2 ± 0.84	ND	86.3 ± 4.2
TCC	ND	105 ± 3	ND	112 ± 2	73.3 ± 1.8	161 ± 6.7

***** Not detected

CHAPTER 4

CONCLUSION

The thesis study progressed in two separate analytical applications of home-made SPME fibers. The first application was speciation of inorganic (Se(IV) and Se(VI)) and organic forms (SeCys and SeMet) of selenium with agarose, CeO_2-agarose, ZrO_2-agarose and nZVI-agarose modified fibers. HPLC-ICPMS was used for the effective separation and detection of analytes.

The main results of this part can be summarized as follows:

Among all fibers prepared, nZVI-agarose fibers demonstrated the best extraction efficiency for all Se species. Homogeneity and coating thickness of the fibers were controlled using capillary tubing as a template and constant stirring of the solution containing agarose and nZVI particles during coating process. At the same the method allowed to prepare various functional groups onto silica fibers. Although iron-based materials are well known for the speciation of inorganic Se(IV) and Se(VI) in aqueous solutions, the extraction of organoselenium species, SeCys and SeMet nZVI coated SPME fibers was reported for the first time in literature. Extracted analytes were separated with anion exchange column and determined with ICP-MS. All selenium species are well separated in 7 minute by isocratic elution program, 1.4 mL/min as flow rate and 10.0 mM citrate buffer (at pH: 5.0) as mobile phase. The optimum extraction parameters after extensive studies have given as; extraction pH: 4.0, agitation speed: 700 rpm, extraction time: 60 min. The fibers have shown reproducible extraction (< 10% rsd), good mechanical strength and good solvent resistivity. The validity of the proposed method was confirmed with the analysis of standard reference materials (SRM 1643e, Natural Water – Trace Elements and TMDA-70 fortified lake water). A good correlation was found between the certified (11.97 μgL^{-1}) and determined (10.93 μgL^{-1}) values for SRM 1643e. In case of TMDA-70 determined value was 21.09 μgL^{-1} while the certified value was 25.8 μgL^{-1}. The heavy matrix of the lake water may cause this difference and some ions may be interfered with Se species. Additional the developed method revealed that it also applicable to real waters such as bottled and tap water.

Moreover this method did not a need a derivatization step to detect inorganic and organic forms of selenium at the same time.

The second application of modified SPME fibers was to separate triclosan and triclocarban and detect them with HPLC-DAD. As in the previous application, nZVI-agarose fiber demonstrated the best extraction efficiency for TCS and TCC. In literature these compounds are separated mostly with commercial polymer coated SPME fibers such as PDMS fibers, but there is no report in literature regarding their extraction with nZVI particles. Extracted analytes were separated with C30 reverse phase column and determined with DAD detector at 280 λ. Analytes are well separated in approximately 7 min. by isocratic elution program, 0.8 mL/min as flow rate, 30 °C as column temperature and use 90 % MeOH-10 % H_2O (adjusted to pH 3.0 with acetic acid) as mobile phase. The optimized parameters for extraction were as; extraction pH: 5.0 and 7.0, agitation speed: 400 rpm, extraction time 60 min. The developed method was applied real waters such as bottled, tap and sea water. Neither of the compounds was detected in bottled or tap water but the spiked samples have shown good results. In sea water, TCS was not detected again, but TCC was determined as 73.3 $\mu g L^{-1}$.

Finally, it can be concluded that right along with commercial fibers and polymer coated fibers in literature, nano zero valent iron coated fibers bring a new approach for the detection of organic compounds as it can be understood from both analytical applications in this study. Sensitivity and detection limits may be not better than the methods used until so far but still the successive extraction of all compounds studied proved that these fibers are worth to take into consideration for further studies in this area.

REFERENCES

Alaejos M.S., F. J. D. Romero, C. D. Romero 2000. Selenium and Cancer: Some Nutritional Aspects. *Nutrition* 16: 376 –383.

Amoako P. O., Uden P.C., Tyson J. F. 2009. Speciation of selenium dietary supplements; formation of S-(methylseleno)cysteine and other selenium compounds. *Analytica Chimica Acta.* 652: 315–323.

Arthur, C.L., J. Pawliszyn. 1990. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry* 62: 2145-2148.

Asiabi H., Y. Yamini, S. Seidi, M. Shamsayei, M. Safari, F. Rezaei 2016. On-line electrochemically controlled in-tube solid phase microextraction of inorganic selenium followed by hydride generation atomic absorption spectrometry. *Analytica Chimica Acta.*922: 37-47.

