

Novel Reactions for the Simple and Sensitive Spectrophotometric Determination of Traces of Selenium in Environmental Samples

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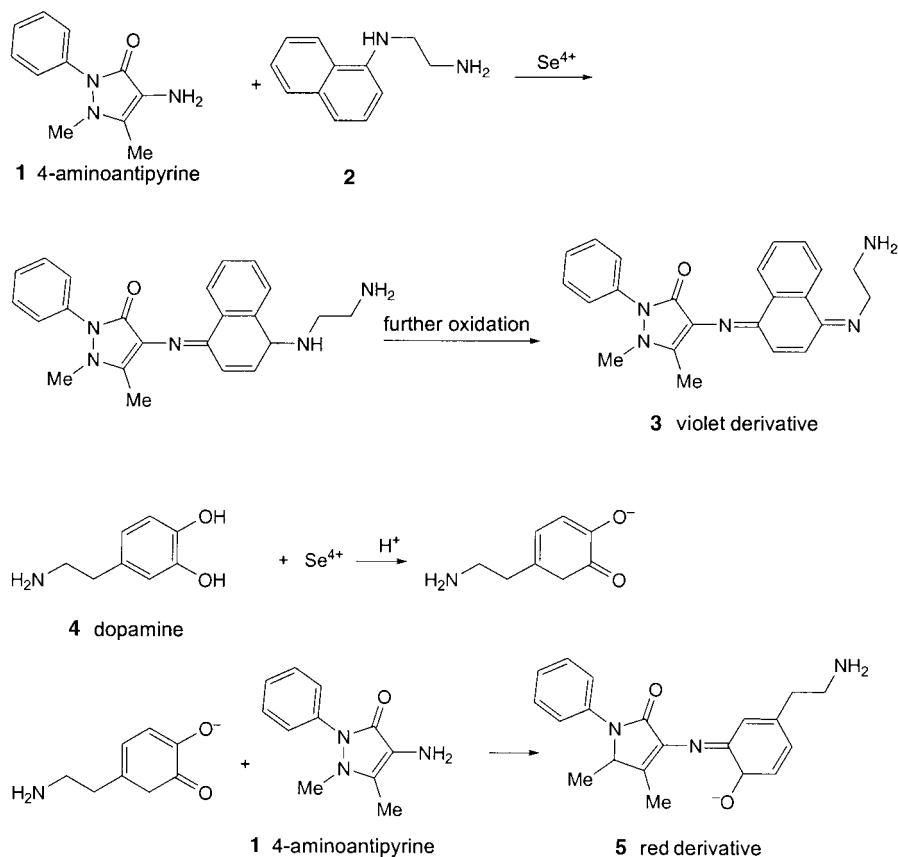
Two rapid, highly sensitive, and selective spectrophotometric methods for the determination of traces of selenium(IV) were studied. The methods are based on either the oxidation of 4-aminoantipyrine (=4-amino-1,2-dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one; 4-AAP; **1**) by selenium in basic medium and coupling with *N*-(naphthalen-1-yl)ethane-1,2-diamine dihydrochloride (NEDA; **2** · 2 HCl) to give a violet derivative **3** or on the oxidation of dopamine hydrochloride (=4-(2-aminoethyl)benzene-1,2-diol hydrochloride; DPH; **4** · HCl) by selenium in H₂SO₄ medium and coupling with **1** to yield a red derivative **5** (see *Scheme*). The violet derivative **3** with λ_{\max} 563 nm is stable for 8 days and the red derivative **5** with λ_{\max} 495 nm for more than a week. Beer's law is obeyed for selenium in the concentration range 0.03–3.5 $\mu\text{g ml}^{-1}$ (violet derivative **3**) and 0.07–2.5 $\mu\text{g ml}^{-1}$ (red derivative **5**), respectively. The optimum reaction conditions and other important analytical parameters were established. Interference due to various non-target ions were also investigated. The proposed methods, were applied to the analysis of selenium in polluted water, natural water, plant material, soil samples and synthetic mixtures. The results of the analyses were superior in precision to those obtained by reported methods.

