



Design of Methodologies for the Determination of Selenium and Mercury in Samples of Environmental Interest by Solid Phase Spectrophotometry

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ABSTRACT

Selenium is a micronutrient with biological, antioxidant, and catalytic properties. Mercury's potential for volatilization and methylation makes its environmental behaviour both interesting and concerning. Because organic compounds can bioaccumulate in the food chain, they are the most toxic. These analytes are extremely rare in nature. It is critical to have an analytical method that combines a species-preserving sample treatment with a system that allows for detection at low concentration levels. There are effective methods and techniques for detecting these species, but they require complex instrumentation and installations, making them impractical for use outside of the lab. Solid phase spectrophotometry is up-front and sensitive method for determining these elements. Overall, the goal of this research was to develop an analytical technique, such as solid phase spectrophotometry, that can measure selenium and mercury in environmental samples in a reliable and safe manner. The technique is to fix a coloured analyte-containing species to a solid support with specific properties. After the coloured complex has been fixed, spectral measurements are taken to quantify the analyte. Organic and inorganic analytes have been measured due to the versatility of these methods. The parameters for determining selenium in foliar samples and mercury in water samples are proposed and standardised using solid phase spectrophotometry. The results of this method are validated to ensure accuracy. The precision, linear range, detection limit, and quantification limit all demonstrate dependability. 1-100 mg/L linear range, the detection limit was 1.92 mg/L, while the quantification limit was 6.41 mg/L. The method's repeatability and reproducibility tests revealed a low degree of dispersion.

Keywords: *Selenium and Mercury, Quantitative analysis, colorimetry methods, method development, Solid Phase Spectrophotometry*

INTRODUCTION

Well-known analytical reactions are frequently optimised or modified in the search for new analytical methods that satisfy the requirements of speed, simplicity, and low cost. In general, chemical systems with adequate sensitivity and selectivity are desired. Because it improves the selectivity of analytical reagents and raises reaction sensitivity, solid phase UV-Vis spectrophotometry (EFS) (Saffaj, 2013) becomes crucial. Additionally crucial are its ease of use and suitability for continuous flow scheme (Kadis, 2011).

According to the large number of bibliographical references, mercury is one of the inorganic elements that has been studied the most in environmental toxicology (Clarkson, 2003). When proposing and designing monitoring systems (often in-situ) in geographically or politically remote regions, simple and reliable techniques for its quantification in various matrices are important. In this situation, semi-quantitative colorimetric techniques can be carried out manually by looking at coloured objects. They offer pertinent and timely information about the situation at the time of the tests despite having low sensitivity (Kompany-Zareh, 2002).

Selenium is a micromineral antioxidant that guards against cardiovascular disease and strengthens the immune system by delaying cellular ageing, which is associated with the prevention of cancer. Burning coal and other fossil fuels releases selenium into the atmosphere (Tinggi U. 2008). In combustion, SO₂, which binds to ash and airborne particles, quickly converts SeO₂ to elemental selenium. The biogeochemical cycle of selenium depends on the atmosphere. In water, soil, and sediments, fungi and bacteria methylate this, creating volatile species like dimethyl selenide: (CH₃)₂Se, which aids in its dispersion (Jenkins DJA, 2020). The solubility and availability of selenium in soil are influenced by chemical reactivity. In oxidising soils and at high pH, elemental selenium and selenides are oxidised to selenates. When the pH is low, selenite is strongly fixed in the soil and precipitates with iron. While some bacteria oxidise selenium to selenate or selenium trioxide, others reduce organic and mineral compounds to H₂Se (SeO₃). Rock weathering and soil leaching, which are influenced by biological and microbiological factors, cause selenium to appear

in water as selenate, selenite, and organometallic forms. (El-Ramady, 2015) The selenium content of groundwater and surface water, which ranges from 0.1 to 100 g/L, is influenced by physical-chemical factors. The concentration is typically lower than 1 g/L in drinking water. Legal potability thresholds range from 8 to 10 g/L, depending on the country. The peroxidation of fats is what gives selenium its carcinogenic effects on human skin, liver, chest, and colon. The element is a part of IARC's group 3. (the agent is not classifiable in relation to its carcinogenicity in humans)(Krystyna Pyrzyńska, 2002)

Atomic absorption⁵, plasma atomic emission spectroscopy⁶, electrochemical methods, etc. are just a few of the instrumental methods available for measuring Mercury (Hg) and Selenium (Se) at trace levels. However, installation and maintenance costs are high. Although UV-vis spectrophotometry in solution is inexpensive for determining Hg and Se, it requires steps to increase sensitivity, which raises the risk of contamination and lengthens measurement times.

It is suggested that trace levels of Hg (II) and selenium be measured using solid phase spectrophotometry (SPE). (Serra, 2010) This technique only needs a UV-vis spectrophotometer and is straightforward, sensitive, adaptable, and simple to use (Soruraddin, 2011).