Augusto, F., E. Carasek, R.G.C. Silva, S.R. Rivellino, A.D. Batista, E. Martendal. 2010. New sorbents for extraction and microextraction techniques. *Journal of Chromatography A* 1217: 2533–2542.

Baranowska I. and I. Wojciechowska 2012. Development of SPE/HPLC-DAD to Determine Residues of Selected Disinfectant Agents in Surface Water *Pol.J. Environ. Stud*. Vol. 21, No. 2, 269-277.

Behera S. K., S. Oh, H. Park 2010. Sorption of triclosan onto activated carbon, kaolinite and montmorillonite: Effects of pH, ionic strength, and humic acid. *Journal of Hazardous Materials* 179: 684–691.

Brausch J. M., G. M. Rand 2011. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. *Chemosphere* 82: 1518–1532.

Budziak, D., E. Martendal, E. Carasek. 2007. Preparation and application of NiTi alloy coated with ZrO_2 as a new fiber for solid-phase microextraction. *Journal of Chromatography A* 1164: 18–24.

Bueno M., Pannier F. 2009. Quantitative analysis of volatile selenium metabolites in normal urine by headspace solid phase microextraction gas chromatography–inductively coupled plasma mass spectrometry. *Talanta* 78: 759–763.

Campillo N., Penalver R., Hernandez-Cordoba M., Perez-Sirvent C., Martınez-Sanchez M. J. 2007. Comparison of two derivatizing agents for the simultaneous determination of selenite and organoselenium species by gas chromatography and atomic emission detection after preconcentration using solid-phase microextraction. *Journal of Chromatography A*. 1165: 191–199.

Canosa P., I. Rodrıguez, E. Rubı, R. Cela 2007. Determination of Parabens and Triclosan in Indoor Dust Using Matrix Solid-Phase Dispersion and Gas Chromatography with Tandem Mass Spectrometry. *Anal. Chem*. 79: 1675-1681.

Cha J., A. M. Cupples 2009. Detection of the antimicrobials triclocarban and triclosan In agricultural soils following land application of municipal biosolids. *Water Research* 43: 2522–253.

Chen B., B. Hu., M. He, X. Mao, W .Zu 2012. Synthesis of mixed coating with multi-functional groups for in-tube hollow fiber solid phase microextraction–high performance liquid chromatography–inductively coupled plasma mass spectrometry speciation of arsenic in human urine. *Journal of Chromatography A* 1227: 19–28.

Cheng C., Y. Wang, W. Ding 2011. Determination of Triclosan in Aqueous Samples Using Solid-phase Extraction Followed by On-line Derivatization Gas Chromatography–Mass Spectrometry. *Analytical Sciences* VOL. 27 197.

Colangelo L. A., K. He, M. A. Whooley, M. L. Daviglus, S. Morris, K. Liu 2014. Selenium exposure and depressive symptoms: The Coronary Artery Risk Development in Young Adults Trace Element Study. *NeuroToxicology* 41: 167–174.

Daughton C. G., T. A. Ternes 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Persp*. 107: 907-938.

Delgado D. R., A. R. Holguin, F. Martínez 2012. Solution Thermodynamics of Triclosan and Triclocarban in Some Volatile Organic Solvents. *Vitae* 19(1)79-92.

Dietz C., Sanz Landaluze J., Ximénez-Embún P., Madrid-Albarrán Y., Cámara C. 2004. Volatile organo-selenium speciation in biological matter by solid phase microextraction–moderate temperature multicapillary gas chromatography with microwave induced plasma atomic emission spectrometry detection. *Analytica Chimica Acta* 501: 157–167.

Dietz, C., J. Sanz, C. Camara. 2006. Recent developments in solid-phase microextraction coatings and related techniques. *Journal of Chromatography A* 1103: 183–192.

Efecan, N., T. Shahwan, A.E. Eroğlu, I. Lieberwirth. 2009. Characterization of the uptake of aqueous Ni(2+) ions on nanoparticles of zero-valent iron (nZVI). Desalination 249: 1048–1054.

Farhadi, K., R. Tahmasebi, R. Maleki. 2009. Preparation and application of the titania sol–gel coated anodized aluminum fibers for headspace solid phase microextraction of aromatic hydrocarbons from water samples. *Talanta* 77: 1285–1289.

