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# Determination of Cr, Se, Ge, and V in Hypothyroidism Patients by Using Graphite Furnace Atomic Absorption Spectrometry and mixture matrix modification

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## Abstract:

A simple, accurate and rapid method was developed for routine determination of trace elements in blood serum. The method based on the direct determination for Cr, Se, Ge, and V in hypothyroidism patients before therapy.

By using atomic absorption spectrometry with graphite surface coated and uncoated pyrolysis GF-AAS, and a mixture of palladium nitrate and magnesium nitrate as the matrix modifier, with deuterium background correction and no sample pretreatment except dilution was necessary. This permitted direct determination hence the risk of sample contamination was reduced. Further, the use of graphite surface-coated GF-AAS decreased the ashing and atomization temperatures of Cr, Ge, and V to values that were lesser than the corresponding values obtained using uncoated pyrolysis GF-AAS, by 100°C; in case of Se, the atomization temperature decreased to a value that was 200°C lesser than that obtained using uncoated pyrolysis GF-AAS.
A mixture of (3µL) palladium nitrate, and (2µL) magnesium nitrate was successfully applied to improve the sensitivity, reproducibility, recovery, limit of detection and the accuracy of the measurements. The correlation coefficients of the calibration curves of Cr, Se, Ge, and V were found to be (0.9999, 0.9999, 0.9995 and 0.9999) respectively, the relative standard deviation of the measurements for Cr, Se, Ge and V were (0.027, 0.075, 0.054 and 0.068) respectively. The statistical analysis of the acquired data showed acceptable accuracy. The analyses thus performed indicated that the levels of serum trace elements Cr, Se, Ge, and V in hypothyroidism patients were lower than those in the control group.

**Key words:** trace elements determination in serum; atomic absorption spectrometry; graphite furnace; hypothyroidism; matrix modification

# تقديرعناصرالكروميوم Cr ,السيلينيوم Se الجرمانيوم Ge والفناديوم Vلمرضى قصورالغدةالدرقية بأستعمال مطيافيةالامتصاص الذري لفرن الجرافيت الكهروحراري لمزيج من معدلات المنشأ

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## الخلاصة:

طورت طريقة تحليلية بسيطة,دقيقة وسريعة لأجراء التحليلات الروتينية للعناصر النزرة في مصل الدم .تعتمد هذه الطريقة على التقدير المباشر لعناصر الكروميوم السيلينيوم الجرمانيوم والفناديوم . لدى ألمرضى المصابين بقصور الغدة الدرقية قبل اعتماد أي معالجة دوائية .بوساطةمطيافيةالامتصاص الذرياالكهروحراريوبأستخدامألأنبوبألكرافيتي ألحراري

المطلي وغيرالمطلي, بوجود مزيج نترات ألبلاديوم ونترات ألمغنيسيوم كمعدل منشأ كما تم أستعمال مصباح ألديتريوم لتصحيح ألخلفية.ألتخفيف كان هوألمعالجةألاولية ألوحيدة للعينة قبل ألتحليل مما قلل مخاطر تلوث العينة وبالتالي سمح بأجراء التحليل بصورة مباشرة. استخدام الانبوب الكرافيتي الحراري المطلي أدى ألى انخفاض درجة حرارة الترميد والتذريةلعناصرالكروميوم,الجرمانيوم و الفناديوم 100 درجة مئوية ,في حين انخفضت درجة حرارة التذرية لعنصر السيلينيوم 200 درجة مئوية مقرنة بأستخدام الانبوب الحراري غير المطلي,كماألتطبيق ألجيد لاستخدام مزيج 3ميكروليتر من نترات البلاديوم و2ميكروليتر نترات المغنيسيوم كمعدل منشأ أدى الى تحسن ألحساسية,ألاستعادية المئوية,ألدقة ,حدود ألكشفوألمديات ألخطية لمنحنيات ألمعايرة. تم رسم منحنيات الترميد والتدرية ومنحنيات المعايرة وكانت معاملات الارتباط0.9999,0.9995,0.9999,0.9999 ) وقيم الانحراف القياسي النسبي ( 0.027,0.075,0.054,0.068 ) للعناصر الكروميوم ,السلينيوم, الجرمانيوم والفناديوم على التوالي .المعالجة الاحصائية جرى تطبيقها لجميع النتائج التي تم الحصول عليها والتي اشارت الى دقة وضبط مقبولين. كما أظهرت النتائج انخفاض تركيز العناصر الاربعة لدى المرضى المصابين بقصور الغدة الرقية مقارنة مع الاشخاص الاصحاء.