Introduction. – Selenium is an essential trace nutrient, and selenium deficiency diseases are well known in veterinary medicine. Above trace levels, ingested selenium is toxic to animals and may be toxic to human beings. The selenium concentrations of most drinking waters and natural waters is less than 10 $\mu\text{g ml}^{-1}$ [1]. Selenium is widely distributed in nature, in relatively small concentrations in rocks, plants, coal, and other fossil fuels. Selenium enters into natural water through seepage from seleniferous soil, chemical weathering, and industrial wastes. Several analytical techniques have been reported for the determination of selenium, which includes spectrofluorometry, electrothermal atomic absorption spectrometry, hydride generation, polarography, cathode-stripping voltammetry, radiochemical neutron activation analysis, and flow-injection techniques, which are reviewed [2]. Few reagents are available for the spectrophotometric determination of selenium such as J-acid [3], leuco crystal violet [4], resazurin [5], sodium salt of hexamethyleneiminecarbodithioate [6], 1-amino-naphthalene-7-sulfonic acid [7], and variamine blue [8]. The above reported methods suffer from poor sensitivity, selectivity, or stability of the color derivative.

In the present study, we report two rapid, facile, sensitive, and selective methods for the determination of traces of selenium(IV). The implied reactions are oxidative couplings in the presence of Se^{IV} of 4-aminoantipyrine (=4-amino-1,2-dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one; 4-AAP; **1**) with *N*-(naphthalen-1-yl)ethane-1,2-diamine dihydrochloride/NEDA, **2** · 2 HCl) and of dopamine hydrochloride (=4-(2-aminoethyl)benzene-1,2-diol hydrochloride; DPH; **4** · HCl) with 4-AAP (**1**), yielding

the highly stable color derivatives **3** and **5**, respectively (see *Scheme*). The methods were successfully employed for the determination of selenium in environmental samples and synthetic mixtures.

Scheme. Oxidative Coupling Reactions of 4-AAP (1) with NEDA (2·2 HCl) and of DPH (4·HCl) with 4-AAP (1) in the Presence of Selenium(IV)



Results and Discussions. – *Absorption Spectra of Oxidative-Coupling Mixtures and Optimized Conditions.* The proposed methods involved the formation, in the presence of Se^{IV} of the violet derivative **3** with a λ_{max} 563 nm or of the red derivative **5** with a λ_{max} 495 nm, and the measurement of their absorption spectra (see *Fig.*). The starting materials **1/2** and **4/1**, respectively (see *Scheme*), had negligible absorptions at the λ_{max} of **3** and **5**.

Under the optimized conditions, although the color developed instantaneously, 5 min were allowed to obtain the maximum and constant absorbance of both colored derivatives **3** and **5**. The violet **3** was stable for 8 days and the red **5** for more than a week. The absorbance varied by $\pm 2\%$ within two days for both **3** and **5**. A temperature of 20–40° was selected as optimum for maximum color development.

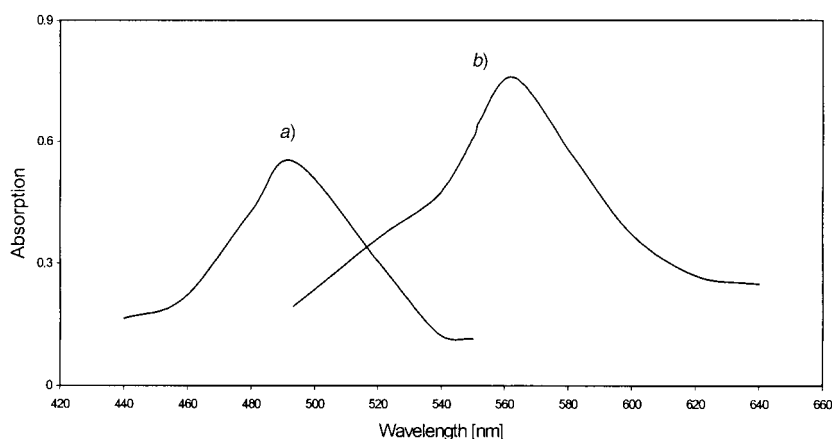


Figure. Absorption spectra of a) the $\text{Se}^{4+}/4\text{-AAP (1)}/\text{NEDA (2}\cdot\text{2 HCl)}$ system and b) the $\text{Se}^{4+}/\text{DPH (4}\cdot\text{HCl)}/4\text{-AAP (1)}$ system

The effect of the **1/2** mixture (\rightarrow **3**) was studied in the range 1–8 ml of a 1.5% (*w/v*) solution of **1/2**·2 HCl 1:2 in H_2O . To achieve the maximum color intensity, a volume of 2–4 ml of this solution was necessary. Hence, 3 ml of 1.5% **1/2**·2 HCl 1:2 in H_2O was selected for further studies, under optimized conditions. The maximum intensity of the violet color was achieved in basic medium.

