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A Chemical Modifier for the Determination of Selenium by Graphite Furnace Atomic Absorption Spectrometry and Its Application to Human Hair and Nails

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ABSTRACT

A method for the determination of total selenium in human hair and nails using graphite furnace atomic absorption spectrometry (GF-AAS) with palladium/ascorbic acid as a chemical modifier is used. The effects of nickel nitrate, palladium/ ascorbic acid, and palladium/ magnesium nitrate as chemical modifiers on the sensitivity for the determination of selenite, selenate and selenomethionine by GF-AAS are compared. The palladium/ ascorbic acid modifier is used for the determination of total selenium in human hair and nails, because the oxidation states of selenium ion are not effective in the analysis. The detection limit of (GF-AAS) is estimated to be 0.8 µg L^{-1} (calculated as 3s of the blank), and the calibration curve is linear for the concentration rang 5-50 µg L^{-1} and the recovery range is from 93.33 to 106 %. The optimal ashing and atomizing temperatures are 1300^{0}C and 2250^{0}C, respectively. The proposed method could be successfully applied to the determination of total selenium in human hair and nails.

Keywords: GF-AAS, Chemical Modifier, Selenium, Human hair* and *Nails.

INTRODUCTION

Selenium is an essential trace element for human organs; however at elevated levels it can be toxic to organisms. Selenium occurs naturally in soils, rocks, and water. Selenium in small amounts is essential for normal growth and health of animals, including humans [(1)]. The use of selenium in human clinical trials is limited, but the outcome indicates that selenium is among the most promising agents to prevent cancer [(2-8)]. The concentrations of selenium in soil vary widely by geographical location, ranging from < 0.1 mg Kg^{-1} to 1.6 mg Kg^{-1}. Selenium stable isotope tracers is now preferred for the bioavailability study of selenium-derived species used in the clinical trials. Because of the low concentration of selenium in many biological samples (< 0.1 mg/Kg), it is essential to establish rapid, precise, and accurate method for the determination of selenium isotope ratios in biological matrices [(9-13)]. Selenium as a nonmetallic chemical element, has received high attention of biologists because of its dual role as an essential trace nutrient and as a toxic element.

For the intake of daily food and drinking water, Se or its salts accumulates in kidney [(12)], liver [(13)], plasma [(14)], blood [(14)], and serum [(14)], or is excreted in the urine [(15)]. The normal levels of urine Se are in the range of 10-10 µg/L [(16)]. Several normal levels of serum Se are reported as follows: 80-270 µg/L from the clinical Guide Laboratory Test [(16)]; 29-139 µg/L from a review [(17)] by collecting the results of healthy subjects from Germany, France, Italy, Ireland, Switzerland, Hungary, Greece, USA, and Spain; 5-160 µg/L from the Glasgow Royal Infirmary Trace Element Unit [(18)]. Smaller concentrations of Se may cause diseases, such as anemia [(19)], heart disease [(15)], or cancer [(15)] whereas large concentrations of Se cause changes in diastolic blood pressure [(19)] higher uric acid [(19)], or

gastrointestinal troubles [15]. Several techniques used for the determination of Se in human hair and nails are atomic absorption spectrometry, especially with the atomization of selenium as a hydride, and graphite-furnace atomic absorption spectrometry, with and without prior sample digestion [20-21], das been useful. Also, neutron activation analysis [22], voltammetry [23], inductively coupled plasma atomic emission and inductively coupled plasma mass spectrometry [24] have been reported over the years.

In this study an accurate method for the determination of total selenium in human hair and nails samples by GF-AAS is proposed. The effect of the different reagents (nickel nitrate, palladium/ ascorbic acid, palladium/ magnesium nitrate), as chemical modifiers, on various oxidation states of selenium was investigated. The optimal ashing and atomizing conditions, limit of detection, and recoveries from samples were studied in order to develop a rapid and precise method for selenium analysis.

