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Determination of Selenium (IV) in Some Iraqi vegetables Samples

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ABSTRACT

A simple and inexpensive spectrophotometric method for determination of trace amount of selenium (IV) was described. This method is based on oxidation of hydroxylamine hydrochloride with selenite ions to nitrous acid, which in turn diazotizes p-amino benzoic acid and is coupled with p-nitro phenol in alkaline medium to form colored azo dye. This azo compound has a maximum absorption at 401 nm. The method obeys Beer's law in the range of 0.04 to 0.4ppm. With R^2 =0.9985. Its molar absorptivity, Sandell's sensitivity, standard deviation and relative standard deviation were found 54885.09 $L.mol^{-1}.cm^{-1}$, 0.0014, 0.001and 0.6024 $\mu g.cm^{-2}$ respectively. All the reaction parameters have been optimized. Interferences between the azo reaction and non-targeted ions often present in plants samples were investigated. The procedure was applied successfully to the determination of selenium (IV) in some Iraqi vegetables samples get from the local market in Hilla.

Keywords: Diazotization, coupling, azo dye, selenium, P-nitro phenol.

INTRODUCTION

Selenium is an element of group VIA and period 4 of the periodic table with Atomic Number Z=34 and Mass Number A=78.96 [1]. It was first isolated in 1817 by the Swedish Chemist Jacob Berzelius Jon's and has long been recognized for its toxicity [2]. Until the 50s Scientist regarded selenium exclusively as toxic element. The importance of selenium in human nutrition was first to make sure of in 1957 [3]. Selenium is present in many chemical species [4] and the four natural oxidation state of selenium are elemental selenium (0), selenide (II), selenite (IV) and selenate (VI). Inorganic selenite and selenate are dominant species in water, while principal species found in vegetables and grains are organo selenium compounds which include selenocysteine and selenomethionine [5]. Selenium is an essential trace element of several major metabolic pathway, including thyroid hormone metabolism, antioxidant defense systems and immune function [6]. Glutathione peroxidase enzyme regulate free radical formation, which protects cell membranes from damage hence prevents aging and disease for this reason, our body use selenium to produce this enzyme [4]. It has come to the public's attention because of the role of selenium in cancer prevention and in decreasing cardiovascular diseases in human beings, which increase the concern of tracing this element all the way from the soil to plant through food chain via animals to human beings [7]. The availability of selenium to plants is also strongly influenced by several soil factors like pH, salinity and the content of $CaCO_3$ [8]. Because of its unique properties, Selenium is considered a link between

metals and nonmetals [9].The range between the concentration in which Selenium is essential and toxic is very narrow [10]. In Nature, selenium found in a relatively small concentration in rocks, coal and other fossil fuels [11]. Volcanic eruption, smelting ceramics, and metallurgical operations, manufacture of pesticides, glass and electronic goods [12]. It is also be present in cigarette paper, tobacco, and various cosmetic samples [13]. The major sources of selenium in many diets are cereals, meat products, and seafood. Only small amount are usually contributed by dairy products, and still less by vegetables and fruits [14]. The beneficial role of micro-amounts of selenium in biological and environmental systems has encouraged the development of analytical strategies for its determination at trace levels [15].

This paper describes the detailed reaction Scheme mechanism of a reliable and sensitive method based on a three step reaction (oxidation, diazotization and coupling) which produces colored azo dye that can be analyzed spectrophotometrically at 401 nm. The advantages of the method are its high sensitivity and the fact that simplicity of measurement and low cost.

MATERIALS AND METHODS

Apparatus: All spectral measurements have been carried out using UV-Visible Spectrophotometer Shimadzu (UV-1650) PC, Japan and UV-Visible Spectrophotometer, single beam, Apel PD-303 UV.
Reagents: All reagents used were of analytical grade chemicals and distilled water is used throughout the experiment.
Selenium solution: 100 ppm stock solution of Se was prepared by dissolving 0.0197 g of SeO2 in 100 mL of distilled water 10 ppm was prepared by appropriate dilution of stock daily.
Hydroxylamine Hydrochloride: 2M of aqueous $NH_2OH.HCl$ was prepared by dissolving 14.0383 g of it in 100mL of distilled water.
P-amino benzoic acid: $2x10^{-2}$ M was prepared by dissolving 0.2769 g of p-amino benzoic acid in 100 mL of 2.5 M HCl.
Hydrochloric Acid: 0.5 mL of concentrated HCl was used for diazotization reaction.
P-nitro phenol: $2x10^{-2}$ M was prepared by dissolving 0.2810 g of p-nitro phenol in 10mL of 2.5M of NaOH and then made up to 100mL by distilled water