Fernández I. P., A. Najafi, V. Pino, J. L. Anderson, J. H. Ayala, A. M. Afonso 2016. Utilization of highly robust and selective crosslinked polymeric ionic liquid based sorbent coatings indirect-immersion solid-phase microextraction and high-performance liquid chromatography for determining polar organic pollutants in waters. *Talanta* 158: 125-133.

Food and Nutrition Board, Institute of Medicine. 2000. Dietary reference intakes from vitamin C, vitamin E, selenium and carcinoids. New York: National Academic Press, 284–324.

Ghasemi E., H. Farahani 2012. Head space solid phase microextraction based on nano-structured lead dioxide: Application to the speciation of volatile organoselenium in environmental and biological samples. *Journal of Chromatography A* 1258: 16–20.

Ghasemia E., Farahani B. 2012. Head space solid phase microextraction based on nano-structured lead dioxide: Application to the speciation of volatile organoselenium in environmental and biological samples. *Journal of Chromatography A* 1258: 16– 20.

Gionfriddo E., Naccarato A., Sindona G., Tagarelli A. 2012. A reliable solid phase microextraction-gas chromatography–triple quadrupole mass spectrometry method for the assay of selenomethionine and selenomethylselenocysteine in aqueous extracts: Difference between selenized and not-enriched selenium potatoes. *Analytica Chimica Acta.* 747: 58– 66.

Gomes da Silva E., Augusto F., Zezzi Arruda M. A. 2012. Determination of Se using a solid-phase micro-extraction device coupled to a graphite furnace and detection by gas chromatography-mass spectrometry. *Analyst* 137: 3841.

Guo J., X. Li, X. Cao, Y. Li , X. Wang, X. Xu 2009. Determination of triclosan, triclocarban and methyl-triclosan in aqueous samples by dispersive liquid–liquid microextraction combined with rapid liquid chromatography. *Journal of Chromatography A,* 1216 : 3038–3043.

Keshan Disease Research Group. 1979. Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. *Chi. Med*. 92: 477-482.

Korba K., L. Pelit, F. Okcu Pelit, K.V. Ozdokur, H. Ertas, A. E. Eroglu, F. N. Ertas. 2013. Preparation and characterization of sodium dodecyl sulfate doped polypyrrole solid phase micro extraction fiber and its application to endocrine disruptor pesticide analysis. *Journal of Chromatography B* 929: 90–96.

Krause R.J., A.A. Elfarra 2009. Reduction of l-methionine selenoxide to seleno-l-methionine by endogenous thiols, ascorbic acid, or methimazole*Biochemical Pharmacology* 77: 134–140.

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.

Mao X., B. Hu, M. He, B. Chen 2012. High polar organic–inorganic hybrid coating stir bar sorptive extraction combined with high performance liquid chromatography–inductively coupled plasma mass spectrometry for the speciation of seleno-amino acids and seleno-oligopeptides in biological samples. *Journal of Chromatography A.* 1256: 32– 39.

Mester, Z., R. Sturgeon, J. Pawliszyn. 2001. Solid phase microextraction as a tool for trace element speciation. *Spectrochimica Acta B* 56: 233-260.

Moreno F., García-Barrera T., Gómez-Ariza J. L. 2013. Simultaneous speciation and preconcentration of ultratrace concentrations of mercury and selenium species in environmental and biological samples by hollow fiber liquid phase microextraction prior to high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry. *Journal of Chromatography A* 1300: 43– 50.

Naccarato A., J. Pawliszyn 2016. Matrix compatible solid phase microextraction coating, a greener approach to sample preparation in vegetable matrices. *Food Chemistry* 206: 67-73.

Nyaba L., J. M. Matong, K. M. Dimpe, P. N. Nomngongo 2016. Speciation of inorganic selenium in environmental samples after suspended dispersive solid phase microextraction combined with inductively coupled plasma spectrometric determination, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2016.06.023

Orvos D.R., D.J. Versteeg , J. Inauen, M. Capdevielle , A. Rothenstein , V. Cunningham 2002. Aquatic toxicity of triclosan. *Environ Toxicol Chem*. 21(7):1338-49.

Pawliszyn J. 1999. Applications of Solid Phase Microextraction. *Royal Society of Chemistry.*

Pawliszyn J. 2000. Theory of Solid-Phase Microextraction. *Journal of Chromatographic Science* 38: 271-278.

Polatajko A., Jakubowski N., Szpunar J. 2006. State of the art report of selenium speciation in biological samples. *J. Anal. Atom Spectro*m. 21: 639-654.