**الكلمات المفتاحية:** تقدير العناصر النزرة في بلازما الدم : مطيافية الامتصاص الذري: الفرن الكرافيتي: قصور الغدة الدرقية: معدلات المنشأ

## Introduction

The thyroid gland plays a crucial role in homeostasis, growth, brain development, nervous, and cardiovascular system function.[1] The main function of thyroid is to synthesis hormones, T4 and T3, which are essential for the regulation of metabolic processes throughout the body.[2] Thyroid function is controlled by the hypothalamic-pituitary-thyroid axis, regulated by thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine(T4).[1] Hypothyroidism is a clinical entity that results from deficiency of thyroid hormones or, in more rare cases, from the impaired activity of these hormones in target tissues.[2] In a patient with hypothyroidism, the basal metabolic rate is decreased and other processes depend on the-thyroid - hormones are adversely affected.[3] Some trace elements and thyroid hormones play essential roles in the human body.[4] Trace elements are essential for different physiological functions, including those of the thyroid gland, which contains a higher concentration of many trace elements than the other tissues.[5] The level of trace elements affects normal thyroid metabolism and function. Also, the thyroid gland affects the secretion and transport of trace element during metabolism. Several minerals and trace elements are essential for normal thyroid-hormone metabolism and coexisting deficiencies of these hormones can impair the thyroid function.[6-7]

Selenium (Se) is a key element in thyroid hormone biosynthesis and metabolism. Thyroid tissue contains the highest concentration of Se. [8] The biological function of (Se) attributes to the selenoproteins that are involved in many diverse biological processes such as DNA synthesis and thyroid hormones metabolism. [9] Chromium (III) is a fundamental trace element for human. Chromium (III) is an ion required for nutritional support for the thyroid gland to enhance insulin through reducing body. [10] Vanadium also may regulate thyroid metabolism. It was found that Vanadium deprivation lead to increase thyroid weight and decreased thyroid peroxidase activity in rats.[11]

The elements Cr, Se Ge, and V were determined by atomic absorption spectrometry (AAS) with a coated curvet furnace. These four elements have been well known as one of the numerable elements used for conventional (AAS) before atomization occurs the formation of refractory carbides and volatile oxides causes a remarkable reduction in the sensitivity and reproducibility [12]. In order to resolve these problems, the use of pyrolytic coated curvet tube has been recommended since the use of this tube increases durability and improves the sensitivity and precision for Cr, Se, Ge, and V determination. [13]

Matrix modification has been attempted to prevent the formation of refractory carbides, and it has been well documented as facilitating significant improvements, especially, in the case of biological fluids such as blood serum and urine.[14] Moreover, a properly designed modification could also affect the interaction of the sample with the graphite surface and/or unify the atomization process of the analyte present in various species.[15].Welz and co-workers proposed the use of a mixture of $Pd(NO_3)_2$ and $Mg(NO_3)_2$ as a universal modifier for 21 elements, including Al, Bi, Cd, Cu, Mn, Pb, Sb, and Se.[16]

Further, a mixture of palladium and magnesium nitrates was found to be a very powerful modifier for the determination of As, Bi, In, Pb, Sb, Se, Sn, Te, and Tl in graphite furnace atomic absorption spectrometry (GF-AAS). [17]

The aim of this study is to use GF-AAS with a mixture of palladium nitrate and magnesium nitrate as the matrix modifier for the determination of Cr, Se, Ge, and V in blood serum. The proposed method is direct because there is no sample pre-treatment other than the dilution of the serum is required. Hence, using the method of standard addition was unnecessary. The proposed mixed modifier is effective as it decreases the matrix interferences, prevents element vaporization during pyrolysis, and minimizes the risk of contamination. Further, palladium and magnesium nitrates can be obtained in high purity. (18) Furthermore, both Pd and Mg are infrequently determined using GF-AAS, and they enhance the analytical signal. The proposed matrix modifier also generates a reproducible signal at high atomization temperatures due to the formation of a Pd–metal compound. [19]

## Materials and Methods

### Apparatus

Two instruments of atomic absorption Shimadzu model, AA-6200, Koyoto, were used for the determination of the trace elements concentration under study. Shimadzu model AA-6200 atomic absorption spectrometer AAS equipped with Shimadzu GFA-2600graphite furnace atomizer for volatilization and atomization in place of flame. Japan sensitive balance 4 digitals, micropipette 10-20µl Eppendorf Germany were used in analysis. Hamamatsu photonics K.K Japan single-element hallow cathode lamps (H.C.L) were used as the spectral sources. Shimadzu AA-6200 auto sample changer, with deuterium background correction, pyrolytically coated and uncoated graphite tubes were used, inert gas (Argon 99.9999%) was used as purge gas with flow rate 1.5 L/min.