A new technique for analysing organic and inorganic analytes is called EFS. Methods based on this technique are highly applicable to real-sample analysis and provide high sensitivity and selectivity, with simple and inexpensive instrumentation. For direct detection, the derivatized analyte is placed inside a cuvette with a 1 mm optical path, fixed to a solid support (typically a resin). With an increase in sample volume, EFS is more sensitive than Solution Molecular Absorption Spectrophotometry. (Gavrilenko, 2017) It is possible to achieve apparent molar absorptivity coefficients of the order of 10⁸ L/mol/cm with 1L sample volumes, which are frequently used in EFS. This implies that EFS has a sensitivity that is 3 to 4 times greater than Solution Molecular Absorption Spectrophotometry and can quantify concentrations as low as 0.1ng/mL. Due to this, elements in very low concentrations can be determined without preconcentrating the analyte.

As previously mentioned, expensive instrumental analytical techniques are required to identify selenium and mercury in environmental samples at low concentrations. This demonstrates the necessity of straightforward analytical techniques. For inexpensive, convenient environmental samples, solid phase spectrophotometry combines sensitivity, selectivity, speed, and reliability.

MATERIALS AND METHODS

Instrumental

Analytical measurements were performed with a single beam UV-Visible Spectrophotometer (200 – 1100 nm), brand METROLAB □ 1700, controlled from a PC, using the METROLAB □ SF170 program. For the readings of the different samples, quartz cuvettes with an optical path of 1 mm and a useful volume of 0.240 mL were used, with an adapter for a cuvette holder. For the pH measurements, a digital pH-meter, combined glass electrode, brand ALTRONIX □ TPX, was used. For the treatment of the samples, a CAVOUR □ VT-32165-DC brand centrifuge was used at 0 – 3500 rpm and a rotating mechanical stirrer at 0 – 200 rpm.

Reagents

The chemical reagents used were analytical grade, all prepared with high purity water.

The exchange resin used as solid support was Dowex 1X8 resin (anion exchanger), 200-400 mesh, to which a washing treatment was applied to remove impurities. Other solid supports such as amberlite-type resins (XAD-4, XAD-7 and XAD-16) and Sephadex C-50 cation exchange resin were tested.

Standard Hg (II) solutions of different concentrations were prepared from a 1000 ppm stock solution (Mercury Atomic Spectroscopy Std., Sigma-Aldrich).

A stock solution of 1,5-diphenylcarbazide (DFC) with a concentration of 5 mg/mL was prepared by dissolving the mass of reagent in acetone. The solution is transparent at the time of preparation, then it takes on a light-yellow color. It should be stored in amber-colored bottles, kept in a refrigerator and discarded when it begins to discolor.

The appropriate pH buffer solution used was a mixture of NaOH/KH₂PO₄.

The Triton X-100 nonionic surfactant solution (octyl phenol-polyester glycol ether) was prepared at 5% v/v from the commercial reagent.

Selenium Standard Solution

Solutions of different concentrations were prepared, at the time of use, from a standard solution of 1.0 g per liter (Selenium Atomic Spectroscopy Standard, Sigma-Aldrich) as SeO₂(s) in nitric acid. Working solutions were obtained by dilution with HNO₃ solution until 6.3% HNO₃(aq) was obtained.

Potassium Iodide Solution 1 M

16.60 g of KI(s) were weighed out and dissolved in distilled water to a final volume of 100 mL.

Diphenylcarbazide Solution (Chromogenic Reagent)

A stock solution of 1,5-diphenylcarbazide (DFC) with a concentration of 5 mg/mL was prepared by dissolving the mass of reagent in acetone. The solution is transparent at the time of preparation, then it takes on a light yellow color. It should be stored in amber-colored bottles, kept in a refrigerator and discarded when it begins to discolor. DFC is an organic compound, whose chemical formula is ((C₆H₅)NHNH)₂CO and has a molar mass of 242.28 g/mol. It is hardly soluble in water, therefore its solutions are prepared by dissolving the compound in organic solvents. 1,5-diphenylcarbazide and diphenylcarbazone react with inorganic mercury compounds, producing violet-colored end products. The specificity of this reaction depends on the pH value in which it develops: at slightly acid or neutral pH these reagents also react with copper, cobalt and iron. (Amin, Alaa. 2014). Working solutions were prepared daily by diluting the stock solution.

Dowex 1X8 Anion Resin

For the development of the Se and Hg (II) sensors, the Fluka brand Dowex 1X8 200/400 mesh anionic resin in chlorinated form with a density of 0.71 g cm⁻³ was used. This is a strong anionic type resin with quaternary ammonium exchange groups (NH₄⁺). The resin was purified and regenerated prior to use.