EXPERIMENTAL

GF-AAS method

All measurements were carried out with a graphite furnace atomic absorption spectrometry (VARIO 6, Analytik Jeana) equipped with a deuterium lamp background correction. Argon with a purity of 99.998% was used as the carrier gas. Hollow cathode lamp of selenium was used as a light source. The instrumental parameters are listed in Table (1).

Table (1): Instrumental parameters for the determination of selenium by GF-AAS with palladium/ascorbic acid as a chemical modifier

Wavelength/nm	**196**
Slit/nm	**1**
Lamp current/mA	**13**
Background correction	**Deuterium lamp**
Calibration mode	**Absorbance, peak height**

Reagents:

All of the reagents used were of analytical grade: concentrated nitric acid, palladium powder, L-ascorbic acid, nickel nitrate, magnesium nitrate and hydrogen peroxide (30%) were purchased from Merck; selenomethionine (United States pharmacopoeia-reference standard); double distilled, deionized water was prepared by Millipore water system (18 µ?).

Solutions:

Stock selenium (IV, VI) solutions (1000 µg/L) were purchased from Merck (as Atomic Absorption standards); a 1000 µg/L stock selenium (-II) solution was prepared by selenomethionine in deionized water, 20 µg/L working selenium (-II, IV, VI) solution were prepared by dilution of the stock selenium (-II, IV, VI) solutions in 2% (v/v) HNO_3, calibration selenium (IV) solutions were prepared by diluting the stock selenium (IV) solutions in 0.67% (w/v) ascorbic acid to give final concentrations of 5-50 µg/L, 1500 mg/L nickel nitrate solution and 2000 mg/L stock magnesium nitrate solution were purchased from Merck. A 2000 mg/L stock palladium solution was prepared by dissolving 20 gm of palladium powder in 1.5 mL of 14 M HNO_3 and 1 mL of concentrated HCl and diluted to 10 mL with deionized water. A 1000 mg/L palladium solution was prepared by diluting the palladium stock solution in 2% HNO_3; 2 and 0.65% (w/v) ascorbic acid aqueous solutions. To produce a palladium/ascorbic acid solution (mixture), the stock palladium solution was diluted to 1:1 with 2%

ascorbic acid. To produce a magnesium nitrate/palladium solution (mixture), the stock palladium solution was diluted 1:1 with the stock magnesium solution. A 20 µL volume of chemical modifier solution followed by 30 µL of samples were injected into the furnace.

Samples:

Human hair and nails samples were taken from sex individuals without any history of environmental exposure. The protocol for the removal of external contamination from this samples involved rinsing with 5 mL of acetone for 10 min. after that the samples washed with dionized water and oven dried at 70 ^{0}C for 8 hours after that milling the samples. Digestion procedure should be designed to avoid and loss of selenium. A portion of 100 mg of the powder sample, with grain size of ~ 200 mesh, is placed in a microwave vessel, to which 3 mL of concentrated nitric acid and 1 mL of 30% hydrogen peroxide are added. Another vessel without sample but contains the same volumes of acids is processed as a blank. The vessels are placed in the microwave system [Mylestone 1200]. The following power-setting program is suitable for the digestion of the hair and nails samples in table 2. Finally, the samples were diluted with water to yield a final volume of 10 mL.

Table (2): The power – setting program

Program stage	Time (min)	Power (watt)
I	**2**	**250**
II	**2**	**0**
III	**6**	**250**
IV	**5**	**400**

RESULTS AND DISCUSSION

Develbpment of a furnace temperature program

One of the most important factors in this method is choosing the optimum temperature program. Selenium is very volatile and can be lost during the ashing stage. Suitable modifiers could be used to form compounds with the selenium, and allow higher ashing temperatures to remove as much of the matrix as possible before the analyte is atomized. The influence of the ashing and atomization temperatures on the determination of total selenium in human hair and nails samples, by GF-AAS with nickel nitrate, palladium/ascorbic acid and palladium/magnesium nitrate as chemical modifiers, was investigated. Working solutions containing 20 µg/L Se (-II, IV, VI) were used to optimize the parameters.