## Methods

This study was applied on fifty patients (20 male, 30 female) with approved hypothyroidism mean aged (50.85±6.36years), who were admitted to the medical city hospital in Baghdad. The samples were collected from the patients before the treatment. The control group consisted of 30 volunteers, (13 male, 17 female). Mean aged (47.89± 12.82 years) healthy individuals who do not have any health problems. They have not been given any medications, vitamins or mineral supplements for at least 2 weeks before sampling.

### Preparation of Stock Standard Solution for (Cr, Ge, Se and V) elements

Preparation of standard stock solutions for each of elements **(**Cr, Ge, Se andV**)** is carried out by the following method:

Stock solution with 100 ppm of the four elements, have been prepared by using the concentrated ampoule (fixinal) that is quantitatively transferred into volumetric flask of 100 ml and diluted to the calibration mark by using re-deionized water

### Palladium and magnesium nitrate solutions

The chemical modifier solutions of Pd $(NO_3)_2$and Mg $(NO_3)_2$ were used. In each measurement, 3µL of 700µg/ml Pd solution, and 2 µL of 500µg/ml Mg $(NO3)_2$ have been added to 10 µl of sample or standard solution, solutions were injected into the graphite tube at 25°C.

### The blood serum

Volume of five milliliters of venous blood from patients and healthy subjects were drawn by utilizing disposable plastic syringes and transferred into sterile test tube for 10-15 minutes at 37°C to clot. The samples then centrifuged at 3000 rpm for 15 min the clear serum was transferred to a plastic tube, by the disposable syringe and topped by a plastic stopper, stored a deep frozen at -20°C then used for estimation serum thyroxine($T_4$), triiodothyronine($T_3$), serum($T_3$), and ($T_4$) concentration level were measured by radioimmunoassay method using ( Gamma center model) and radio immune technique kit as shown in Table (1), before analysis to determine of selenium, chromium, vanadium and germanium by atomic absorption spectrophotometer. The serum was diluted by re-deionized water and measured directly against an aqueous standard made from certified standard solution.

Re-deionized water was used to ensure that there is no leaching of any trace elements to the standards and serum.

**Table (1): Thyroid hormones levels of the patients and controls**

| Parameters | Hypothyroidism | Controls |
|---|---|---|
| T3 | 1.642±0.593 | 2.085±0.316 |
| T4 | 119±61.009 | 108±0.15.866 |
| TSH | 2.347±0.005 | 1.342±0.004 |

### Determination of (Cr,Ge,Se And V)by using Coated Graphite Furnace GF-AAS

The concentration of elements (Cr, Ge, Se and V) in blood serum have been determined by electro thermal atomic absorption spectrophotometer through employing of coated graphite tubes GF-AAS pyrolysis with the existence of palladium nitrate and magnesium nitrate as a matrix modifier and deuterium correction background.

After setting up GF-AAS according to the optimum instrumental conditions described in the Table (2). The calibration curves for (Cr, Ge, Se and V) were carrying out by injected (10µl) from standard solution or blood serum that contains 3 µl of Pd $(NO_3)_2$ and 2µl of Mg $(NO_3)_2$ solution as mixture matrix modifier, to the furnace and start the cycle heating where the absorbance was recorded by reading the peak height.