To do this, a mass of resin to be purified was taken and suspended in an equivalent volume of water. The system was shaken and allowed to stand for 24 hours. It was then washed repeatedly, separating the supernatant by centrifugation. This cycle was repeated at least 10 times. Subsequently, the solid phase was suspended for 2 hours in a 4 M HCl(aq) solution to regenerate its chlorinated form. Finally, it was washed repeatedly with distilled water until reaching a neutral pH value. In all steps, high purity water, free of ions, was used. Finally, it was placed in an oven to remove moisture (110°C).

Resin functionalization

To 5 g of purified resin, 100 mL of diphenylcarbazide solution in acetone (at different concentrations of ligand reagent) was added, and the system was allowed to stand for at least 24 hours. In a later step, the solid support was separated by centrifugation and dried in an oven at 40°C.

Subsequently, the proportion of reagent (DFC) fixed on the resin was studied. 80 mg of functionalized resin was reacted with 50 mL of an aqueous solution containing 10 µg of Hg (II), in the presence of a surfactant (Triton X-100) at a suitable pH, to obtain the colored complex on the resin. Then, the absorbance was measured directly on the solid support, using a quartz cuvette with an optical path of 1 mm, at 540 nm. High absorbance values indicate greater binding reagent fixation on the resin. On the other hand, color measurement tests were carried out by visual colorimetry ("naked-eye method"), the results of which are presented later.

pH Measurements and Adjustments

An Orion model 701-A digital pH meter equipped with a combined glass electrode, with internal Ag-AgCl reference, ALTRONIX® TPX brand, was used.

Sample Treatment

A CAVOUR® VT-32165-DC 0 – 3500 rpm centrifuge, a rotating mechanical stirrer 0 – 200

rpm and an analytical balance capable of discriminating ± 0.1 mg as a minimum were used.

RESULTS AND DISCUSSION

Determination of Mercury by Solid Phase Spectrophotometry

Resin Selection

Several qualitative fixation tests of the compound were carried out with adsorption resins (Silica Gel, Amberlite XAD 4, XAD 7 and XAD 16) and ionic resins (Dowex 1X8 and Sephadex C-50). They were carried out on a touch plate, simultaneously performing a blank test for each one. Dowex 1X8 anion exchange resin was chosen, given its ability to fix the ligand in the appropriate medium for its reaction with the analyte. With the rest of the solid supports, the fixation was not complete or they presented a high opacity to the passage of light at the working wavelengths.

Influence of reagent concentration

Different concentrations of ligand were tested on the chosen support. All resin studies were performed directly on the solid phase by solid phase spectrophotometry (SPE) (Yoshimura and Waki, 1985), using quartz cells with 1 mm path length. The optimal diphenylcarbazide solution concentration was 0.5 mg/mL. Higher concentrations of ligand made its spectral reading impossible due to the strong intensity of the color of the complex formed, while lower concentrations affected the formation of the complex. On the other hand, it was necessary to add a non-ionic surfactant (Triton X-100) to the solution (0.1% v/v final concentration), to improve contact between phases.

pH dependence

One of the main factors governing the formation of the Hg-DFC complex is the regulation of pH in the samples. Hence the importance of studying this parameter. The tests were carried out on the functionalized resin which was reacted with a dilute solution of mercury and small amounts of surfactant. The different pH values were adjusted with HCl and NaOH solutions, leaving the amount of resin and Hg (II) concentration

constant for each of the tests. Of the tests carried out, the maximum absorbance was recorded at pH close to 7, where the color developed by the complex is violet. Above or below said pH, the

development of the color of the complex is very weak or non-existent, so it can be inferred that the desired reaction does not occur. The results are shown in Figure 1.

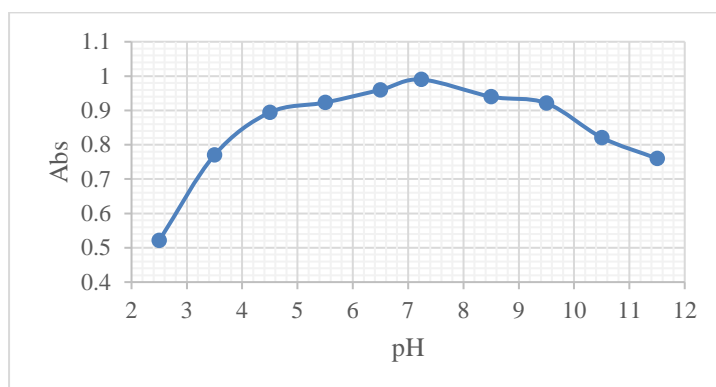


FIGURE 1: Determination of the optimal pH

Influence of the amount of resin

In plastic centrifuge tubes with screw caps, a standard solution was placed, containing at least 50 ppb of Hg(II), then the following reagents were added: 0.5 mL of Triton X-100 5% v/v and 1 mL of buffer, bringing to a final volume of 50 mL. Increasing amounts of R-DFC were then added with stirring for 20 minutes. The resin was packed in the cuvettes, measuring the absorbance

of both the samples and their respective blanks. The graph shows the values obtained that can be seen in Figure 2. From the graph it can be seen that with 80 mg of resin the maximum absorbance is produced. This mass contains a sufficient concentration of ligand to completely bind the analyte present in solution and at the same time cover the optical path of the light beam.

