Detection of Trace Amounts of Selenium Ions in Water and Blood Samples by Solid Phase Extraction Using Thiolated Nanosilica

Fereshteh Keyghobadi (MSc)
Department of Analytical Chemistry, Golestan University of Medical Sciences, Gorgan, Iran

Nader Bahramifar (PhD)
Department of Analytical Chemistry, Tarbiat Modares University, Noor, Iran

Elahe Gharekhani (MSc)
Department of Toxicology, Golestan University of Medical Sciences, Gorgan, Iran

Seyyedeh Marzieh Kia (MSc)
Department of Food Sciences, Golestan University of Medical Sciences, Gorgan, Iran

Corresponding author: Fereshteh keyghobadi
Email: fereshteh_keyghobadi@yahoo.com
Tel: +98-919668393
Address: Golestan University of Medical Sciences, Gorgan, Iran

Received: 31 Oct 2018
Revised: 05 Jan 2019
Accepted: 13 Jan 2019

This work is licensed under a Creative Commons Attribution 4.0 License.

ABSTRACT

Background and Objectives: In this study, nanosilica modified with HS-SiO$_2$ thiol groups was utilized as adsorbent for solid phase extraction, as a fast and reliable method of preconcentration and separation of very small quantities of selenium ions from water and blood samples.

Methods: The samples included four natural water samples and one biological sample (blood serum) prepared in volumes of 25, 100, 200, 300, 400 and 500 ml. The samples were analyzed by solid phase microextraction, using thiolated-nanosilica (as adsorbent), ultraviolet-visible spectrophotometry and atomic absorption spectroscopy.

Results: Optimized conditions for preconcentration of a 25 ml 0.2 mg/l selenium solution were pH 5, 40 mg of adsorbent, sample-adsorbent mixing time of 15 minutes and 5 ml of 2N sulfuric acid as detergent. The volume limit and concentration factor were 400 and 80, respectively. Limit of detection and relative standard deviation of the method were 0.46 μg/l and 0.9%, respectively.

Conclusion: This study is the first to successfully utilize thiolated nanosilica for measuring low selenium levels. Thiolation of the absorbent increases selenium adsorption by thiolated-silica compared to SiO$_2$.

Keywords: Solid phase extraction, Selenium, Preconcentration, Nano, UV-visible spectrophotometry.
INTRODUCTION

Selenium is a trace element essential for human health. Nowadays, selenium supplementation is approved as part of the public health policies in selenium-deficient regions (1, 2). Reduced selenium levels in the body weaken the immune system and results in the formation of toxic substances in the blood and vulnerability of humans to various diseases. The main sources of selenium in the environment are industrial resources for agricultural practices. The tendency of this element to bioaccumulate at base of the food chain, from sediment to marine plants and then to animals, has raised some concerns (3). The maximum safe dietary selenium intake is about 800 μg/day, while daily intake of 400 μg selenium prevents poisoning and regulates the amount of nutrients (4). Due to the importance of this element, various methods such as solid phase microextraction have been proposed for its measurement (5). The aforementioned method uses different adsorbents as a stationary phase. In recent years, scientists have focused on the use of nanomaterial-based adsorbents for preconcentration and separation of metal ions. These materials have compelling characteristics including large surface area to volume ratio and large number of active analyte adsorption sites. Thus, nanomaterials can selectively adsorb metal ions due to the presence of suitable surface functional groups. Similar to nanomaterials including SiO₂, Al₂O₃, TiO₂, ZrO₂ and CeO₂, metal oxides show a good level of surface activity. In addition, adsorbent preparation is very simple and inexpensive compared to other commonly used solid metals. Functionalization of silica nanoparticles with various suitable reagents can notably improve the adsorbent-analyte interactions by increasing both sensitivity and selectivity. Mofidi et al. used thiolated mesoporous mcm-41 as a new adsorbent for preconcentration of mercury using solid phase extraction and spectrophotometric determination. In this study, we used thiolated nanosilica adsorbents as stationary phase in solid phase extraction, for separation and preconcentration of low selenium levels from biological and water samples. We aimed to increase selenium adsorption capacity from these samples by functionalizing carbon nanotubes with thiol groups. The most important reasons for the use of thiolated nanosilica adsorbents are large surface area, selectivity, high adsorption capacity, stability and reusability.