**Table (2): Optimum parameters for coated GF-AAS for determination (Cr,Se,Ge and V)**

| Parameters | Cr | Se | Ge | V |
|---|---|---|---|---|
| Wave length (nm) | 357.9 | 196.0 | 265.1 | 318.4 |
| Spectral band width (nm) | 5 | 1.2 | 0.5 | 0.8 |
| H.C.L curent (mA) | 0.8 | 6 | 6 | 6 |
| Drying temp°C ˙/sec | 150/30 | 150/30 | 150/30 | 150/30 |
| Ashingtemp°C ˙/sec | 1000/20 | 1000/20 | 1200/20 | 1200/20 |
| Atomization temp °C/ sec | 2300/3 | 1900/3 | 2400/3 | 2500/3 |
| Cleaning a temp°C ˙/sec | 2500/3 | 2100/3 | 2600/3 | 2700/3 |
| Gas flow (L/ $min^{-1}$) | 1.5 | 1.5 | 1.5 | 1.5 |
| Signal mode | Peakheight | Peakheight | Peakheight | Peak height |
| Sample volume (µl) | 10 | 10 | 10 | 10 |

## Results and Discussion

### Matrix modifier

The main problems of Se ET-AAS are related to the high volatility of the element and subsequent loss during the ashing step. [19] Some experiments indicated the formation of Cr and V carbides, i.e., $Cr_3C_2$ and VC, respectively. These carbides are not stable and decompose into their elements at temperatures higher than 2200°C. [20] The losses of atomic vapor above this temperature are responsible for the reduction of the corresponding absorbance signals. [(21] Ge is oxidized to volatile GeO at temperatures higher than 1200°C. The loss of these oxides at low temperatures reduces the absorbance signal of Ge. [(21]

Many modifiers have been tested to improve the thermal stabilization of Cr, Se, Ge, and V. The obtained data indicate that the use of a mixture of palladium and magnesium nitrates plays an important role in the elimination or moderation of adverse chemical processes, e.g., carbide and oxide formation, and significantly improves the sensitivity, reproducibility, recoveries, accuracy, [14] and the limit of detection. The aforementioned mixture seems to be most effective as a modifier for the stabilization of the absorbance signals of Cr, Se, Ge, and V. This may be due to the formation of a thermally stable compound of palladium and metals, thus resulting in a decrease in the loss of elements during the pre-atomization stage. Low recoveries are avoided by the addition of magnesium nitrate.

### Effect of ashing and atomization temperatures

Table 3 shows the best ashing and atomization temperatures for 10 µL of aqueous standard solution of Cr, Se, Ge, and V in the presence of a mixture of Pd $(NO_3)_2$ and Mg $(NO_3)_2$ as a matrix modifier when using uncoated pyrolytic GF-AAS. Figures 1–4 show the effect of ashing and atomization temperature on the AAS signal of 10 µL of the above mentioned aqueous standard solution. The ashing temperature curves were obtained by fixing the atomization temperatures. Ashing temperatures of 900, 1000, 1100, and 1100°C were found to be optimum temperatures for Cr, Se, Ge, and V, respectively. This allows for the maximum removal of the serum matrix without any loss of the analyte. The atomization temperature curves were obtained by fixing the ashing temperatures and varying the applied atomization temperatures. The most suitable observation height was used to achieve the maximum sensitivity. The stopped-flow mode (and a gas flow rate of 1.5 L/min) was used during the atomization of the elements to increase the sensitivity by enhancing the residence time of the atoms of the elements in the furnace. Figures 1–4 show the effect of atomization temperatures on the absorbance of (Cr, Se, Ge, and V) under optimum ashing temperature. As shown in the figures, when the temperature increases, the absorbance increases until it reaching a plateau. Such a plateau indicates the beginning of the stable atomization temperatures at which all the elements have been transformed into free atoms, and hence, the temperature selected for the element analysis is fixed as per the atomizing temperature. Further, increasing the atomization temperatures causes an increase in the peak height of the constructed atomization curves; this shows that the curves reach maximum peak points when equilibrium is established between the rate of free atom supply and the corresponding rate of removal of these atoms. Atomization temperatures of 2200°C, 1900°C, 2300°C, and 2400°C were consequently chosen as optimum temperatures for Cr, Se, Ge, and V respectively.

**Table (3): Ashing and atomization temperatures for uncoated GF-AAS**

| Temperatures | Cr | Se | Ge | V |
|---|---|---|---|---|
| Drying temp°C /̇sec | 150/30 | 150/30 | 150/30 | 150/30 |
| Ashingtemp°C /̇sec | 1100/20 | 1100/20 | 1300/20 | 1300/20 |
| Atomization temp °C/ sec | 2400/3 | 2100/3 | 2500/3 | 2600/3 |
| Cleaning temp°C /̇sec | 2600/3 | 2300/3 | 2700/3 | 2800/3 |