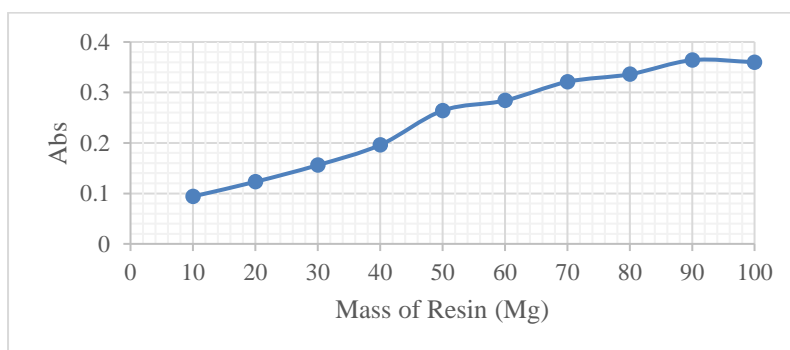


FIGURE 2: Influence of the Resin Mass

Influence of Stirring Time

To determine the minimum time from which the maximum signal is reached, the determination of Hg(II) was repeated in samples of the same concentration but subjected to different agitation times. For this study, the necessary volume of standard Hg(II) solution to reach 50 ppb Hg(II) was placed in plastic centrifuge tubes with a screw cap, and 0.5 mL of Hg(II) was then added to each of the tubes. Triton X-100 5% v/v and 1 mL of buffer solution and brought to a final

volume of 50 mL with bidistilled water. Finally, 80 mg of R-DFC were added, and stirred according to the different times tested, finally the absorbance was measured against the respective blank, once the resin was packed. The results obtained are similar to those corresponding to the determination of Se(IV) with RB, so it can be stated that for the complex of Hg(II) to be correctly formed with DFC fixed to the Dowex 1X8 resin, it is necessary to stir for at least 20 min on a rotary shaker.

Interference

The most important interfering analytes are ions such as Cr(VI), Fe(III), and Pb(II). Chromium practically does not react to form color with the reagent at the specified pH. If it does, the intensity is much lower than for mercury, so the interference it can cause is negligible. Iron and lead can react with the functionalized resin, but if the absorbance is measured at the appropriate wavelength (540 nm), the relative error introduced is less than $\pm 5\%$, so the effect on the measurement is considered negligible.

Possible applications of the functionalized resin

Hg (II) Solid Phase Spectrophotometry: The functionalized resin was reacted with solutions with different concentrations of mercury, maintaining the appropriate pH for complex formation with a buffer prepared from NaOH and KH₂PO₄. With a reaction time of 20 minutes (development), the spectral scan was performed, detecting the maximum absorbance at a wavelength of 540 nm, following the methodology used for the measurement of absorbance in solid phase (Pellerano et al., 2007). A calibration curve was made with different standards at the selected wavelength. It is shown in Figure 3.

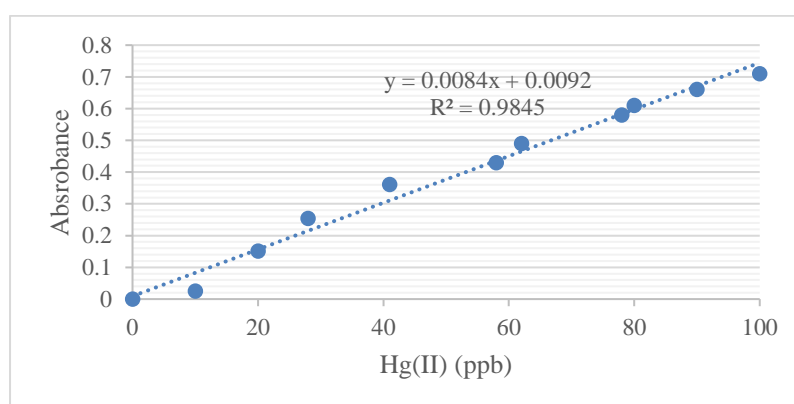


FIGURE 3: Calibration curve

Visual (semi-quantitative) colorimetry of Hg (II): As can be seen in Figure 4, the intensity of the color of the functionalized resin depends on the concentration of Hg (II) in the sample solutions (adsorbed complex concentration). This becomes important when performing a rapid semi-

quantitative analysis or simply when the necessary inputs for spectrophotometric determination are not available. In these cases, "naked eyes" techniques can be used, which have become relevant in recent times (Wu et al., 2008), thus providing a method low cost simple and fast detection.