Selenium determination method by GF-AAS with nickel nitrate.

A solution of 20 µL of a 1500 mg/L nickel nitrate as a chemical modifier was injected into the furnace. Two drying steps at 90 ^{0}C and 120 ^{0}C ensured to dry the samples without boiling. The pre-ashing temperature was set at 500 ^{0}C, and held for 30 s for the complete removal of smoke. Ashing temperatures between 700-1400 ^{0}C were investigated (Fig. 1). The optimum ashing temperature was found to be 1100 ^{0}C when the atomizing temperature at 2100 ^{0}C was used. The maximum selenium (IV) absorbance was 0.43 for 1000-1100 ^{0}C and decreasing at 0.29 for an ashing temperature of 1200 ^{0}C. The absorbance of selenium (II) and (VI) is 0.29 and 0.36, respectively, at 1100 ^{0}C. In conclusion, when nickel nitrate is used as a chemical modifier, the signal intensity depends on the oxidation states of selenium.

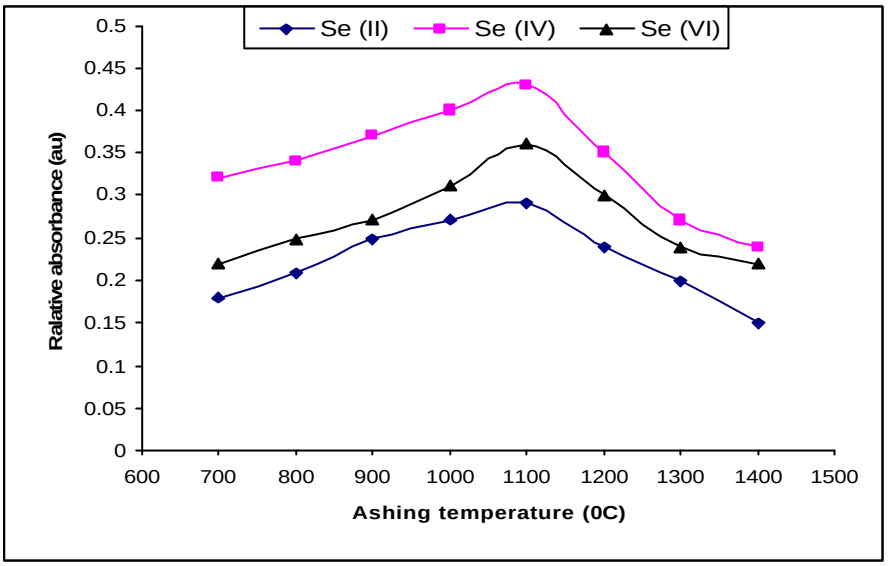

Fig. (1): Effect of the ashing temperature (atomization fixed at 2100 ^{0}C) on the absorption signals of 20 µg/L Se (II), Se (IV), and Se (VI), using nickel nitrate as a chemical modifier.

Selenium determination method by GF-AAS with Pd/magnesium nitrate.

A 20 µL volume of a palladium/magnesium nitrate solution (mixture) as a chemical modifier was injected into a furnace. Ashing temperatures between 700-1400 ^{0}C were investigated. The optimal ashing temperature was found to be 1100-1200 ^{0}C when atomizing temperature at 2100 ^{0}C was used (Fig. 2). Keeping the ashing temperature fixed, the furnace was programmed for a variation of the atomization temperature (1800-2500 ^{0}C). The optimal atomizing temperature was found to be 2100 ^{0}C when an ashing temperature at 1200 ^{0}C was use, where the maximum selenium (II) absorbance was 0.38.