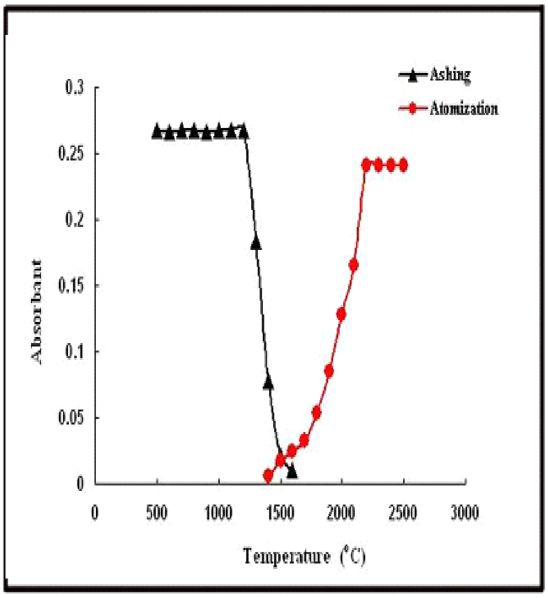

Fig1: Ashing and Atomization curve for Chromium

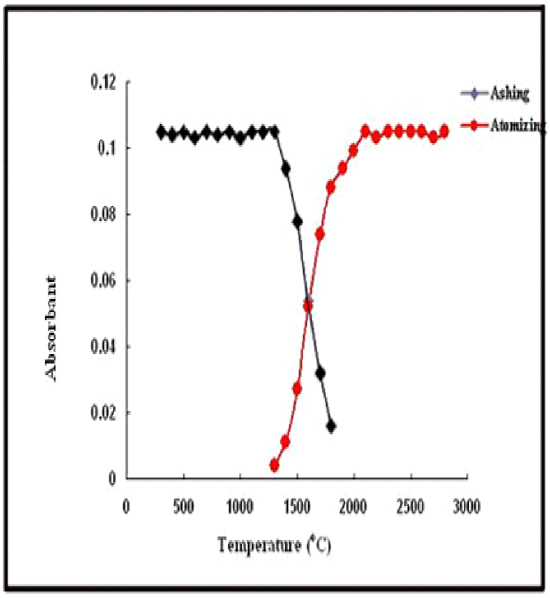

Fig2: Ashing and Atomization curve for Selenium

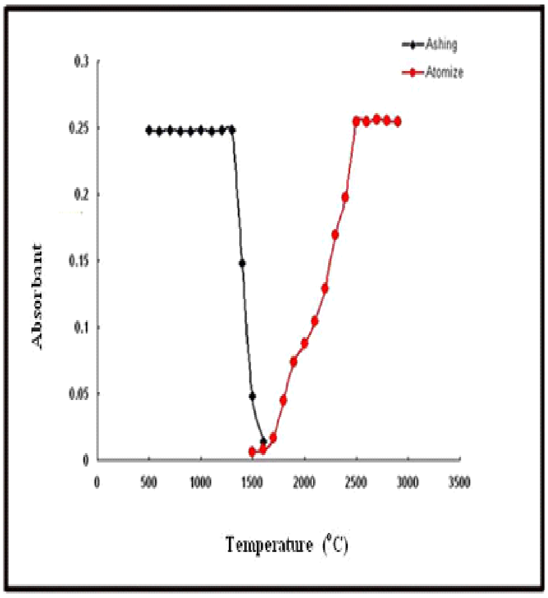

Fig3: Ashing and Atomization curve for Germanium

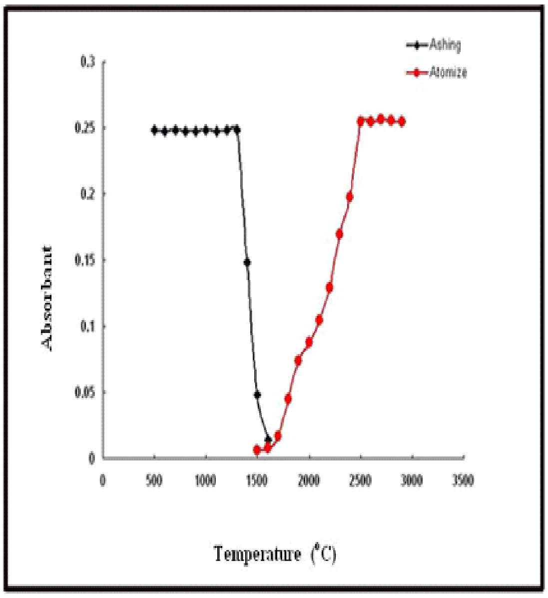

Fig4: Ashing and Atomization curve for vanadium

## Calibration curve

Figures 5–8 show the calibration curves for Cr, Se, Ge, and V, which were constructed using the coated pyrolytic graphite tube and the mixture of the matrix modifier at the optimum conditions listed in Table (2). Calibration curves for the elements have been obtained for 10 µL injections at several concentrations of the standard solutions of the elements with a mixture of Pd $(NO_3)_2$ and Mg $(NO_3)_2$ as the matrix modifier; the element solutions with different concentrations were prepared using deionized water and by employing the triplicate mode for each set standard.

A significant improvement in the accuracy of the absorbance measurements as well as good reproducibility in terms of the determination the elements in aqueous solution and blood samples can be seen from Figures 5–8. This indicates that the proposed method of preparing the standard solutions is done in good methodology.

### Statistical parameters

The results of statistical treatment for the obtained calibration curves are summarized in Table 4. The results prove that the validity of the calibration curves in performing analysis with minute amounts of elements, and the detection limit estimation and the repeatability of standard solutions were optimal. The values of relative standard deviation and correlation coefficient are suitable for using standard curves for analysis purposes and for achieving accurate results. In addition, the results demonstrate that it is possible to enhance the sensitivity of the analyses, which means the detection limit corresponded to low concentrations.

**Table (4): Analytical values of statistical treatments for calibration curves for (Cr,Se,GeandV)**

| Parameters | Cr | Se | Ge | V |
|---|---|---|---|---|
| Correlation coefficient r | 0.9999 | 0.9999 | 0.9995 | 0.9999 |
| $r^2$ linearly | 0.99994 | 0.99994 | 0.99974 | 0.99994 |
| Linearity percentage r % | 99.99 | 99.99 | 99.95 | 99.99 |
| Regression equation | Y=9.856x-0.0212 | Y=4.58x-0.0029 | Y=6.591x+0.0079 | Y=4.69x-0.0029 |
| Slope (µg\ml) | 9.856 | 4.58 | 6.591 | 4.6924 |
| intercept | -0.0212 | -0.0029 | 0.0079 | -0.0029 |
| Standard deviation | 0.027 | 0.075 | 0.054 | 0.068 |
| Limit of detection (µg\ml) | 0.01312 | 0.001606 | 0.0264 | 0.0014 |
| Sensitivity (µg\ml) | 0.0027 | 0.00956 | 0.00631 | 0.00809 |