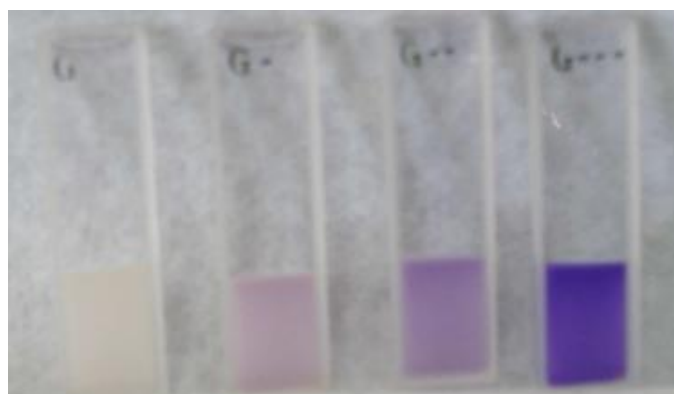


FIGURE 4: Adsorbed Hg (II)-DFC complex. From left to right: reagent blank, Hg concentration of 30, 60 and 100 ppb, respectively.

Determination of Selenium by Solid Phase Spectrophotometry

Se (IV) oxidises iodide anion in a strongly acidic medium, forming the triiodide complex in aqueous solution, which later forms a coloured, less soluble ionic complex with Rhodamine B, which binds to the resin and is measured spectrophotometrically at 560nm. Rhodamine B was chosen to determine Se (IV) because of its high sensitivity, ease of access, and stable solutions and complexes. Rhodamine B and its complex are poorly water-soluble, so a surface-active agent is added. Triton X-100 at 0.05% v/v produced the best results. Best acidity was between 1.60 and 2.10 pH. Acids were tested to reach the desired pH. 1mL 6 mol/L hydrochloric acid gave the best results.

The reagent addition order should keep the following sequence

In solid phase spectrophotometry, the first objective that arises is the choice of the appropriate support on which the compound under study is fixed. After testing different solid supports, the Dowex 1X8 anionic support was

chosen, since fast and complete fixations of the previously formed ion pair were achieved. The concentration of Rhodamine B, volume of Triton X-100 and potassium iodide solution were simultaneously optimized by applying the Box-Behnken method. The optimal values calculated are the following:

[RB] = 2.08×10^{-3} mol /L, volume of Triton X-100 at 5% v/v = 0.47 mL and volume of KI 1mol /L . = 2.18mL.

The amount of resin that provides maximum absorbance in this sensor is 0.0700 g, being an amount easily transferable with a dropper. It was established that a stirring time of 20 minutes is sufficient to reach the maximum absorbance. The intensity of the coloration of the complexes fixed to the resin does not show changes for at least 2 hours. Then the calibration curve is made. The graph corresponding to the calibration curve was linear, with a correlation coefficient of 0.9921 and a sensitivity of 0.0068 UAbs (Figure 5). Linearity was maintained from values close to the detection limit and up to at least 100 µg /L. the detection limit value obtained for a 50 mL sample of a Se (IV) solution under the previously specified conditions was 1.92 µg /L.

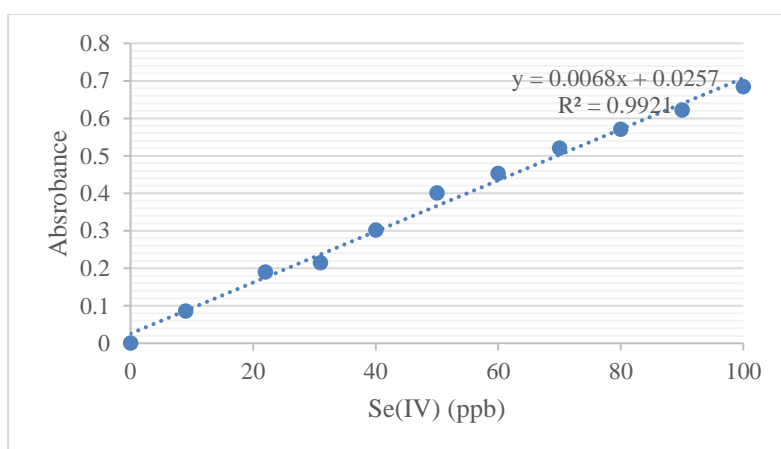


FIGURE 5: Calibration Curve

Parameters Affecting Complex Formation

Se (IV) oxidises iodide anion in a strongly acidic medium, forming the triiodide complex in aqueous solution, which forms a less soluble, coloured ionic complex with Rhodamine B. Triiodide-Rhodamine B was measured at 560 nm. In this case, a surface-active agent was needed to maintain a homogeneous chelate system and increase sensitivity.

pH influence

Since complex formation occurs in solution before solidification, the study was conducted in solution (resin). The ionic pair is produced in a strongly acid medium, according to previous research. 50 mL of sample containing 10 mg/L of Se (IV) was taken, 2 mL of KI(aq) 1 mol/L was

added, and it was left for 15 minutes before adding 0.5 mL Triton X-100 (5% v/v). Hydrochloric acid, phosphoric acid, sulfuric acid, and acetic acid were tested to determine the best acid for the reaction. 0.5 mL of 2.10×10^{-3} mol/L RB solution was added. Hydrochloric acid favours complex formation, giving the coordination

compound the highest absorbance and stability. Using different concentrations of hydrochloric acid, experimental data showed that the optimal pH for formation and fixation of complex species is 1.60 to 2.10 pH units (Figure 6). All subsequent studies were conducted at pH 1.70.