Procedure: 4 mL of freshly prepared Se (IV) solution are mixed with 0.5 mL of 2M hydroxylamine hydrochloride (NH2OH.HCl), 0.5 mL of concentrated HCl and 0.5 mL of 2 x 10^{-2} M p-amino benzoic acid. The solutions are shaken and heated at (50 ºC for 50 min. Next, the mixture was added to 0.5 mL of 2 x 10^{-2} M p-nitro phenol in alkaline medium. The solution is heated at 50 ºC for 15 min. This leads to the formation of colored azo dye and after dilution with the distilled water in 25 mL volumetric flask, the colored complex is measured with a spectrophotometer at 401 nm as shown by figure 1.

RESULTS AND DISCUSSION

Reaction mechanism: The color reaction for the proposed system may be as follows [16]

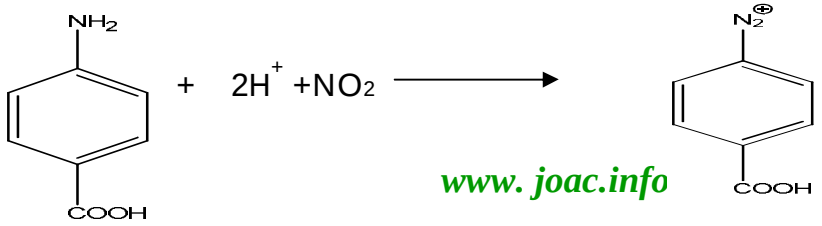
Step I: Selenite ions Se (IV) oxidizes NH2OH.HCl to HNO2

$$SeO_2 + H_2O \longleftrightarrow H_2SeO_3$$

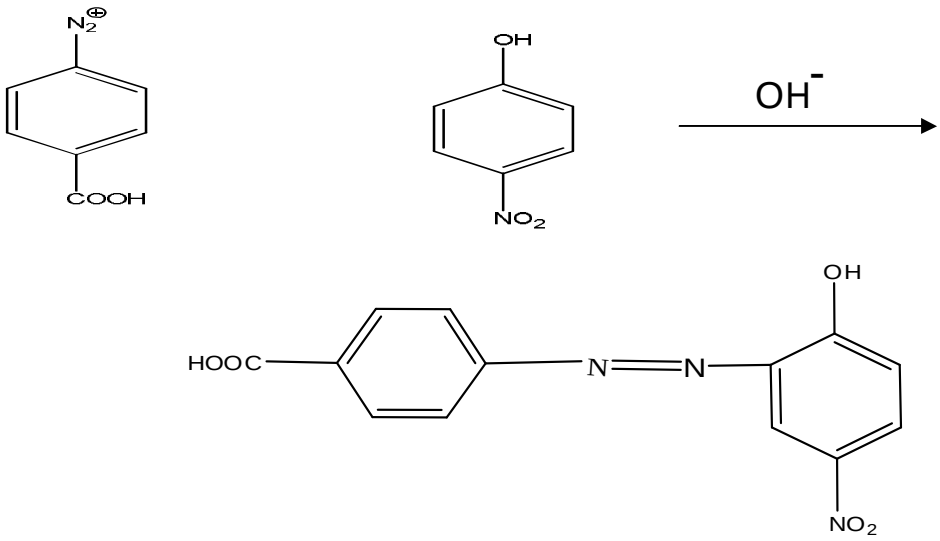
$$H_2SeO_3 \longleftrightarrow HSeO_3^- + H^+$$

$$NH_2OH.HCl + HSeO_3^- + H^+ \longleftrightarrow Se + HNO_2 + 2H_2O$$

Step II: HNO_2 produced diazotizes p –amino benzoic acid producing a diazonium salt .

