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Validation of spectrophotometric method for Se(IV) determination: analytical applications

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Abstract As selenium is an important part of the antioxidant enzymes and also because there are several studies suggesting a possible link between cancer and selenium deficiency, this paper presents a spectrophotometric method for the assay of Se(IV), using *N*,*N*-diethyl-*p*-phenylenediamine monohydrochloride as reagent. The proposed method is based on the reaction between the selenium and potassium iodide in low acidic medium, when iodine is released. This last product will further oxidise the new reagent. The final obtained product is strongly coloured in red and has an absorption maximum at 552 nm and molar extinction coefficient (ε) of 6.1×10^4 L mol^{-1} cm^{-1}. The optimum working conditions were established, and the developed method was validated, being characterised by a good linearity (in the range of 0.5–3.0 μg/mL), a limit of detection (0.0573 μg/mL) and a limit of quantification (0.1737 μg/mL). At the same time, the repeatability, the precision of the method and the accuracy were established. The proposed and validated method was applied with good results for the determination of Se(IV) in spring and bottled water from Iasi and also in pharmaceutical and cosmetic products.

Keywords Selenium · Spectrophotometric method · Validation

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Introduction

Selenium is an essential element for the human body (daily normal intake should be at about 200 μg), being an important part of the antioxidant enzymes which protect the cells against the effects of free radicals produced during the oxidative metabolism (Food and Nutrition Board 2000). The main sources of selenium are plants, water, sea fruits, fish and pharmaceutical products containing it (Whange 2002). Besides its role as an antioxidant alongside vitamin E, selenium maintains the elasticity of the tissues and slows down the aging process, and it is used in the treatment and prevention of dandruff and ensures normal growth and development of children (Revanasiddappa and Kiran Kumar 2001).

Many studies indicate associations between low levels of selenium and some diseases such as lung cancer (Knekt et al. 1998), colon rectal and prostate cancer (Lippman et al. 2009; Combs and Clark 2001), heart diseases (Neve 1996; Levander and Beck 1997) and rheumatoid arthritis (Kose et al. 1996). Also, selenium increases the immune

function (Beck et al. 2003), influences the HIV progression (Terry et al. 2000; Singhal and Austin 2002) and binds arsenic, cadmium and mercury in order to decrease their harmful effects (Sasakura and Suzuki 1998). The exceeding tolerable upper intake level of 400 μg per day can lead to selenosis (Hathcock 1997; Goldhaber 2003).

Spectrophotometric methods for the determination of metals are very common, especially the direct determination of inorganic metal compounds. Selenium(IV) can be determined quantitatively and indirectly through a spectrophotometric method in VIS domain. The method consists of a catalytic reaction of selenium ions with different redox reagents such as *p*-hydrazinebenzosulfonic acid, phenylhydrazine and 3-fluorophenylhydrazine after amine coupling to azides or reduction of sulphates (Niedzielski and Siepak 2003).

In this paper, a novel spectrophotometric quantitative determination method for selenium using *N*,*N*-diethyl-*p*-phenylenediamine hydrochlorate as reagent is presented. The proposed method is based on the reaction of Se(IV) with potassium iodide in acid medium, iodine being released. This further oxidises the reagent resulting in a bright red compound with an absorption maximum at 552 nm.

Further, the optimum conditions for the oxidation reaction when using *N*,*N*-diethyl-*p*-phenylenediamine monohydrochloride were established. At the same time, the effect of reagent, potassium iodide, HCl and sodium acetate concentrations; the reaction time; compound stability; and the influence of interferers were determined. After the validation, this spectrophotometric method was applied for the pharmaceutical products and water.

Materials and method

Reagents

All chemicals used for the reaction: HCl (0.1 M), potassium iodide (0.2 M), H_2SO_4 (0.5 M), HNO_3 (0.1 M), $CH_3COONa \cdot 3H_2O$, NaOH (10 %*w*/*v*) and $Na_2SeO_3 \cdot 5H_2O$ were reagent grade (Merck Darmstadt, Germany). Bi-distilled deionised water (non-absorbing under visible radiation) was also used.

First, a 0.1 mg/mL Se(IV) solution was prepared by dissolving 0.0333 g $Na_2SeO_3 \cdot 5H_2O$ in 100 mL bidistilled water. After, the above-mentioned solution was diluted in different standard solutions with concentrations between 0.5 and 3.0 μg/mL.

Reagent solution 0.01 % (*w*/*v*), 0.01 g of *N*,*N*-diethyl-*p*-phenylenediamine monohydrochloride (monohydrate), was dissolved in 100 mL bidistilled water.