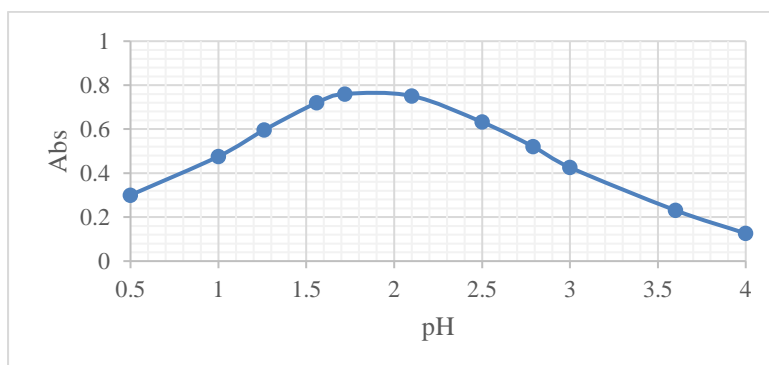


FIGURE 6: Determination of the Optimum pH

Dowex 1X8 was selected for its high stability in strongly acidic media, long shelf life and almost colorless.

Study of the Influence of Chemical Variables

Once the pH, composition, and concentration of the buffer and the order of addition of the reagents have been chosen, three variables that affect Se quantification should be considered. RB reagent concentration, Triton X-100 nonionic surfactant volume, and potassium iodide reagent volume (needed to form $[RB] + [I_3^-]$): An initial mapping or screening study is performed to determine how these three variables affect maximum absorbance. All experiments were done in triplicate, and the absorbances used are the averages. With the data, an Analysis of Variance is performed for each variable to determine its influence on response variance. Said ANOVA-type analysis uses a randomised factorial design for all three variables. The model's F-value of 92.51 indicates significance. All factors and interactions whose ANOVA P value is less than or equal to 0.05 are considered significant when a 95% confidence interval is applied. RB concentration, KI volume, and their interactions are statistically significant. Lack of fit with a F value of 2.87 is not significant in

relation to error (it could occur due to noise). The predicted R² of 0.9200 matches the adjusted R² of 0.9833. This model can be used to optimise, given the above.

Influence of the amount of resin

In any heterogeneous system, a decrease in the volume or mass of the phases results in an increase in the solute concentration at equilibrium in that phase. This experiment was carried out to determine the influence that the amount of resin used exerts on the absorbance value and to determine, at the same time, the minimum amount of resin that, without greatly increasing the stirring time, produces a maximum absorbance. In plastic screw cap centrifuge tubes, 50 µg of Se (IV) and 1 mL HCl(aq) 6 mol/L were placed. After 15 minutes, 2 mL of KI(aq) 1 mol/L, 0.5 mL Triton X-100 (5% v/v) and 0.5 mL of RB solution 2×10^{-3} mol/L were added. It was brought to a final volume of 50 mL in a volumetric flask and then increasing amounts of resin were added, stirring for 20 minutes. The resin was packed in the cuvettes, measuring the absorbance of both the samples and that of their respective blanks (Figure 7).

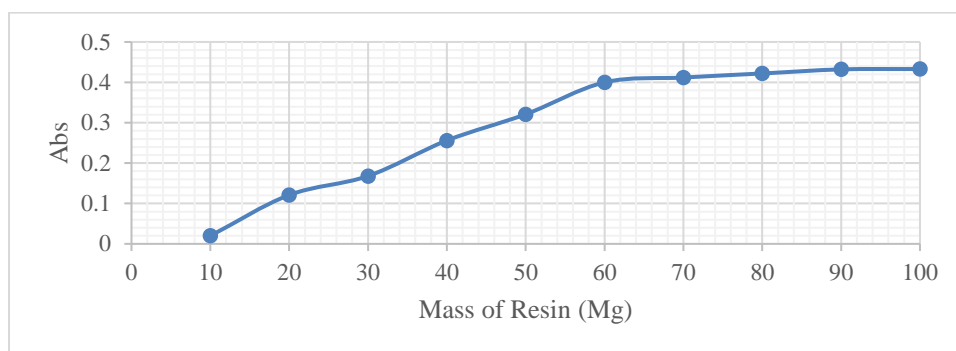


FIGURE 7: Influence of the Resin Mass

It was observed that with 70 mg of resin the maximum absorbance is obtained. This mass is enough to cover the optical path of the light beam in the readings, being an amount easily transferable to the work cuvette when packing the resin.