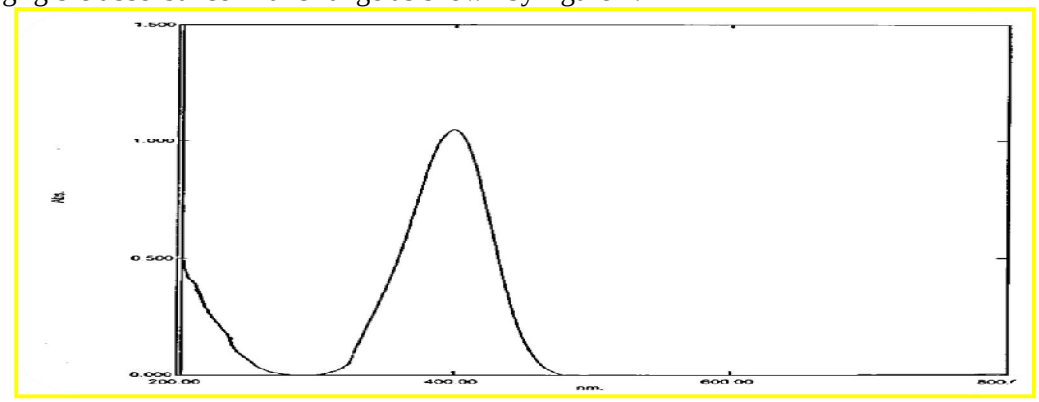


Step III: Coupling between p-nitro phenol and diazotized p-amino benzoic acid in alkaline medium, thus forming a stable colored dye. The method is based on the oxidizing property of selenite ions in acidic medium



Colored solution of azo dye

Absorption spectra: The colored dye formed exhibit maximum absorbance at 401nm. Reagent blank shows negligible absorbance in this range as shown by figure 1.



D= Dyes, B=Blank

Figure 1. Absorption spectra of sample with the blank,

Study of the reaction optimum conditions

Reagents: The optimum concentration of reagents used in the reaction were found.

Selenium solution: 2 ppm was prepared by appropriate dilution of the stock daily**.**

Hydroxylamine Hydrochloride: 0.8 M was prepared by appropriate dilution of stock solution daily**.**

P-amino benzoic acid: 1x 10^{-3} M was prepared by appropriate dilution of the stock solution daily.

Hydrochloric Acid: 1 M was prepared by appropriate dilution of the concentrated acid daily**.**

P-nitro phenol: 8 x10^{-3} M was prepared by appropriate dilution of the stock solution daily.

Optimization of the reaction conditions

Effect of Time on diazotization reaction: The time required for complete diazotization reaction was investigated. The results indicated that 30 min was needed to give complete diazotization process as shown by figure 2.

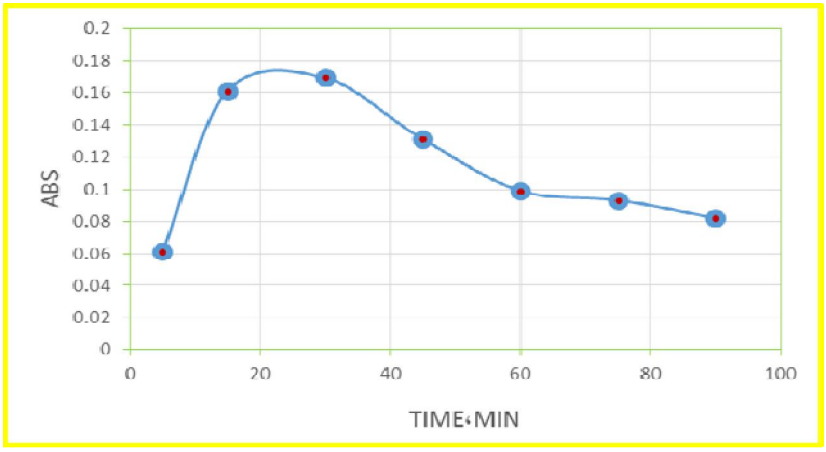


Figure 2. Effect of Time on diazotization reaction

Effect of temperature on diazotization reaction: The reaction was studied for the temperature range 15 – 70 ºC .The results indicated that the optimum absorbance is obtained at 50 ºC. It is therefore recommended that the diazotization reaction should be carried out at 50 ºC as shown in figure 3

