

**MODIFICATION SPECTROPHOTOMETER METHOD TO EVALUATE
SELENIUM LEVELS IN THE IRAQI MARINE WATERS**

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Abstract

A Spectrophotometric method has been modified for determination of selenite using 4-methyl-o-phenylene diamine as a Piazselenol complex in acidify media about (pH=1.5) , Adjust pH of solution by concentration (0.1N) Hydrochloric acid, Identified optimal condition as a temperature, formation time of the complex, volume of sample and amount of MOPDA . The detection limit for this method is 0.535 ($\mu\text{g} / \text{l}$) ,the relative standard deviation for eight replicate measurements is 0.66% for 40 ($\mu\text{g} / \text{l}$) of selenium. This study applied to samples from the Iraqi marine waters to estimate the concentration of selenite (selenium IV) and total selenium. In this study Selenite concentration was recorded about (14-34 $\mu\text{g}/\text{l}$), while total selenium was recorded about (15-73 $\mu\text{g}/\text{l}$) .

Keywords: Spectrophotometry , 4-MOPDA, ICP analysis

Introduction

Selenium is a complex class due to its properties of being both essential and toxic, leaving a tight optimal range within which intake amounts are healthy. Both excesses and deficiencies in Selenium are known to cause endemic disease (Stoica et al., 2002, Sargar et al., 2011, Baqir and Ameer 2014 and Trevisan et al., 2011). The four natural oxidation states of selenium are Elemental selenium (0), selenide (II) selenite (IV), and selenate (VI) (Soruraddin et al., 2011 and Bao et al., 2012). Inorganic selenite and selenate are dominant class in waters while organoselenium compound, selenocysteine and selenomethionine, are the main species found in vegetables and grains (Baqir and Ameer, 2014 and Terol et al., 2015). Both selenite and selenate are highly available forms of selenium for living organisms (Schneider et al. 2015). Selenium can accumulate in the body tissues of organisms and can pass through the food chain (Schneider et al. 2015 and Shabani et al. 2013). A variety of analytical methods can be used to determine trace concentrations (ng/g) of selenium. These include fluorometry, neutron activation analysis (NAA), atomic absorption spectroscopy (AAS), inductively plasma-atomic emission spectrometry (ICP-AES), coupling mass spectrometry with inductively coupled plasma (ICP-MS), the gas chromatography (GC), spectrophotometry and fluorescence

analysis of x-ray (Baqir and Ameer,2014,Trevisan et al.,2011, Soruraddin et al.,2011,Valencia et al.,1999 , Niedzielski and Siepak,2003). Popular spectrophotometric determination reagents selenium are dithizon , o-phenylenediamine and chromotropic acid. Based methods require the use of amines long reaction time (~ 2 hours),these methods are not selective and they are subjected to various interferences. A new reagent for the spectrophotometric determination of selenium is 6-amino- Naphtholo-1-sulfonic acid , forming Selenium (IV) to pH 1-2.5 a yellow complex showing a maximum absorption at 392 nm.The method is substantially free of interference and achieves a detection limit of 80ng/ml (Ramachandran et al.,1993). Selenium reacts with the 2,3-diaminenaphthalene presence of bromide ions acting as a catalyst, forming a complex that can be extracted by cyclohexane at an acidic environment. The absorption maximum of the complex is at 378.5 nm and the detection limit of the method is 12 ng/ml(Ramachandran and Kumar ,1996). In this study, we aim at developing a previous method (Kartal et al.,2010) that depends on the reaction of Selenium (IV) in acidify media(pH 1.5) as selenous acid with 4-methyl- o-phenylenediamine leading to the formation of the 5-methyl-piazselenol complex (see Fig 1) , affecting the parameters determined to the optimal conditions: such as pH, time of formation of the complex , MOPDA amount, ionic strength, sample volume.

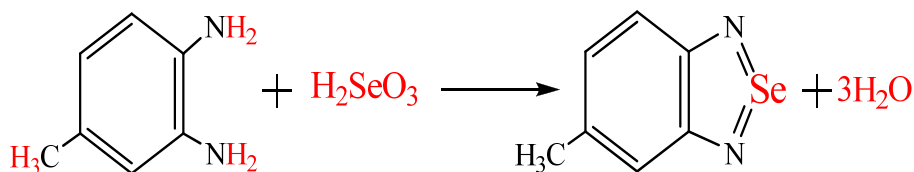


Fig.1 Formation of the5-methyl-piazselenol complex

Experimental

Instrumentation

Selenium determination after formation of the complex by Shimadzu double- beam UV-Vis spectrophotometer (model 1800 PC , Japan) with 1.0 cm quartz cell was used for all spectral measurements and total selenium was determined also by ICP-OES (HORIBA JY 2000-2). Magnetic stirrer was used to mix all samples.All samples of water were collected by instrument of water sampler collection.