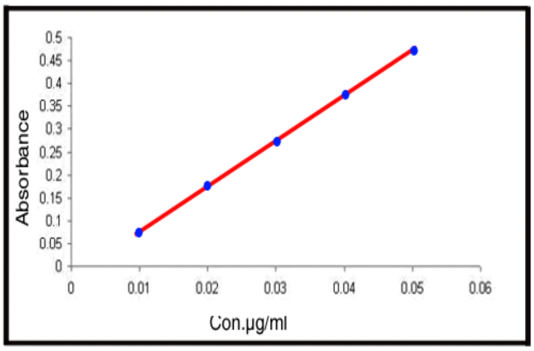

Fig5: calibration curve of GF-AAS wit mixture matrix modifier for chromium

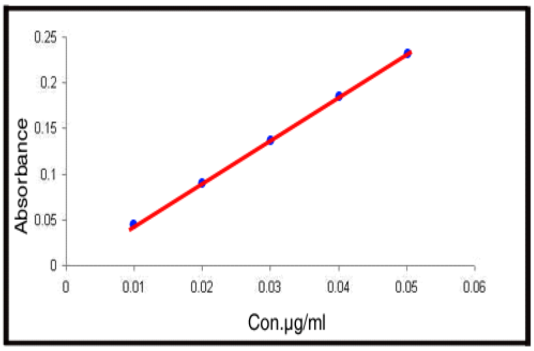

Fig6: calibration curve of GF-AAS wit mixture matrix modifier for Selenium

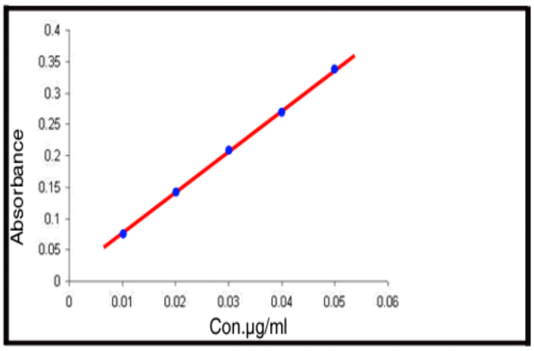

Fig7: calibration curve of GF-AAS wit mixture matrix modifier for Germanium

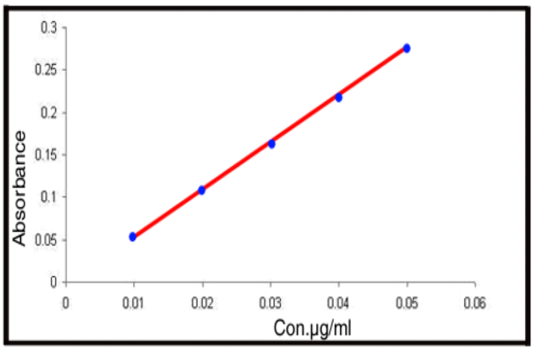

Fig8: calibration curve of GF-AAS wit mixture matrix modifier for vanadium

**Table (5): Analytical values of Correlation coefficient (r) for calibration curves for (Cr, Se, Ge and V)**

| Parameters | Cr | Se | Ge | V |
|---|---|---|---|---|
| Correlation coefficient r | 0.9999 | 0.9999 | 0.9995 | 0.9999 |

## Sensitivity

Sensitivity is defined as the concentration of a solution (typically in µg/mL) of an element needed to produce a signal of 0.0044 absorbance units, which is equivalent to a 1% decrease in the transmitted radiation. The sensitivity values calculated from the data obtained in this study, under optimum conditions, for the analysis of standard solutions were: 0.0027, 0.00956, 0.00631, and 0.00809 µg/mL for Cr, Se, Ge, and V, respectively.

## Limit of detection (LOD)

The detection limits for atomization were calculated. The limits give an estimation of the lowest concentration and the higher sensitivity reached by using the coated-tube GF-AAS with the mixture matrix modifier. The obtained limit of detection corresponds to a probability level of 99%, which is calculated for a given concentration by using the following statistical formula: DL = 2 × SD × C/x. The LOD for each element in units of concentration (µg/mL) obtained from their calibration curves were 0.01312, 0.00106, 0.0264, and 0.0014 µg/mL for Cr, Se, Ge, and V, respectively.

### Linearity

The linearity was evaluated by analyzing standard aqueous solutions of Se, Ge, and V with concentrations of 0.01 to 0.05 µg/mL and of Cr with a concentration of 0.005 to 0.025 µg/mL. The calibration graphs were linear within a linear percentage of ≥95%. Thus, these calibration curves possess excellent linearity.