Influence of Stirring Time

To determine the minimum time from which the maximum signal is reached, the determination of Se (IV) was repeated in samples of the same concentration but subjected to different agitation

times. For this study, 50 μg of Se (IV), 1 mL of HCl(aq) 6 mol /L, 2 mL of KI(aq) 1 mol /L, and 50 μg of Se (IV) were placed in plastic screw cap tubes, and left let the system rest for 15 minutes. Then 0.5 mL Triton X-100 (5% v/v) and 0.5 mL of RB solution 2×10^{-3} mol /L were added. It was brought to a final volume of 50 mL in a volumetric flask and then 70 mg of resin were added. The tubes were shaken according to the different times tested, finally the absorbance was measured against the respective blank, once the resin was packed. The values obtained are represented graphically in Figure 8.

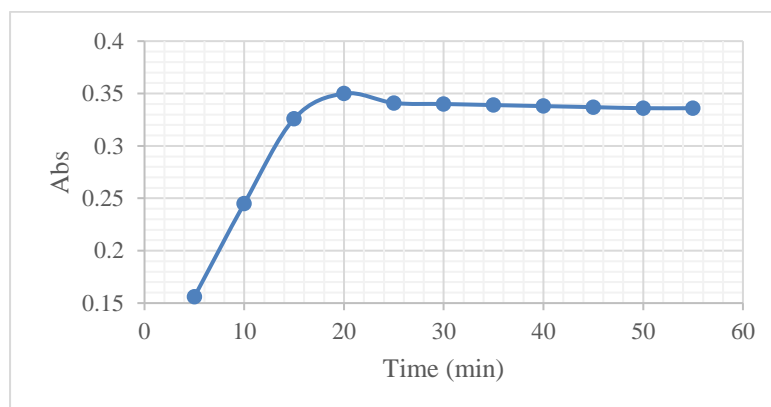


FIGURE 8: Influence of the Stirring Time

From the graph it is deduced that a stirring time of 20 minutes is sufficient to reach the maximum absorbance. This time will be used successively for the other experiences.

Complex Stability

To determine the stability of the complex once formed and fixed on the resin, 50 mL of solution containing 50 μg of Se (IV) were taken in a

plastic centrifuge tube and added in the following order: 1 mL HCl(aq) 6 mol /L, 2 mL of KI(aq) 1 mol /L. After 15 minutes, 0.5 mL Triton X-100 (5% v/v) and 0.5 mL of RB solution 2×10^{-3} mol /L. Next, 70 mg of resin were added, stirred for 20 minutes, then the resin was packed in the cell and the net absorbance was measured at different times, against the blank, at the selected wavelengths (Figure 9.).

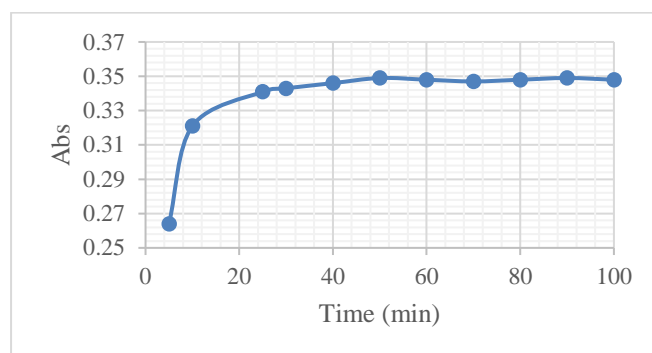


FIGURE 9: Complex Stability

According to the results obtained, it can be affirmed that the compound fixed in the solid phase is stable (at least for 2 hours), sufficient time to carry out all the operations corresponding to the EFS.

Summary of the Conditions Selected for the Determination of Se (IV)

The optimal conditions for the determination of Se (IV) by EFS are described below (Table 1.)

TABLE 1. Conditions for the Determination of Se (IV) by EFS

| | |
|-----------------------------|-----------|
| λ (nm) | 560 |
| Sample Volume (mL) | 50 |
| Solid Support | Dowex 1X8 |
| Resin mass (mg) | 70 |
| pH range | 1.6 – 2.1 |
| Selected Acid | HCl |
| Stirring Time (minutes) | 20 |
| Volume of Triton X-100 (mL) | 0.5 |

Validation and Application of the Method for Selenium Determination

Valeriana officinalis, Tilia x moltkei, and Melissa officinalis were the three foliar samples from which the method was used. Wash samples with tap water then deionized water. Dry small plant samples at 65°C. Before weighing 1.0 g of ground leaf, add 65% HNO₃, 70% HClO₄, and 98% H₂SO₄ (l). Leave overnight at room temperature. Once the solution is 3 mL, heat it for 3 hours at 220°C. Heat the digested sample at 120 °C for 20 minutes while adding 12% HCl(aq) (V). Cool, filter, and dilute with double-distilled water to 100 mL. The filtrate is aliquoted into 50 mL plastic tubes with screw-on caps and 1 mL HCl (aq) Each tube contains 1 mol/L and 6 mol/L KI(aq). After 15 minutes, 0.5 mL of RB (210-3 mol/L) and 0.5 mL of Triton X-100 (5% v/v) are added to each tube. Using double-distilled water, add 70 mg of resin to make 50 mL. Shake mixtures for 20 minutes, centrifuge at 2500 rpm for 1 minute, and separate supernatant with a dropper. A UV-Vis spectrophotometer at 560 nm