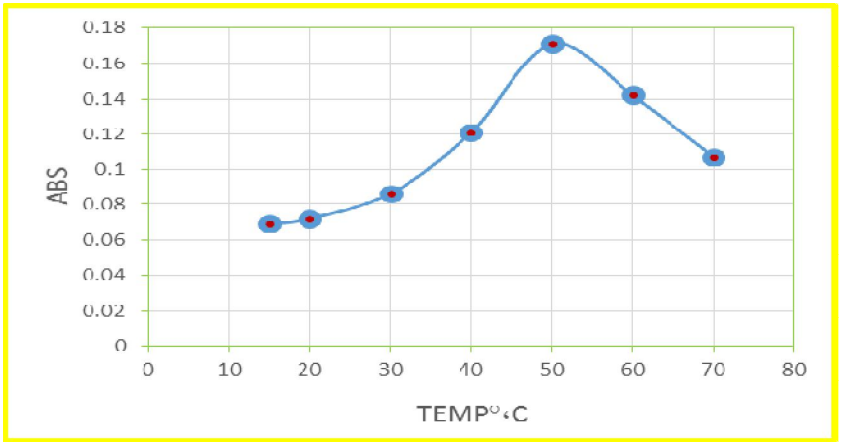


Figure 3. Effect of Temperature on diazotization reaction

Effect of acid type and quantity of acid used on the diazotization reaction: Different amounts of various acids have been studied in diazotization reaction. The experimental results showed that 2 mL of 1 M HCl solution was selected for the reaction because it gave the highest color intensity of the formed product and good color contrast with the other acids as shown in table1.

Table 1. Effect of quality and quantity of acid used for diazotization reaction

Amount / 1 M of acid	Absorbance					
	0.5 mL	1 mL	1.5 mL	2 mL	2.5 mL	3 mL
HCl	0.171	0.173	0.178	0.182	0.172	0.169
H_2SO_4	0.082	0.083	0.098	0.099	0.092	0.085
HCOOH	0.071	0.078	0.085	0.087	0.082	0.079
CH_3COOH	0.064	0.074	0.080	0.083	0.081	0.063

Effect of base type and quantity of base media on the coupling reaction: The preliminary experiments have shown that the colored dye can be developed only in alkaline medium. Many bases solutions with different amount are examined and the results indicated that 2 mL of 2.5 M NaOH produces the highest intensity of the dye and better color as shown in table 2.

Table 2. Effect of quality and quantity of base media for coupling reaction

Amount / 2.5 M of base	Absorbance			
	1.5 mL	2 mL	2.5 mL	3 mL
NaOH	0.113	0.193	0.161	0.159
NH_4OH	0.013	0.018	0.014	0.011
CH_3COONa	0.019	0.028	0.014	0.013
Na_2CO_3	0.021	0.029	0.027	0.025

Effect of p-amino benzoic acid amount for diazotization reaction : The effect of different amount on the absorbance has been studied. The results in figure 4 show that 1.5 mL of 1x 10^{-3} M p-amino benzoic acid was optimum and it was recommended for the subsequent experiments.

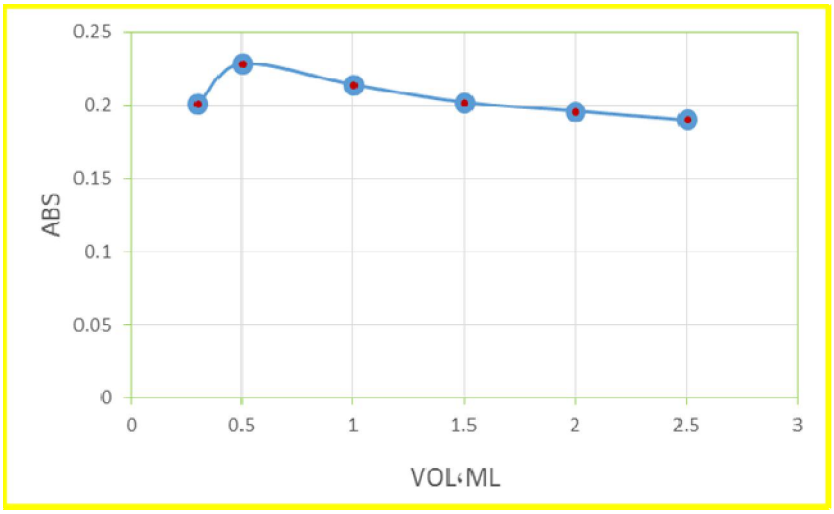


Figure 4. Effect of p-amino benzoic acid amount for diazotization reaction

Effect of hydroxylamine hydrochloride amount for diazotization reaction: It was found from the experimental results that 0.5 mL of 0.8 M $NH_2OH.HCl$ was more suitable to give highest absorbance for the azo compound formed as shown in figure 5.