Material and Chemicals

Selenium dioxide (Purity 99.8%) and KSCN were supplied by Sigma-Aldrich. Nitric acid (65%) and Hydrochloric acid (37%) were obtained from Scharlau, Hexan was purchased from J.T.Baker, 4-MOPDA was supplied by Merck, Hydrogen Peroxide (35%) was obtained from Riedel-de Haen (Germany), EDTA Na₂ was obtained from G.C.C and Sodium hydroxide was obtained from Himeda. Deionized water was used for preparation of all solutions.

Preparation of solution

The Stock solution of selenium(IV) (1000 ppm) was prepared by dissolving (1.405 mg) from SeO₂ and diluted to 100 ml by acidify water (10% HCl). From this solution serial dilutions were made to obtain different concentration levels of (0.2, 0.5, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppb) of selenium (IV). MOPDA solution (8.185 × 10⁻³ M, 0.1% w/v) was prepared by dissolving (0.1 gm) from 4-MOPDA in small volume of HCL diluted and complements to 100 ml by 0.1 N of HCl solution. EDTA Na₂ Solution (0.125 M): An exact amount of 4.65 gm of EDTA di sodium was weighed and dissolved in small volume of water, and was complemented to 100 ml with deionized water, from this solution 0.02 M was prepared and complement to 100 ml by deionized water. Preparation of KSCN Solution (2M): 9.7 gm of KSCN was weighed and dissolved in water, then complemented to 50 ml by deionized water and prepared from this solution (0.05 M) in 100 ml deionized water.

Recommended Procedure

Solution volume of sample about (25 ml), Hydrochloric acid (0.1 N) is used for adjusting pH solution about (1.5 = pH). Potassium thiocyanate (0.02 M) and EDTA disodium (0.05 M) were used as complexing agents for eliminating interferences with ferric, Zinc and copper ions. They were added to the solution before adding (1 ml) MOPDA reagent and reaction time necessary to form the 5-methyl selenol complex. The formed complex was extracted into the organic phase with 10 mL of *n*-hexane, and finally the measurements of absorbance were performed by UV-Vis spectrophotometry at 332 nm (Kartal et al., 2010), while the estimate of total selenium should digest the samples with Hydrogen peroxide 30%, to convert all selenium forms in samples to selenium(VI), it has been using the method described (3500-Se B.3) for digestion with Hydrogen peroxide (APHA., 1999). After that, the solution was digested by concentrated Hydrochloric acid, to convert Selenium (VI) to Selenium (IV), 25 ml of the sample solution was mixed with 25 ml of concentrated HCl. The solution was put on hotplate at 85°C in an open system (almost to dry). After being cooled, the solution was

diluted with HCl(0.1N) to constant volume (APHA.,1999 and Cacho et al.,2013), and then conducted in the same manner as described above.The total selenium for Z_1 sample was determined with ICP instrument as standard method .

Results and Discussion

Effect of Shaking Time on Complex Formation

To determine the optimal shaking time for a complex formation, 25 ml of solution containing 100 $\mu\text{g/l}$ of selenium was added to 1 ml from complex agent MOPDA ,this solution was shaken at different time . The formed complex was extracted with 10 ml n-hexane ,then measured in UV-Vis Spectrophotometer at 332 nm , the results are given in (Fig 2). The maximum absorbance values were obtained after 10 min of shaking as shown in Fig 2.