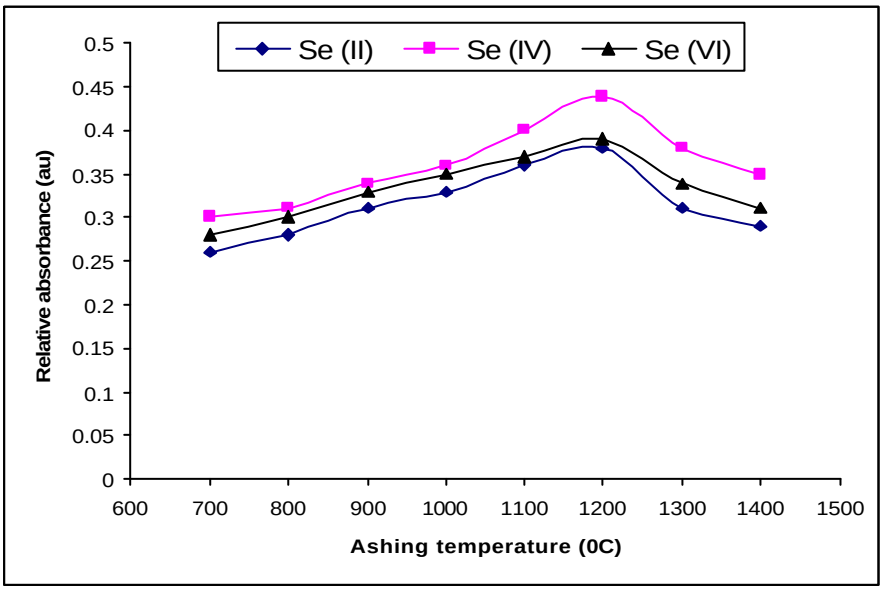

Fig. (2): Effect of the ashing temperature (atomization fixed at 2100 ^{0}C) on the absorption signals of 20 µg/L Se (II), Se (IV), and Se (VI), using Pd/magnesium nitrate as a chemical modifier.

Selenium determination method by GF-AAS with Pd/ascorbic acid.

20 µL of a palladium/ ascorbic acid solution (mixture) as a chemical modifier was injected into the furnace. After drying, the sample was slowly heated to 500 ^{0}C. A preashing step at 500 ^{0}C allowed the palladium modifier to be reduced to elemental palladium, and react with selenium to form more stable species [25-26].Using ascorbic acid as a reducing agent for palladium, the optimum ashing temperature of 2100 ^{0}C was used (Fig. 3). Atomizing temperature of between 1800-2500 ^{0}C was investigated, when the ashing temperature at 1300 ^{0}C was used. The plateau of the atomization temperature was observed over 2100-2300 ^{0}C; therefore, an atomization temperature of 2250 ^{0}C was selected. The maximum selenium absorbance was between 0.43-0.50 for different oxidation states. When Pd/ascorbic acid were used as a chemical modifier, the oxidation states of selenium were not significant in the determination. Undoubtedly, these results indicate that Pd/ascorbic acid can enhance the sensitivity for the analyte element. The application of this modifier showed the best result in terms of the sensitivity and signal intensity independent of the selenium species. Peak-shapes and appearance times of the atomizing signal is equal for the three selenium species with this modifier. The repeatability from 10 consecutive measurements of 20 µg/L working selenium (II, IV, VI) solution was calculated. The repeatabilities, expressed as a relative standard deviation (RSD), were 6.8, 7.2 and 7.5 %, respectively. As can be seen, this repeatability is not very good.

The result led to the presumption that precipitation of palladium can occur in the presence of ascorbic acid, which is a reducing agent for palladium. Adding ascorbic acid to the samples after digestion and to the standard solutions can solve this problem. Under these conditions, 20 µL of a 1000 mg/L palladium solution as a chemical modifier, followed by 30 µL of a 20 µg/L calibration selenium (IV) solution containing 0.67% ascorbic acid were injected into the furnace. The optimal ashing and atomizing temperature were 1300 ^{0}C and 2250 ^{0}C, respectively. The repeatability from 10 consecutive measurements of 20 µg/L calibration selenium (IV) solution in ascorbic acid was 1.4%. The limit of the detection was 1 µg/L (calculated as 3 s of the blank) and the calibration curve was linear for the concentration range between 5-50 µg/L with a slope of 0.015 with the temperature program I Table 3.