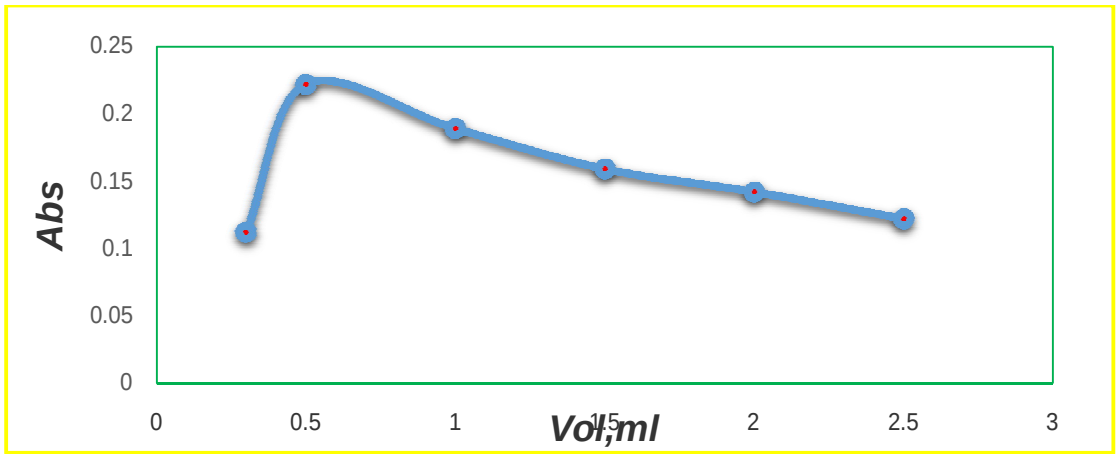


Figure 5. Effect of hydroxylamine hydrochloride amount on the diazotization reaction

Effect of p-nitro phenol amount for coupling reaction: The effect of p-nitro phenol amount on the absorbance of the azo dye has been studied. It was observed from the results that 0.5 mL of solution 8 x10 $^{-3}$ M was more suitable to give the highest intensity value for the azo dye as shown in the figure 6.

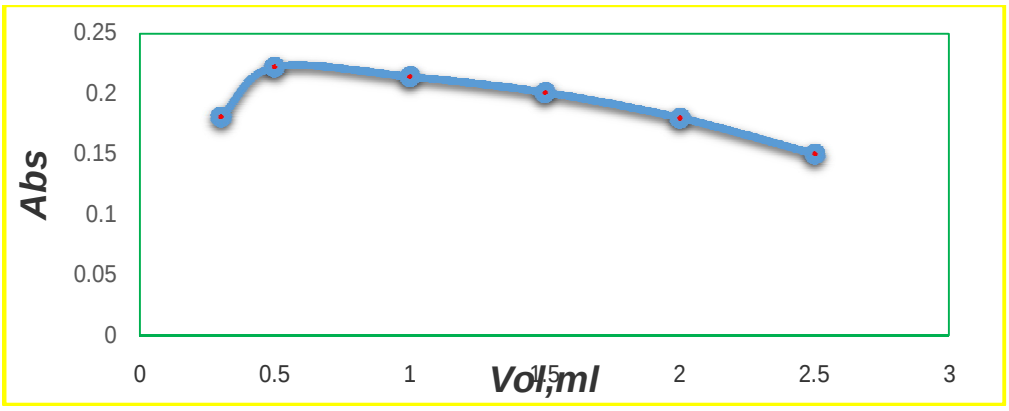


Figure 6. Effect of p-nitro phenol amount for coupling reaction

Effect of Time on coupling reaction: The time required for complete coupling reaction was tested. The results indicated that 20 min was needed to give the complete coupling process**.**