MATERIAL AND METHODS

We used 3-mercaptopropyl trimethoxysilane to prepare nanosilica. To remove organic impurities, 1g of SiO₂ nanoparticles was added to 40 ml of toluene, and the solution was refluxed for 24 hours at 80-90 °C. After centrifugation at room temperature, supernatant was discarded and 40 ml of toluene was added two times to the sediment. The mixture was refluxed at 80-90 °C for 24 hours and then filtered through Whatman filter papers (grade 42). The solid matter was dried at room temperature after washing with 20 ml of toluene several times. One g of prepared nanosilica powder was added to 40 ml of toluene and 2 g of reagent (3-[Methoxy(dimethyl)silyl]-1-propanethiol). The solution was refluxed for 24 hours at 80-90 °C. After reaching room temperature, the solution was filtered using Whatman filter papers (grade 42). After washing with 20 ml of toluene and drying at room temperature, the solid matter was used as the solid phase in the solid phase extraction method. Functional groups created at the surface of carbon nanotubes were identified using KBr tablets and Fourier transform infrared spectroscopy (Shimadzu FT-IR 8400, Japan) (Figure 1). The vario EL elemental analyzer (Germany) was used for quantitative analysis of the elements in the crude and thiolated carbon nanotubes. Experiments were performed in a discontinuous system with thiolated nanosilica adsorbents. The amount of adsorbent, contact time, impact of acid detergent concentration and pH were investigated. The effect of these parameters was studied by keeping all parameters constant except for one. To measure selenium in natural water samples, various parameters affecting the extraction efficiency was optimized using different prepared solutions of selenium. Parameters including pH, amount of adsorbent, time required for mixing of adsorbent and specimen, concentration and volume of sulfuric acid, the limit volume, limit of detection (LOD) and precision of the method were investigated to determine optimal conditions for evaluating the efficiency of the proposed pre-concentration method. Selenium
Selenium was measured in serum samples based on a specific method and using a furnace atomic absorption spectrophotometer. Effect of different pH levels (3-7) was investigated. The contact time (5, 10 and 15 minutes) and time of adsorbent mixing (20 minutes) were determined. The amount of adsorbent (10, 20, 30, 40 and 50 mg) was determined. The effect of different concentrations (0.2, 0.5, 1, 1.5, 2 and 3 N) of detergent was also investigated. Adsorption of selenium ion was carried out using thiolated nanosilica. The concentration of aqueous solutions was measured using a Shimadzu UV-160PC spectrophotometer. Selenium standard stock solution (1000 mg/ml) was used to prepare standard solutions containing selenium ion (0.2, 0.4, 0.6, 0.8, 1 and 1.2 mg/ml).

Under optimal conditions, the solid phase extraction process was performed for preconcentration of the standard solutions. Finally, absorption of each solution was determined by a UV-spectrophotometer (Figure 1).

**Figure 1** - Calibration curve for spectrophotometric measurement of selenium

![Figure 1 - Calibration curve for spectrophotometric measurement of selenium](image1)

**Figure 2** - IR spectrum of the nanosilica (Si) and thiolated nanosilica (HS-Si) absorbent

![Figure 2 - IR spectrum of the nanosilica (Si) and thiolated nanosilica (HS-Si) absorbent](image2)
RESULTS

The structural properties of thiolated carbon nanotubes

Figure 2 shows the results of FTIR analysis for the crude absorbent and the thiolated nanosilica. As shown in Figure 2, a C-S stretching band at 1686 cm\(^{-1}\) is visible for the spectrum of thiolated adsorbent, which was absent in the Si spectrum. The sharp absorption peaks at 1093 cm\(^{-1}\) and 810 cm\(^{-1}\) were related to the symmetric and asymmetric Si-O-Si stretching vibrations, respectively. In addition, the Si-O-Si bending vibrations at 460 cm\(^{-1}\) is visible in both spectra (7). There was a visible but weak peak related to the SH stretching vibrations in the 2569 cm\(^{-1}\) region of the SH-Si spectrum, which indicates the bonding of the thiol group to the nanosilica. The bands at 2854 cm\(^{-1}\) and 2923 cm\(^{-1}\) were related to symmetric and asymmetric C-H stretching, respectively, which are increased in the SH-Si spectrum (7). Moreover, the broad band observed at 3440 cm\(^{-1}\) was related to surface silanol groups and water molecules, which are reduced in the SH-Si because of the replacement of SH group with OH (8).

Effect of the solution’s pH on selenium adsorption by thiolated nanosilica

One of the factors affecting the solid phase extraction is pH, which affects the quantitative inhibition of analytes on the stationary phase and the chemical properties of the adsorbent and metal ions, highlighting the utmost importance of optimization. Table 1 shows the results obtained for the effect of pH. Effect of adsorbent level on the pattern of selenium adsorption by thiolated nanosilica

The amount of adsorbent is another important factor affecting the inhibition of metal cations and the pre-concentration process. The optimal amount of adsorbent was determined to be 40 mg, which produced higher adsorption capacity and metal ion removal rate. Table 1 shows the effect of adsorbent level on selenium recovery.