Instrumentation

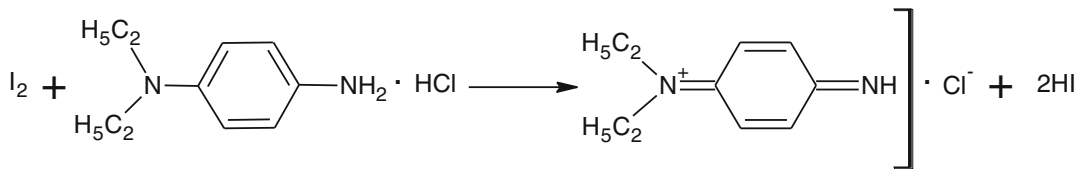
The electronic spectra were obtained using a Hewlett-Packard 8453 UV–Visible spectrophotometer, and the pH value of solutions was monitored using the Hanna Instruments 300 Series pH meter.

Method

The proposed method consisted in the release of iodine, which further oxidised the reagent in the presence of CH_3COONa (1 M), resulting a bright red compound with an absorption maximum at 552 nm.

The reactions took place as follows:

$$SeO_3^{2-} + 4I^- + 6H^+ \rightarrow Se + 2I_2 + 3H_2O$$



One millilitre solution containing 5.0–30.0 μg of Se(IV) was mixed with 1 mL potassium iodide (0.2 M) and 1 mL of HCl (0.1 M). After 10 min, other 1 mL of CH_3COONa (1 M) and 0.5 mL reagent (0.01 % *w*/*v*) were added. The obtained mixture was diluted with bidistilled water up to a volume of 10 mL. After another 10 min, the absorbance at 552 nm was measured against a reference sample.

The proposed method was applied to the determination of Se from tablets, lipstick and spring and bottled water from Iasi, Romania.

Results and discussions

The absorption spectra of the Se(IV) solution showed a spectral band with a maximum at 552 nm. The reagent used as a reference did not show any absorbance at this wavelength. The specific absorbance coefficients of the Se(IV) solution and the reaction product were $A^{1\,\%}{}_{1\ cm,\ 552\ nm}$=418 absorbance units (a.u.) and $A^{1\,\%}{}_{1\ cm,\ 552\ nm}$= 7,730 a.u., respectively.

In order to take a full advantage of the procedure, the reagent concentrations and reaction conditions must be optimised, to ensure that the optimum concentration of each component will give the smallest relative standard deviation (RSD). The effect of reaction variables such as the pH value and the concentration of HCl and sodium acetate were studied in detail by changing each variable in turn while keeping all the others constant.

The effect of the volume of HCl (0.1 M) used was studied by varying it between 0.5 and 2.0 mL, and the results are shown in Fig. 1. It was observed that the absorbance increased by increasing HCl volume up to 1 mL and then decreased when higher volumes were used. Therefore, 1 mL HCl solution (0.1 M) was considered the optimum volume, being used for further determinations.

According to the reaction between Se(IV) and KI, the concentration of the obtained iodine is proportional with Se(IV) concentration. In order to evaluate the effect of KI concentration on the absorbance, the volume of KI (0.2 M) was modified from 0.5 to 2.0 mL. From Fig. 1, it can be observed that a rapid increase of the absorbance occurred up to 1 mL KI (0.2 M), followed by a slow decrease when higher volumes were used.

The reaction between iodine and *N*,*N*-diethyl-*p*-phenylenediamine monohydrochloride occurred in low acidic medium. For optimising the reaction conditions, the effect of the volume of the sodium acetate (1 M) on the rate of reaction was studied in the range of 0.5–2.0 mL (Fig. 1). The results showed that the reaction rate increased when CH_3COONa volume increased up to 1 mL and decreased slowly for higher volumes, and the pH value necessary for the formation of the complex varies between 5.6 and 6.0. In consequence, 1 mL CH_3COONa (1 M) solution was selected for all determinations.

In order to evaluate the volume of the new reagent solution necessary for the stoichiometric reaction with the iodine, various volumes between 0.25 and 2.0 mL were used. The results showed an increase of the absorbance up to 0.5 mL reagent; between 0.5 and 1.0 mL, the absorbance was almost constant, and after those values, by increasing the reagent volume, the absorbance decreased. Thus, a reagent volume between 0.5 and 1.0 mL was suitable for the reaction.

The stability in time of the final reaction product obtained in the optimum conditions was investigated over 30 min. It was established that the optimum moment to measure the absorbance was between 10 and 25 min after the adding *N*,*N*-diethyl-*p*-phenylenediamine monohydrochloride.

The combination rate of Se/reagent was established by isomolar series method (Job's method) and is presented in Fig. 2.