### Accuracy and precision

To evaluate the precision and accuracy of the method, we performed a series of determination of two element-containing materials: a standard reference material (SRM) and blood serum that was appropriately prepared and labeled. The four serum samples were fortified with a known concentration of an aqueous standard solution of the elements under study before performing the absorbance. Both methods give good accuracy under the optimum conditions. The results of the determination of these materials are presented in Tables 6 and 7. Each sample contains a mixture of 3 µL Pd $(NO_3)_2$ and 2 µL Mg $(NO_3)_2$ as the matrix modifier. The accuracy and precision of the measurements were established by five replicates for each concentration, and they were defined as the percentage values of E%, RSC%, and RSD%. The relative error and the relative standard deviation were less than 0.1, which indicates the high accuracy and precision of the proposed method.

**Table (6): Accuracy and Precision for determination (Cr, Se, Ge, and V) in aqueous solution**

| Cr con µg\ml. | | | | |
|---|---|---|---|---|
| **present** | **found** | **Error%** | **REC%** | **RSD%** |
| 0.014 | 0.0143 | 1.210 | 101.210 | 0.0654 |
| 0.019 | 0.019 | 1.104 | 101.104 | 0.0343 |
| 0.026 | 0.026 | 1.352 | 101.352 | 0.0428 |
| **Se con. µg \ml** | | | | |
| **present** | **Found** | **Error%** | **REC%** | **RSD%** |
| 0.0150 | 0.0152 | 1.321 | 101.321 | 0.0485 |
| 0.0250 | 0.0256 | 1.054 | 101.054 | 0.0726 |
| 0.0350 | 0.0354 | 1.131 | 101.131 | 0.0563 |
| **Ge con. µg \ml** | | | | |
| **present** | **found** | **Error%** | **REC%** | **RSD%** |
| 0.0150 | 0.0153 | 1.112 | 101.112 | 0.0849 |
| 0.0250 | 0.0252 | 1.037 | 101.037 | 0.0599 |
| 0.0350 | 0.0356 | 1.144 | 101.144 | 0.0678 |
| **V con. µg \ml** | | | | |
| **present** | **found** | **Error%** | **REC%** | **RSD%** |
| 0.0150 | 0.0152 | 1.032 | 101.032 | 0.0972 |
| 0.0250 | 0.0252 | 1.117 | 101.117 | 0.0537 |
| 0.0350 | 0.0354 | 1.182 | 101.182 | 0.0642 |