reads coloured resin packed in 1 mm quartz cuvettes. The suggested method is specific, selective, and linear in the concentration range. Low dispersion in repeatability and reproducibility tests shows the method's accuracy. During interference testing, it was determined that the method's selectivity for Se (IV) is adequate for its intended use. Strong correlation between both determinations showed the method's validity. The ICP-OES was compared.

Determination of Mercury by Solid Phase Spectrophotometry

In the presence of a non-ionic surfactant (Triton X-100), Hg(II) from its aqueous solutions reacts with a functionalized resin containing diphenylcarbazide to form a violet-blue complex. This complex develops fully after 20 minutes of promoting the reaction in a weakly acidic or neutral medium, and it is measured spectrophotometrically at 540 nm.

Diphenylcarbazide was chosen as the reagent for the measurement of Hg(II), taking into account the high degree of sensitivity attained, its accessibility, and the fact that both its solutions and the complexes it forms have a high degree of stability. The range of pH values close to 7 was found to have the best acidity conditions. The ability of various buffer solutions to achieve the specified pH value was tested. The use of 1 mL of KHPO₄/KH₂PO₄ buffer produced the best results. Similar to the analysis of the selenium element, Dowex 1X8 anionic resin was selected as the support after testing various solid supports due to the complex's quick and thorough fixation. It became necessary to change the way solid phase spectrophotometry is carried out. It was decided to functionalize the resin by fixing the reagent to it in order to put it in contact with solutions containing the analyte in a later stage. The steps below were taken in order to functionalize the resin: After conducting studies to determine the ideal concentration of diphenylcarbazide to be used (0.5 mg/mL), 5 g of Dowex 1X8 resin is taken, and 100 mL of diphenylcarbazide solution in acetone is added. The mixture is then allowed to stand for 24 hours to allow the complexing agent to fix to the resin. The solid phase is separated by centrifugation and repeatedly cleaned with deionized water. Finally, it is allowed to dry in a 40°C oven before being kept at room temperature and without light in polyethylene bottles. It was necessary to add a surfactant solution to enhance the contact of the problem solutions with the functionalized resin. Triton X-100 was the surfactant of choice, and its optimal concentration was around 0.05% v/v. This sensor requires 0.0800 g of resin to be packed, which is a quantity that can be transferred with ease using an eyedropper. It was determined that 20 minutes of stirring was sufficient to achieve the highest absorbance. For at least two hours, the complexes fixed to the resin's coloration intensity does not change. For the Hg(II) determination, the following sequence of reagent additions must be maintained: The calibration curve is created next. The calibration curve's output was a linear graph with a correlation coefficient of 0.9845 and a sensitivity of 0.0084 UAbs. Up to at least 100 g/L and from values near the detection limit, linearity was preserved. Under the predetermined conditions, a

50 mL sample of a Se(IV) solution yielded a detection limit value of 3.93 g/L. The suggested method exhibits good linearity across the range of concentrations examined and is selective and specific. The tests for repeatability and reproducibility yielded results with low dispersion, demonstrating the method's accuracy. When potential interferences were studied, it was possible to see that the method's selectivity for Hg(II) was suitable for the application. Finally, the proposed method was compared to the CV-AAS technique to assess its dependability; the results showed a strong correlation between the two determinations.

CONCLUSIONS

The quantification of selenium in plant samples and mercury in water using conventional spectrophotometry (UV Visible) in the solid phase modality is possible in this research work, demonstrating that it is adequate and reliable for low concentrations and extremely helpful for low complexity laboratories. The simplicity and low cost of the equipment and reagents needed (along with the adequate sensitivity) are two of the proposed methodology's main advantages because they make it simple to use for the distribution and control of vestiges of environmental importance. It was made possible to functionalize the Dowex 1X8 ion exchange resin with the ligand diphenylcarbazide, increasing the specificity of the method for detecting Hg (II) in aqueous solutions. On the other hand, some real-world uses for the quantification of Hg (II) are suggested, utilising sensitive and trustworthy methods that also stand out for their adaptability and ease of use in medium- or low-complexity laboratories (Solid Phase Spectrophotometry and Visual Colorimetry). The functionalized resin can also be used in samples with a complex matrix or with very low analyte concentrations for pre-concentration of Hg (II) prior to analysis with particular instrumental techniques (atomic absorption/emission). The reagent concentration of 0.5 mg.m/L, the contact time of at least 24 hours between the resin and the reagent, and the drying temperature of 40°C for the functionalized resin are the ideal conditions for the functionalization of the resin.

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