For evidencing the influence of the Se(IV), reactive ratios on the stoichiometry of reaction, the volumes of these were varied in order to obtain ratio values between 0.2 and 2.0. From Fig. 2, one can observe that a ratio of

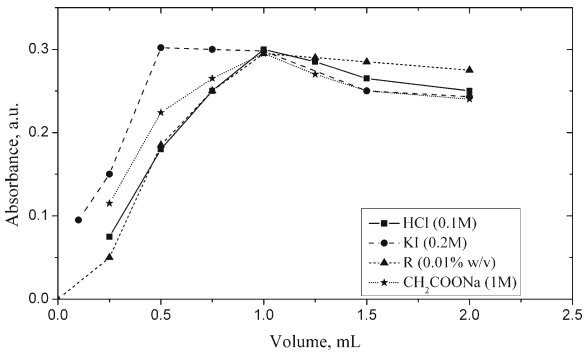


Fig. 1 The setting of the volume for HCl (0.1 M) solution, reagent (*R*), KI (0.2 M) and sodium acetate necessary for the Se(IV)–KI–R system

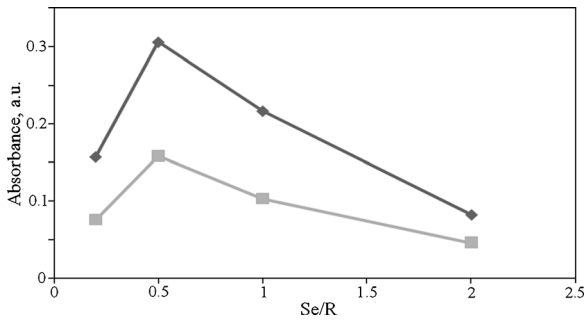


Fig. 2 Molar ratio Se/R

Table 1 Linearity of the method

No.	Concentration (μg/mL)	Absorbance				
		I	II	III	IV	Average
1	0.5	0.07843	0.07714	0.07709	0.07974	0.07810
2	1.0	0.15155	0.15461	0.14848	0.17300	0.15691
3	1.5	0.22765	0.22521	0.22276	0.23042	0.22651
4	2.0	0.30590	0.30255	0.30681	0.30866	0.30598
5	2.5	0.37890	0.38288	0.37101	0.37905	0.37797
6	3.0	0.46285	0.45601	0.45775	0.45387	0.45762

0.5 was the optimum value for the maximum rate of reaction.

Validation of the method

Further, the linearity of the method was evaluated, and for that (according to the procedure of the method), the samples were prepared, corresponding to the concentration range 0.5–3.0 μg/mL of Se(IV). The absorbance of each sample was measured against a reference sample at 552 nm. For each concentration, four determinations were made, and for further evaluation, the average value was used. After the processing of the data (shown in Table 1) through mathematical regression, the calibration curve was obtained (Fig. 3).

The equation of the calibration curve calculated through mathematical regression was as follows:

$$\text{Absorbance} = 0.1509 \times \text{Concentration} + 0.0032. \quad (1)$$

The limit of detection (LOD) and the limit of quantification (LOQ) were determined using the following equations:

$$\text{LOD} = 3.3 \times \frac{\text{SE}}{\text{Slope}} = 0.05731\ \mu\text{g/mL} \quad (2)$$

$$\text{LOQ} = 10 \times \frac{\text{SE}}{\text{Slope}} = 0.17370\ \mu\text{g/mL} \quad (3)$$

where SE is the regression standard error.

Using Eq. (1) for the calibration curve, the sample concentration was calculated. The RSD was of 1.50 % for the first set, 1.64 % for the second set and 1.57 % for both sets; these values were close to the system precision (RSD=1.3226 %). The statistical evaluation of the linearity revealed the correlation coefficient (r) of 0.9999, regression coefficient (r^2) of 0.9997, SE of 0.00262, intercept of 0.003159±0.002439 and a slope of 0.150869±0.001253. All these values confirmed the precision of the proposed method.

Accuracy was evaluated through the recovery percent of analysed substance.

From Table 2, one can observe that in the concentration range of 0.5–3.0 μg/mL of Se(IV), the mean recovery is 99.96 % (minimum 98.13 % and maximum 102.47 %). These values proved that the determining method of the Se(IV) was accurate.