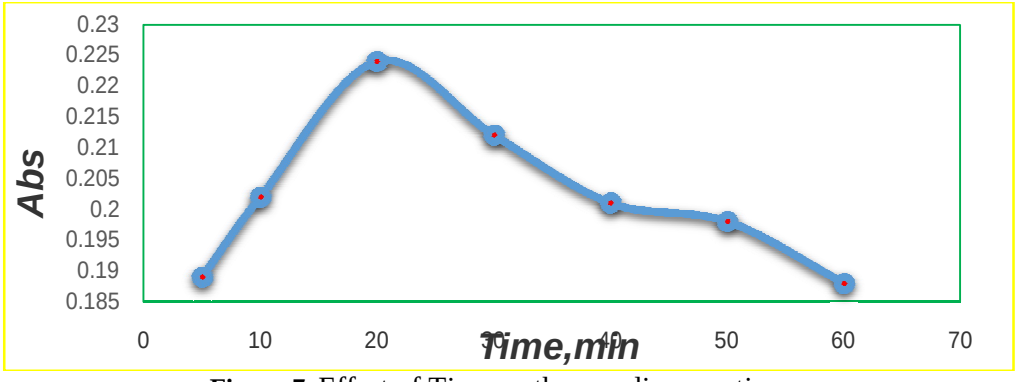


Figure 7. Effect of Time on the coupling reaction

Effect of temperature on coupling reaction: The reaction was studied by varying the reaction temperature for the range 10 - 70 ºC .The results indicated that the maximum absorbance get was at 50 C. Therefore it is recommended that the coupling reaction should be carried out at 50 ºC as shown in figure 8.

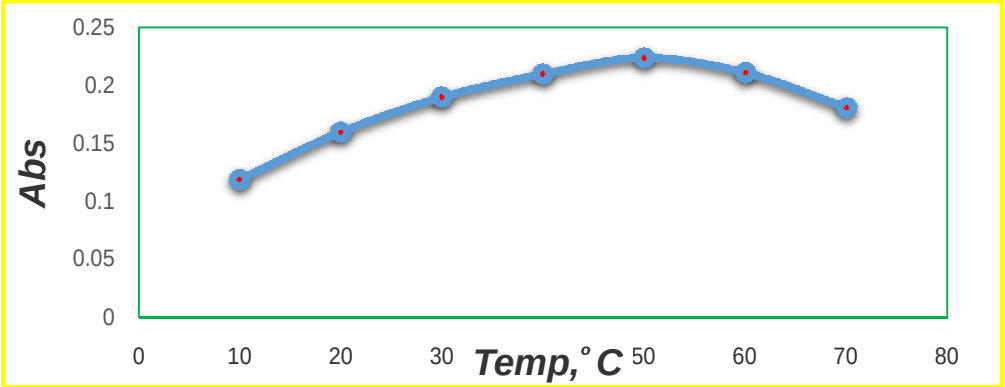


Figure 8. Effect of temperature on coupling reaction

Calibration Curve: Under the recommended conditions described above and mentioned in general assay procedure, a linear calibration curve (Figure 9) for selenium was obtained, which shows that Beers law obeyed the concentration range of 0.04 – 0.4 ppm with a correlation coefficient of 0.9985 .The conditional molar absorptivity was found to be 54885.09 L.mol $^{-1}$ .cm $^{-1}$ and sandells' sensitivity was 0.0014 µg.cm^{-2} .