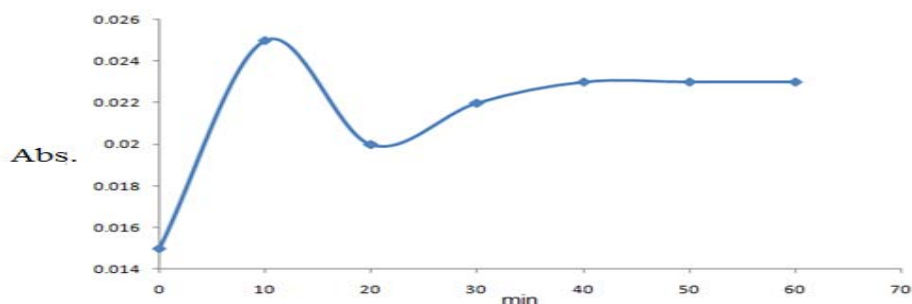
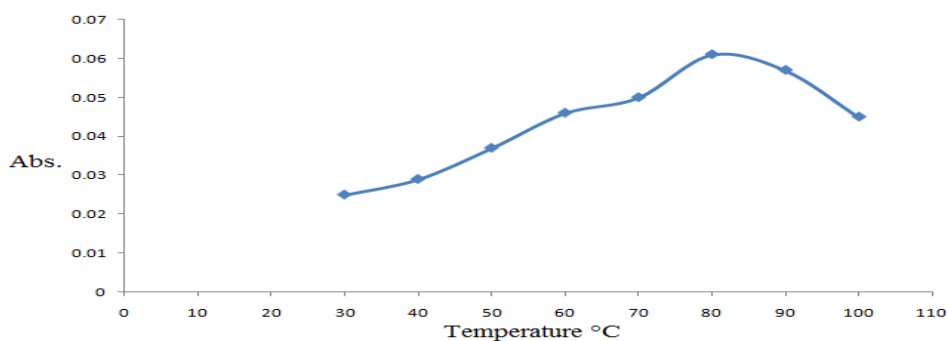


Fig.2 Effect Shaking time on complex formation

Effect Temperature on complex formation

Optimal temperature was selected in complex formation . About 25 ml from selenium solution containing 100 $\mu\text{g/l}$ was taken and added 1ml of MOPDA .The solution was left in constant stirrer about 10 minutes , This solution was shaken at different temperatures begining from room temperature to 100°C , after then the formed complex was extracted with n-hexane and measured by UV-Vis spectrophotometer . The results are given in (Fig 3). The maximum absorbance values were obtained at range 70-80 °C . The optimal temperature is shown in Fig 3.



Analytical Performance

Analytical characteristics for determination of selenium were studied under optimum conditions. Calibration curve was obtained by 25mL of the solution containing 0.2, 0.5,1,5,10, 20, 30, 40, 50, 60, 70,80,90 and100 µg/l of selenium (IV) under the optimal experimental conditions. Calibration curve is shown in fig.4 , the result obtained from the calibration curve appears extent of Beer’s law (1-40) µg/l. In order to determine detection limits for this method , it was evaluated with blank solution (n=8) . The detection limits was calculated by equation [$DL=(2SD \times \text{Conc. of metal}) / \text{means of Abs.}$, where SD is standard deviation] (Radhy,2010) . Analytical parameters for the determination of the selenium complex are presented in table 1 . The relative standard deviation was for six replicate of standard selenium solution 40 µg/l.

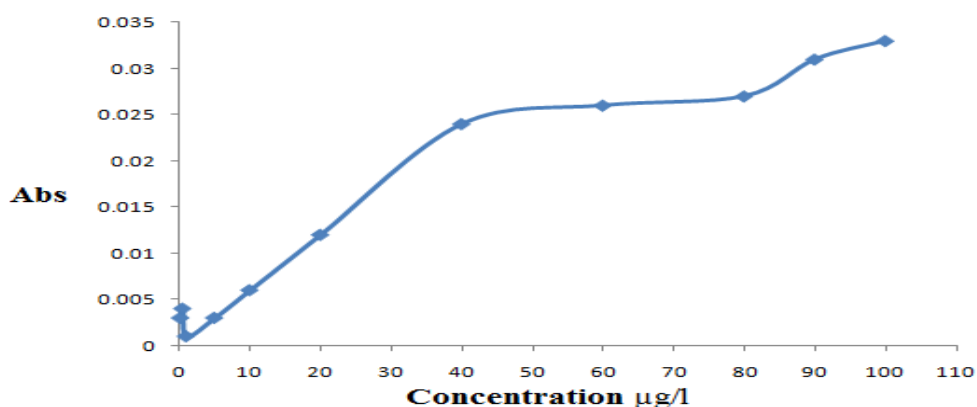


Fig.4 Standard curve of Selenium (IV) Solution

Table 1: Analytical parameters for the determination of the selenium complex.