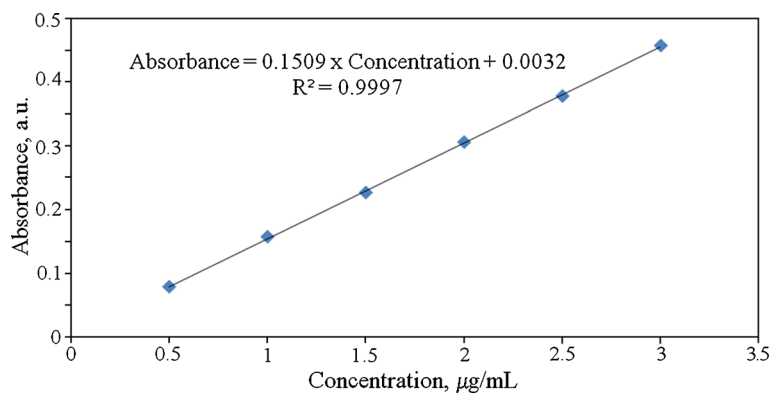


Fig. 3 Calibration curve

Table 2 Absorbance and recovery for the precision and accuracy methods applied to the studied system

Se(IV) (μg/mL)	Method precision		Intermediate precision		Accuracy	
	Absorbance	Recovery %	Absorbance	Recovery %	Absorbance	Recovery %
1.5	0.22651	98.66	0.23495	102.39	0.22868	99.62
	0.23441	102.15	0.22868	99.62	0.22651	98.66
	0.22765	99.16	0.23516	102.48	0.23516	102.48
2.0	0.30866	101.21	0.29835	97.80	0.30850	101.16
	0.29861	97.88	0.30899	101.32	0.29835	98.13
	0.30241	99.14	0.30850	101,16	0.30255	99.19
2.5	0.37905	99.63	0.38655	101.62	0.37986	99.84
	0.38775	101.94	0.37492	98.53	0.37875	99.55
	0.38288	100.64	0.38452	101.08	0.38402	101.08
Statistic	Mean=100.05 RSD=1.50 %		Mean=100.67 RSD=1.64 %		Mean=99.96 98.13–102.47 %	

Applications

The validated method was applied for the spectrophotometric determination of Se(IV) from selenium tablets, cosmetic sample (lipstick) and spring and bottled water (from Iasi, Romania).

In the first case, samples of 0.30–0.35 g of powdered tablets were mixed with 10 mL nitric acid (65 %) and then heated at 60 °C; 25 mL bidistilled water was added to the suspension and then filtrated using a quantitative filtering paper. The filtrate was brought with bidistilled water up to 50 mL. One millilitre sample was collected and processed according to the procedure of the spectrophotometric determination method of Se(IV).

Cosmetic product (0.1 g) (lipstick) was dissolved in alcohol to extract all organic substances. The residue was heated with 10 mL of concentrated nitric acid for 10 min and then cooled. After that, 10 mL of HCl was added, and the solution was boiled for 10 min. The sample residue was cooled, leached with 5 mL of H_2SO_4 (0.5 M), neutralised with NaOH (10 %) solution and diluted up to 25 mL with bidistilled water. One millilitre sample was analysed using the established procedure.

The obtained results are presented in Table 3.

Using the same method, Se(IV) was quantified from two water sources: spring water and bottled water from Iasi. In order to prepare the samples, 5 mL of water was treated with 0.5 mL NaOH (1 M). The solution was centrifuged until a fine precipitate appeared. The content was processed according to the method, and it was observed that both water samples did not contain selenium. The results obtained by adding the method are presented in Table 3. As can be observed, there is a good correlation between certified and found values of selenium.

Conclusions

A novel method for the assay of the selenium was proposed based on the oxidation reaction of potassium iodide by Se(IV). The released iodine further oxidised the new chemical reagent, *N*,*N*-diethyl-*p*-phenylenediamine

Table 3 Spectrophotometric determination of Se(IV)

No.	Product analysed	Certified value of selenium (μg)	Found value of selenium (μg)	Recovery (%)	RSD (%)
1.	Selenium tablets	50	50.55±0.262	101.11	0.4166
2.	Cosmetic product (lipstick 2.5 g)	Not known	2.48±0.035	99.50	1.32
3	Spring water	2.0	1.985±0.244	99.25	0.982
4	Bottled water	2.0	1.990±0.0165	99.50	0.833

hydrochlorate, resulting in a bright red compound which showed a maximum absorption at 552 nm.

The analysis method has been validated, establishing the optimum wavelength of detection (552 nm), the linearity (in the range of 0.5–3.0 μg/mL), the correlation coefficient (r^2=0.9997), repeatability (RSD=1.32 %), precision of the method (RSD=1.50 %) and the accuracy (mean recovery=99.96 %).

This offers the advantage of an increased sensitivity, as the detection and the quantification limits were established to be 0.0573 and 0.1737 μg/mL, respectively.

The proposed method was linear, precise and accurate, simple and fast, and it was applied with good results for the assay of Se(IV) from different samples: pharmaceutical and cosmetic products and also water.

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