**Table (7): Accuracy and precision of determination of (Cr,Se,Ge,andV) in blood serum**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Cr** | **Present µg\ml** | **Add µg\ml** | **Found µg\ml** | **Error%** | **REC%** | **RSD%** |
| | 0.025 | 0.0015 | 0.033 | 1.380 | 101.380 | 0.011 |
| | 0.026 | 0.001 | 0.0314 | 1.153 | 101.153 | 0.0118 |
| | 0.022 | 0.002 | 0.0324 | 1.14 | 101.14 | 0.0124 |
| | 0.0285 | 0.0025 | 0.0415 | 1.142 | 101.142 | 0.0865 |
| **Se** | **Present µg\ml** | **Add µg\ml** | **Found µg\ml** | **Error%** | **REC%** | **RSD%** |
| | 0.0391 | 0.003 | 0.0431 | 1.246 | 101.246 | 0.0135 |
| | 0.0430 | 0.001 | 0.0444 | 1.204 | 101.204 | 0.0126 |
| | 0.0452 | 0.002 | 0.0475 | 1.152 | 101.152 | 0.0120 |
| | 0.0464 | 0.0015 | 0.0486 | 1.136 | 101.136 | 0.0132 |
| **Ge** | **Present µg\ml** | **Add µg\ml** | **Found µg\ml** | **Error%** | **REC%** | **RSD %** |
| | 0.0218 | 0.005 | 0.0273 | 1.226 | 101.226 | 0.0189 |
| | 0.0230 | 0.001 | 0.0242 | 1.204 | 101.204 | 0.021 |
| | 0.0255 | 0.0015 | 0.0276 | 1.135 | 101.135 | 0.0237 |
| | 0.0272 | 0.002 | 0.0299 | 1.140 | 101.140 | 0.0153 |
| **V** | **Present µg\ml** | **Add µg\ml** | **Found µg\ml** | **Error%** | **REC%** | **RSD%** |
| | 0.0280 | 0.005 | 0.0430 | 1.112 | 101.112 | 0.0279 |
| | 0.0250 | 0.009 | 0.0460 | 1.105 | 101.105 | 0.0286 |
| | 0.0268 | 0.007 | 0.0413 | 1.113 | 101.113 | 0.0315 |
| | 0.0220 | 0.01 | 0.0428 | 1.138 | 101.138 | 0.0301 |

### Determination of Cr, Se, Ge, and V in healthy control group and in patient group

The mean ± SD concentrations of serum Cr, Se, Ge, and V in patients with hypothyroidism was lower than that in the control group, as shown in Table 8. The lower concentration of trace elements Cr, Se, Ge, and V in the blood serum of the patients suggests a correlation between these elements and the activity of thyroid hormones. Therefore, the concentration of these trace elements may play an important physiological role in hypothyroidism.

**Table (8): Serum concentration in hypothyroidism and control subjects of (Cr, Se, Ge and V)**

| serum elements level ng/ml | hypothyroidism | controls |
|---|---|---|
| Cr | 43±2.8 | 49±1.3 |
| Se | 100± 5 | 106±1.6 |
| Ge | 28 ±3.7 | 48±1.5 |
| V | 30 ±2.1 | 50±1.3 |

## Conclusion

A simple, rapid, and inexpensive method was developed to improve the sensitivity, recovery, reproducibility, and limit of detection for trace elements determination in blood serum. The main advantages of the developed method are the decreased sample handling and shorter analysis time. Sample pre-treatment, which could cause contamination and significantly delay sample throughput, is thus unnecessary for trace elements determination in blood serum. Furthermore, using the standard additions method was avoided giving another time-consuming technique.

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