Parameters	Selenium complex
λ_{max} (nm)	332
Beer’s law range (µg / l)	1 - 40(µg / l)
Relative standard deviation (RSD) %	0.66
Detection limits (DL)	0.535(µg / l)
Standard deviation (SD)	2.1×10^{-4}
Correlation coefficient (r)	0.998

Effect of Various cations on complex formation

This study focuses on interference with cations such as ferric , zinc and copper ions. Because these ions interfered more with the measurement of selenium (Kartal et al.,2010) , The effect

of ions on the determination of 100 µg /l of selenium (IV) was studied, and the results are summarized in table 2 . The results show the interfere ferric ion with measurement of selenium (IV) in high concentration of ferric ions when be tenfold or more of selenium concentration . This effect can be removed using solution of KSCN(0.05M) . While, noting the concentration effect of Zinc and copper ions on determination of selenium(IV) up to twofold for copper ion and fivefold for zinc ion for selenium concentration in solution, this effect can be removed by EDTA disodium solution(0.02M) , through various volumes from adding complexing agents to solution that contains 100 µg/l of selenium(IV). The results shown in table 3 . Those results showed high recovery ratio for selenium absorbance in solution . This refer to the complete removing for interfere ions . This appear evidently when mixed ions in solution were used .

Table 2 : Effect of cations on the determination of 100 µg /l of selenium (IV)

Sample taken (ml)	Selenium (µg/ l)	Abs. of Solution before addition ions	Metal Ion added	Absorbance of Solution after addition ions		
				Two fold	Five fold	Ten fold
25	100	0.040	-	-	-	-
25	100	0.040	Fe ⁺³	0.041	0.042	0.448
25	100	0.040	Zn ⁺²	0.044	0.052	0.053
25	100	0.040	Cu ⁺²	0.055	0.053	0.053

Table 3: eliminate interferences with measurement of Selenium

Sample taken (ml)	Abs. of Se(100 µg/l) solution before addition	Ion interference	Conc. Ion in solution µg/l	Abs. of Se solution with interfere of ion	Vol. of KSCN (0.05M) added (ml)	Vol. of EDTA di Na (0.02M) added	Abs. after addition	% Recovery
25	0.040	Fe ⁺³	1000	0.448	1	-	0.0490	122.5
					2	-	0.0462	115.5
					3	-	0.0421	105.25
					4	-	0.0399	99.75
					5	-	0.0360	90
25	0.040	Zn ⁺²	1000	0.053	-	1	0.0510	127.5
					-	2	0.0500	125.0
					-	3	0.0470	117.5
					-	4	0.0441	110.25
					-	5	0.0389	97.25

25	0.040	Cu ⁺²	1000	0.053	-	1	0.0490	122.5
					-	2	0.0460	115
					-	3	0.0446	111.5
					-	4	0.0423	105.75
					-	5	0.0400	100
25	0.040	Ions mixed Fe ⁺³ Zn ⁺² Cu ⁺²	1000 1000 1000	0.051	4	5	0.040	100

Application of the proposed method to real samples

In order to evaluate the analytical applicability, the method was applied for the determination of selenium in the Iraqi marine water. These samples were collected from various regions in winter season in December, 2014 . Eight sites were Selected along the Iraqi Marine waters extending from Fao to Khor alZubair port. This area is important in marine navigation for large number and different loads of Ships pass through it to Arabian gulf. Fig.(5) are shown below describing the selected sites in the Iraqi marine waters.