Regiart M., J. L. Magallanes, D. Barrera, J. Villarroel-Rocha, K. Sapag, J. Raba, F. A. Bertolino 2016. An ordered mesoporous carbon modified electrochemical sensor for solid-phase microextraction and determination of triclosan in environmental samples. *Sensors and Actuators B* 232: 765–772.

Rita A., M. Silva , J.M.F. Nogueira 2008. New approach on trace analysis of triclosan in personal care products, biological and environmental matrices. *Talanta* 74: 1498–1504.

Seby F., Potin-Gautier M., Giffaut E., Borge G., Donard O.F.X. 2001. A critical review of thermodynamic data for selenium species at 25°C. *Chemical Geology*. 171: 173-194.

Shahdousti P., Alizadeh N. 2011. Headspace-solid phase microextraction of selenium(IV) from human blood and water samples using polypyrrole film and analysis with ion mobility spectrometry. *Analytica Chimica Acta* 684: 67–71.

Shen J. Y., M. S. Chang, S. Yang, G. J. Wu 2011. Simultaneous and Rapid Determination of Triclosan, Triclocarban and Their Four Related Transformation Products In Water Samples Using SPME-HPLC-DAD DOI: 10.1080/10826076.2011.631258

Shi K., Wang X., Guo Z., Wang S., Wu W. 2009. Se(IV) sorption on TiO_2: Sorption kinetics and surface complexation modeling. *Colloid and Surface: Physicochem. Eng. Aspects.* 349: 90-95.

Su Y., H. Chen, Y. Gao, X. Li, X. Hou, Y. Lv 2008. A novel HPLC-UV/nano-TiO_2-chemiluminescence system for the determination of selenocystine and selenomethionine. *Journal of Chromatography B* 870: 216–221.

Tyburska A., Jankowski K., Rodzik A. 2011. Determination of arsenic and selenium by hydride generation and headspace solid phase microextraction coupled with optical emission spectrometry. *Spectrochimica Acta Part B* 66: 517–521.

Venkatesan A. K., B. F. G. Pycke, L. B. Barber, K. E. Lee, R. U. Halden 2012. Occurrence of triclosan, triclocarban, and its lesser chlorinated congeners in Minnesota freshwater sediments collected near wastewater treatment plants. *Journal of Hazardous Materials* 229– 230: 29– 35.

Vonderheide A. P., Montes-Bayon M., Caruso J. A. 2002. Solid-phase microextraction as a sample preparation strategy for the analysis of seleno amino acids by gas chromatography-inductively coupled plasma mass spectrometry. *Analyst* 127: 49–53.

Wang J., S. Mannino, C. Camera, M.P. Chatrathi, M. Scampicchio, J. Zima 2005. Microchip capillary electrophoresis with amperometric detection for rapid separation and detection of seleno amino acids. *Chromatography A* 1091: 177–182.

Zhang N., N. Fu, Z. Fang, Y. Feng, L. Ke 2011. Simultaneous multi-channel hydride generation atomic fluorescence spectrometry determination of arsenic, bismuth, tellurium and selenium in tea leaves. *Food Chemistry* 124: 1185–1188.

Zhang Y., B. Chen, S. Wu, M. He, B. Hu 2016. Grapheneoxide – TiO_2 Composite solid phase extraction combined with graphite furnace atomic absorption spectrometry for the speciation of inorganic selenium in water samples. *Talanta* 154: 474–480.

Zhang, Z., M.J. Yang, J. Pawliszyn. 1994. Solid-phase microextraction. *Analytical Chemistry* 66 (17): 844-8563.

Zhao J., G. Ying, Y. Liu, F. Chen, J. Yang, L. Wang 2010. Occurrence and risks of triclosan and triclocarban in the Pearl River system, South China: From source to the receiving environment. *Journal of Hazardous Materials* 179: 215–222.

Zhao R., X. Wang, J. Sun, C. Hu, X. Wang 2011. Determination of triclosan and triclocarban in environmental water samples with ionic liquid/ionic liquid dispersive liquid-liquid microextraction prior to HPLC-ESI-MS/MS. *Microchim Acta* 174:145–151.

Zhu, Y., S. Zhang, Y. Tang, M. Guo, C. Jin, T. Qi. 2010. Electrochemical solid-phase nanoextraction of copper(II) on a magnesium oxinate-modified carbon paste electrode by cyclic voltammetry. *Journal of Solid State Electrochemistry* 14: 1609–1614.

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