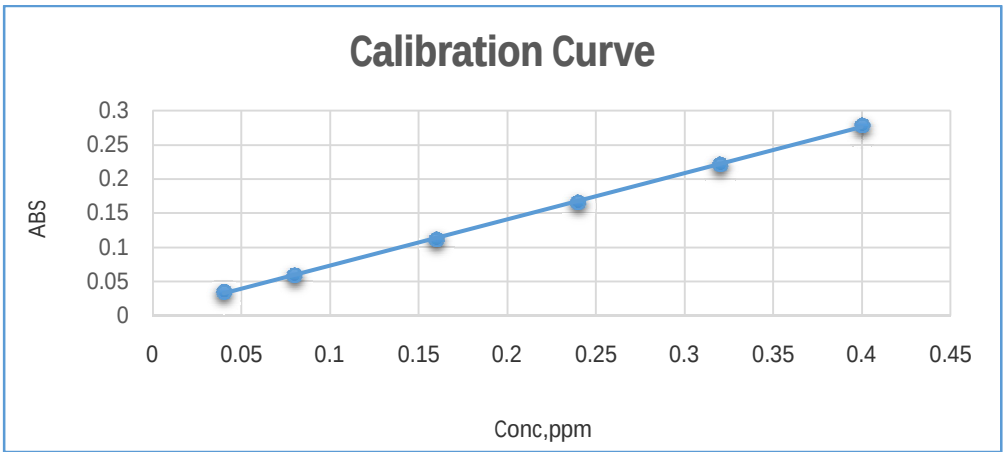


Figure 9. Calibration Curve

Accuracy and precision: To determine the accuracy and precision of the method, Selenium was determined in seven times at the same concentration 0.24 ppm. The relative standard deviations, recovery and all the analytical data obtained from this method were summarized in table 3.

Table 3. Analytical data obtained from proposed method

parameters	value
Beer's law limits (µg.mL^{-1})	0.04-0.4
Molar absorbtivity (L.mol $^{-1}$ .cm $^{-1}$)	54885.09
Sandell's sensitivity (µg.cm^{-2})	0.0014
Slope (a)	0.6951

Correlation coefficient	0.9985
λmax (nm)	401
RSD (%)	0.6024%
Regression equation	Y=0.6951x
LOD ($\mu g.mL^{-1}$)	0.0047
LOQ ($\mu g.mL^{-1}$)	0.0143
S.D	0.001
Recovery%	97.9166

Effect of interfering ionic species: The method has been checked for its validity in the presence of various interfering ionic species when exist in fifty and hundred times more than the concentration of Selenium. Interferences of Fe^{2+}, Fe^{3+} and Cu^{2+} ions are showing interference effect where it is overcome by masking with EDTA prior to analysis. The results obtained were given in table 4.

Table 4. The recovery of various interfering ionic species

S. No	Interfering ions	Re% fifty times the concentration of selenium	Re% hundred times the concentration of selenium
1	**Na^{+}**	**99.095**	**100.495**
2	**Sn^{++}**	**102.707**	**102.989**
3	**Ni^{++}**	**97.809**	**98.417**
4	**Mn^{++}**	**99.286**	**99.889**
5	**Hg^{++}**	**98.939**	**99.549**
6	**Sr^{++}**	**102.508**	**101.106**
7	**Pb^{++}**	**99.893**	**100.506**
8	**Cl^{-}**	**98.091**	**98.696**
9	**SO_4^{--}**	**100.098**	**102.473**
10	**NO_2^{-}**	**98.203**	**00.176**
11	**CO_3^{--}**	**98.543**	**99.944**
12	**NO_3^{-}**	**100.928**	**102.019**

APPLICATIONS

Iraqi vegetables samples get from the local grocery market in Babylon Governorate were cleaned, dried and digested by acid (5 g of tissue and 10 mL conc HNO3 + 10 mL of HClO4) .Soaked overnight and then digested for 30 min at 50 ºC. 10 mL of water was added to the cooled residue and heated again for 10 min .Then 5 mL of conc. HCl was added and heating continued for 10 min. All samples were filtered using filter paper. The contents were diluted to 50 mL after adding 6 mL of EDTA Solution. An aliquot of 3 mL of samples was taken and selenium was determined as in the recommended procedure.

Table 5. Selenium found in environmental samples

S. No	sample	Selenium found (ppm)
1	Garlic	0.302
2	Potatoes	0.256
3	Tomatoes	0.201
4	Cucumber	0.205
5	Lettuce	0.185
6	cauliflower	0.129

Comparison of the proposed method with the other Quantitative methods: Several previous analytical methods have been reported for determination of selenium in this paper. These methods include Graphite Furnace Atomic Absorption Spectrometry [17], Coupled Ion Chromatography- Hydride Generation Atomic Absorption Spectrometry 18], Hydride generation –atomic fluorescence Spectrometry (HG-AFS) [19] and table 6 summarizes some of these methods.