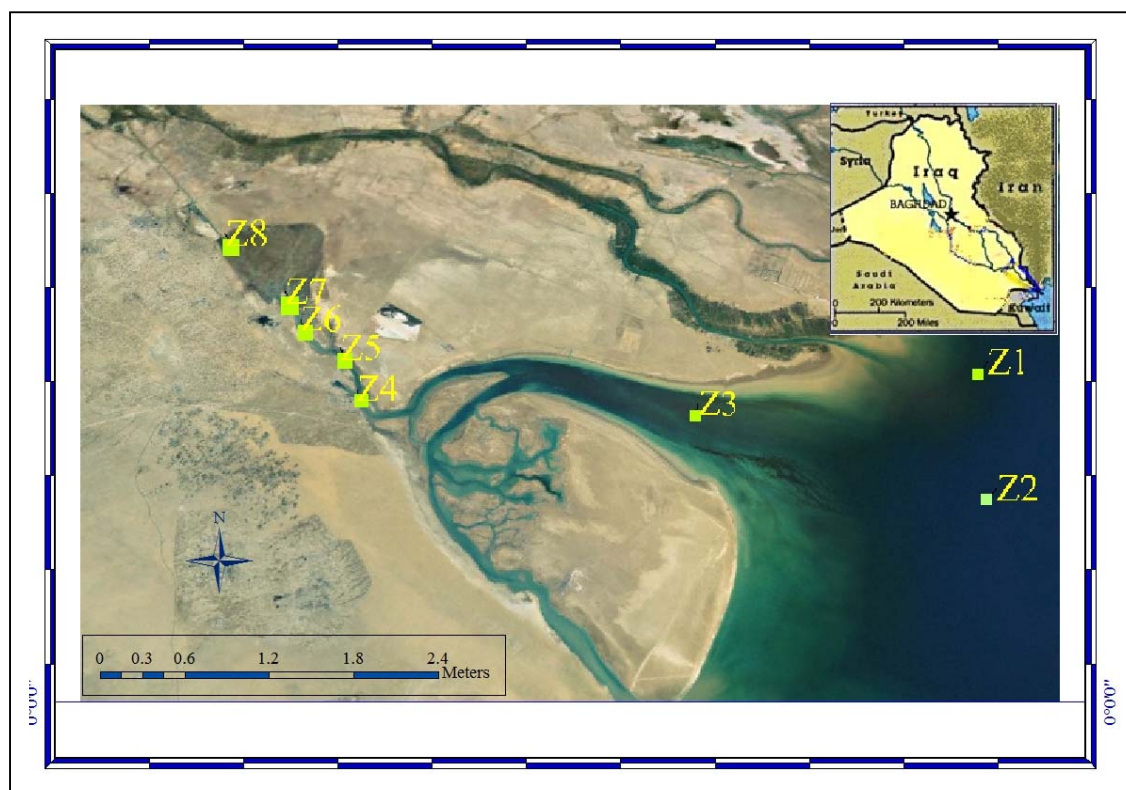


Fig 5 : Locations the sample selected in Iraqi marine water

All the samples were collected in polyethylene containers and kept for cooling . After that they were transferred into the laboratory for carrying multiple operations on samples such as filtration , digestion and analysis as described above in the procedure .Selenium (IV) and total Selenium were measured by UV-Vis instrument and also total selenium for Z₁ sample was measured by ICP-OES instrument for compared with the obtained results from Uv-Vis data. The results are given in the table 4 and 5. These results show the contamination of high concentrations for Selenium in sea water compared with other studies (Kartal et al.,2010 and Kutty et al.,1995). Selenium (IV) of the Iraqi marine waters which was recorded in this study was about (14-34 µg/l), while total selenium was recorded about (15-73 µg/l) , also gradual increase for measured values of selenium was noted from Fao towards Khor alZubair port . That may be due to marine navigation and the loading and unloading operations of commercial loads and oil inside the ports. The most important sources of selenium pollution of surface water through weathering operations, combustion coal and oil , movement of wind, irrigation and drainage operations, wastewater and industrial waste. All these processes cause the ecosystem pollution of selenium (Schneider et al.,2015 and Staicu et al.,2015). Therefore maximum concentration was recorded in nearby zone from the confluence of the Shatt al-Basra canal with Khor al-Zubair (Z₈) , because of the Shatt al-Basra canal is a drainage channel, where wastewater and industrial waste are released into canal without treatment, selenium concentration in canal will be increased. This is another reason to increase the concentration of selenium in the confluence zone with channel of Khor al-Zubair compared with Um-Qaser zone (Z₄) . Also the results of total Selenium concentration were compared with World Health Organization (WHO) data and Canadian Drink Water Guidelines (CGL) data for drink water (WHO.,2011 and CGL.,2014) , the results shows four stations within acceptable limits for WHO and CGL , while four other stations highest than the acceptable limit, as shown as in table 5.

Table 4: The results Compared of Total Selenium concentration

No.	Symbols of Sample	Data of UV-Vis	Data of ICP
		Conc. of total Se µg/l	Conc. of total Se µg/l
1	Z ₁	19.94138	19.943

Table 5: The results of Selenium concentration in Iraqi marine water .

No.	Symbols of Sample	Data of UV-Vis	
		Conc. of Selenite $\mu\text{g/l}$	Conc. of total Se $\mu\text{g/l}$
1	Z ₁	17.70517	19.94138
2	Z ₂	14.54828	15.78448
3	Z ₃	20.86207	21.9931
4	Z ₄	27.75517	52.64138
5	Z ₅	14.99483	17.46724
6	Z ₆	32.09655	67.37414
7	Z ₇	34.64828	67.63793
8	Z ₈	31.5431	73.34655
9	WHO 2011	-	40
10	CGL 2014	-	50

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