Effect of contact time of analyte with solid phase on the adsorption of selenium by thiolated nanosilica

Contact time is an important factor for the proper use of an adsorbent in applied fields and indicates the rate of adsorption. The results showed that selenium is quickly adsorbed, so that 90% of adsorption equilibrium was achieved within 20 minutes. The favorable recovery of analytes depends on the duration of contact with the solid phase and requires a brief ion pre-concentration period (10). Table 1 shows the effect of contact time of analyte with solid phase on the adsorption of selenium.

Effect of type and amount of detergent on selenium adsorption by thiolated nanosilica

As shown in Table 1, the selenium recovery percentage was highest when using H\(_2\)SO\(_4\) as detergent.

Table 1: Effect of the solution’s parameters on selenium adsorption by thiolated nanosilica

<table>
<thead>
<tr>
<th>Optimization factor</th>
<th>Amount</th>
<th>Recovery percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>51</td>
</tr>
<tr>
<td>HNO(_3)</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>HCl</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>H(_3)PO(_4)</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Type of detergent</td>
<td>10</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>103</td>
</tr>
<tr>
<td>Amount of detergent (ppm)</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>Contact time of analyte with the solid phase (minutes)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>
**Effect of detergent concentration on adsorption of selenium by thiolated nanosilica**

The acidic solution was chosen as the optimal washing solvent. The results of selenium washing with 0.2, 0.5, 1, 2, and 3 normal sulfuric acid solutions are shown in Table 1. LOD is defined as the lowest concentration of analyte that can be measured at a specified level of confidence (11).

To determine the LOD, 10 blank samples (without selenium) were prepared and extracted based on the method explained previously, and the absorption of each solution was determined by UV-spectrophotometry.

<table>
<thead>
<tr>
<th>Concentration factor</th>
<th>Recovery percentage</th>
<th>RDS</th>
<th>LOD for the device (µg/l)</th>
<th>LOD (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>97%</td>
<td>0.9%</td>
<td>36.8</td>
<td>0.46</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Increasing pH from 3 to 5 enhanced the selenium ion removal rate, an effect that was reduced at pH 7. The maximum selenium ion removal rate was recorded at pH 5. Depending on the type of adsorbent, functional groups and surface charge, pH can have different effects on the pattern of adsorption. Moreover, the higher adsorption of selenium in pH 4-6 may be due to the electrostatic interaction between the negatively charged anions and the positively charged adsorbent surface. The decrease in adsorption in higher pH values could be due to the abundance of OH⁻ ions and generation of a repulsive force between the negatively charged surface and anionic molecules. In highly acidic pH values, the tendency for adsorption is reduced on the surface because selenium anions tend to interact with H⁺ instead of the surface (8).

The amount of adsorbent is another important factor affecting the inhibition of metal cations and the pre-concentration process. At a constant concentration of metal ions, increasing the amount of adsorbent increases the number of adsorption sites, while at a very low amount, the adsorbent’s surface becomes saturated with metal ions, resulting in the incomplete removal of metal ions from the solution (9). In this study, the complete adsorption time was 15 minutes, which can be attributed to the high level of nano-adsorbents used in the study. It is important to note that a suitable detergent should be able to wash the analyte thoroughly without damaging the adsorbent’s structure (11). The selenium recovery percentage was highest when using H₂SO₄ as detergent. In concentrations less than 2 normal, the recovery percentage was low due to presence of insufficient acidic protons for replacing the selenium adsorbed onto the adsorbent’s surface.

**CONCLUSION**

This study is the first to successfully utilize thiolated nanosilica for measuring low selenium levels. The solid phase extraction method was used to preconcentrate and determine low selenium quantities by thiolated SiO₂ nanosilica absorbent and UV-visible spectrophotometry. Optimized conditions for preconcentration of a 25 ml 0.2 mg/l selenium solution are pH of 5, 40 mg of adsorbent, sample-adsorbent mixing time of 15 minutes and 5 ml of 2N sulfuric acid as detergent. Thiolation of the adsorbent increases selenium adsorption by thiolated-silica compared to SiO₂.

**ACKNOWLEDGEMENTS**

This project has been partly funded by a joint research grant from Golestan University of Medical Science and Gorgan University of Agricultural Sciences and Natural Resources.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest regarding publication of this article.
REFERENCES