Table 6. Comparison of proposed method with the other reported methods

S. No.	Reagents used	Method	λmax (nm)	Range of Determination	reference
1	Dithizone	Cloud point extraction	424	5-100 ppb	(10)
2	4-methyl-o-phenylenediamine	spectrphotometry	322	0.5-4 ppb	(5)
3	Methylene blue and sodium sulfide	Kinetic Catalytic Spectrophotometry	-	2.5-30 ppb	(20)
4	2,3-diaminonaphthalene	Solid phase extraction - multisyringe flow injection	λex=380 λem=595	5.7-1290 ppb Absorptiometry 10-850 ppb Fluorometry	(21)
5	Hydroxylamine hydrochloride,sulfanilic acid,N-(1-naphthyl) Ethylene diamine dihydrochloride	spectrphotometry	550	0.05-0.26 ppm	(12)
6	Hydroxylamine hydrochloride ,p-nitro phenol,p-amino benzoic acid	spectrphotometry	401	0.04-0.4 ppm	proposed method

CONCLUSIONS

A simple, rapid and sensitive spectrophotometric method has been evaluated for the determination of selenium in some Iraqi vegetables samples. The method is based on diazotization-coupling reaction to form yellow azo dye which is stable for more than ten hours and has a maximum absorption at 401 nm. The proposed method has been successfully applied to the determination of selenium and the experimental results refer that the concentration of selenium in the studied samples can be arranged as follow: Garlic Potatoes Cucumber Tomatoes Lettuce Cauliflower.

REFERENCES

[1] D.T.Win," Selenium: Atomic Number 34, Mass Number 78.96", Auj.T, **2003,** 7, 1-7.
[2] Y.Mehdi, J.Hornick, L.Istasse and I.Dfrasne, *Molecules*, **2013,** 18, 3291-3311.
[3] T.Kurkova, L. Skrypnik and E. Zalieckiene, *CHEMIJA*, **2008,** 40-43.
[4] S.Sounderajan, G.K.Kumar and A.C .Udas, *Journal of Hazardous Materials*, **2010,** 175, 666 -672.
[5] S.Kartal, T. Oymak and S. Tokalioglu, **2010,** 65, 1250-1256.
[6] H.Stosnach, *Spectrochimica Acta Part B*, **2010,** 65, 859 – 863.
[7] R. Wakim, I.Bashour, M.Sidahmed and I.Toufeili, *Journal of Geochemical Exploration*, **2010,** 107, 94-99.
[8] P.Smrkolj and V.Stibilj, *Analytica Chimica Acta*, **2004,** 512, 11-17.
[9] D.R.Sharma and A.Sulbha, *International Journal of Research in Chemistry and Environment*, **2012,** 2, 88-95.
[10] M.H.Soruraddin, R.Heydari, M. Puladvand and M .M, Zahedi, *International Journal of Analytical Chemistry*, **2011,** 1-8.
[11] A.S.Bashammakh, *International Journal of Chemistry*, **2010,** 2, 91-97.

[12] S.R.Dubey, J.Smita and A. Sulbha, *Research Journal of Recent Sciences*, **2012,** 10, 51-54.
[13] K.Lina and A. Joumaa, *World Journal of Pharmacy and Pharmaceutical Sciences*, **2012,** 1, 820-830.
[14] F. C. Silva,G.Marques, C.C Matos, A.I.R.N.A.Barros and F.M Nunes, *Food Chemistry*, **2011,**126, 91-96.
[15] J. Pedro, Andrade D.Magni, M .Tudino and A, Bonivardi, *Analytica Chimica Acta*, **2004,** 516, 229 -236.
[16] A.Matamoros and L.G.Benning, *Mineralogical Magazine*, **2008,** 72, 451-454.
[17] A.M.Abdel-Lateef, *Arab Journal of Nuclear Science and Application*, **2013,** 46, 144-151.
[18] J.B.Garcia, M.Krachler, B.Chen and W.Shotyk, *Analytical Chimica Acta*, **2005,** 534, 255-261.
[19] S.Goldberg, D.A.Martens, H.S.Forster and M.J.Herbel, *Soil SCI.Soc.AM.J.*, **2006,** 70, 41-47.
[20] A.Songsasen,P.Aukkaray unyong ,S.Bangkedphol and W.Sasomsap,*Kasetsart J.(Nat.Sci.)*, **2002,** 36,103-109.
[21] A.M. Serra, J.M.Estela, B.Coulomb, J.L.Boudenne and Cerda**,** *Talanta*, **2010,** 81, 572 -577**.**