Table 3: Temperature program for the determination of total selenium with palladium/ ascorbic acid as a chemical modifier

step	Temp./^{0}C	Ramp/^{0}C s^{-1}	Time/s	Gas flow/L min^{-1}
Drying	**90**	**5**	**10**	**3**
Drying	**120**	**10**	**40**	**3**
Pre-ashing	**500**	**20**	**30**	**3**
Ashing	**1300**	**20**	**15**	**3**
Cooling	**20**	**1**	**15**	**3**
Atomizing	**2250**	**0**	**5**	**0**
Cleaning	**2600**	**1**	**5**	**3**

Matrix interferences

The influence of matrix interference on the selenium determination in human hair and nails was evaluated based on the ratios of the slopes of the calibration graphs (the slope of calibration curve for aqueous standard solution and the slope of calibration curve obtaining by the standard-addition method). To make standard Se (IV) solutions for the standard addition method, 1 mL of sample

solution was spiked with selenium (IV) standard solution, and then diluted to obtained final selenium concentrations of 10, 20, 30, and 40 µg/L with 0.67% (w/v) ascorbic acid. The slope of the calibration curve obtained by the standard-addition is 0.021, which are significantly different from the slope of the calibration curve for aqueous standard solution. This indicates that a standard method is needed in the given matrix.

The proposed method for the determination of total selenium was applied to the human hair and nails samples. The results obtained with the proposed method are presented in table (4). The relative standard deviations (RSDs) of this method ranged from 2.1% to 4.53%. The accuracy [27] of the analytical method was established by determining the selenium in human hair and nails samples, and quantitative recovery studies of the amounts of selenium (IV) added to the sample. The data for the recovery studies are presented in Table 5 and 6.

Table (4): Total selenium contents in human hair and nails samples.

Sample no.	Human hair Se/ mg Kg^{-1}	RSD%	Nails Se/ mg Kg^{-1}	RSD%
1	**0.60**	**2.10**	**0.69**	**3.26**
2	**0.73**	**2.75**	**0.81**	**4.16**
3	**0.61**	**3.24**	**0.63**	**2.83**
4	**0.60**	**4.53**	**0.85**	**3.92**
5	**0.66**	**4.16**	**0.79**	**3.66**
6	**0.64**	**3.84**	**0.91**	**2.27**

Table (5): Recovery of total selenium from human hair samples.

Sample no.	Se present/ mg Kg^{-1}	Se added/ mg Kg^{-1}	Se found/ mg Kg^{-1}	Recovery, %
1	**0.60**	**0.15**	**0.745**	**96.66**
2	**0.73**	**0.15**	**0.88**	**100.0**
3	**0.61**	**0.15**	**0.757**	**98.00**
4	**0.60**	**0.15**	**0.752**	**101.33**
5	**0.66**	**0.15**	**0.80**	**93.33**
6	**0.64**	**0.15**	**0.788**	**98.66**

Table (6): Recovery of total selenium from nails samples.

Sample no.	Se present/ mg Kg^{-1}	Se added/ mg Kg^{-1}	Se found/ mg Kg^{-1}	Recovery, %
1	**0.69**	**0.10**	**0.788**	**98.00**
2	**0.81**	**0.10**	**0.910**	**100.0**
3	**0.63**	**0.10**	**0.729**	**99.00**
4	**0.85**	**0.10**	**0.956**	**106.0**
5	**0.79**	**0.10**	**0.893**	**103.0**
6	**0.91**	**0.10**	**1.007**	**97.00**

CONCLUSION

Determination the concentration of Se species is more important than concentration of total Se. nickel nitrate, palladium/ ascorbic acid, and palladium/ magnesium nitrate modifiers could be used to form compounds with the selenium, and allow higher ashing temperatures to remove as much of the matrix as possible before the analyte is atomized . GF-AAS is used for determination of Se species because its Rapid and precise method for selenium analysis.

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