The maximum intensity of the red color was achieved in the range of 3–5 ml of conc. sulfuric acid for **4/1** (\rightarrow **5**). Therefore, 4 ml of conc. sulfuric acid was used for best results. A range of 2–4 ml of a 0.5% (*w/v*) solution of **4**·HCl in H_2O and 3–5 ml of a 0.5% (*w/v*) solution of **1** in H_2O were necessary to achieve the maximum color intensity. Hence, 2 ml of 0.5% (*w/v*) **4**·HCl in H_2O and 4 ml of 0.5% **1** in H_2O were employed for the experiments under optimized conditions.

Beer's law range, molar absorptivity, *Sandell's* sensitivity, and other parameters of the oxidative-coupling mixtures are given in *Table 1*. The precision and accuracy of the method was studied by analyzing the coupling solution containing known amounts of the cited reagents within *Beer's* law limit. The low values of the relative standard deviation (in %) and the percentages of error indicated the high accuracy of the two experiments.

Reaction Mechanism. Under the optimized reaction conditions, 4-AAP (**1**) loses probably 2 e^- and a proton on oxidation with Se^{IV} in basic medium to form an electrophilic intermediate (active coupling species), which couples with **2** to give the violet derivative **3** (see *Scheme*).

Similarly, DPH (**4**) is oxidized first with Se^{IV} , which is mildly oxidizing in conc. sulfuric acid medium. Then the formed keto intermediate is condensed with 4-AAP (**1**) to give the red derivative **5** (see *Scheme*). Since phenolic groups are prone to oxidation, **4** is oxidized first and not **1**.

Interference of Non-Target Ions and Applications. The effects of various species on the determination of Se^{IV} was investigated. The tolerance limit was taken as the amount that caused a $\pm 2\%$ absorbance error in the determination of $2 \mu\text{g ml}^{-1}$ (**1/2** method) and $1.2 \mu\text{g ml}^{-1}$ (**4/1** method) of Se^{IV} . The results are shown in *Tables 2* and *3*.

Table 1. *Optical Characteristics of 3 and 5 in the Oxidative-Coupling Mixtures and Precision Data^{a)}*

	3 (from 4-AAP/NEDA)	5 (from DPH/4-AAP)
Color	violet	red
λ_{\max} [nm]	563	495
Beer's law range [$\mu\text{g ml}^{-1}$]	0.03–3.5	0.07–2.5
Molar absorptivity [$\text{l mol}^{-1} \text{cm}^{-1}$]	$3.10 \cdot 10^4$	$2.85 \cdot 10^4$
Sandell's sensitivity [$\mu\text{g cm}^{-2}$]	0.00259	0.00268
Regression equation (Y) ^{b)}		
Slope <i>a</i>	0.18734	0.34865
Intercept <i>b</i>	0.0148	– 0.0124
Correlation coefficient <i>r</i>	0.99971	0.99975
Relative standard deviation [%] ^{c)}	0.8957	0.3035
Range of error (95 % confidence level)	± 1.2425	± 0.4213
Detection limit [$\mu\text{g ml}^{-1}$]	0.0165	0.0335
% Error	0.1427	0.0745

^{a)} Experiments performed under optimized conditions (see text) with 1 ml of 0.014% aqueous SeO₂ solution.

^{b)} $Y = ax + b$, where *x* is the concentration of selenium in $\mu\text{g ml}^{-1}$. ^{c)} $n = 5$.

Table 2. *Effects of Foreign Species on the Determination of 2 $\mu\text{g ml}^{-1}$ of Selenium(IV) in the 4-AAP/NEDA (1/2) Method.*

Species	Tolerance limit [$\mu\text{g ml}^{-1}$]
EDTA	10500
Na ⁺ , Mg ²⁺ , Cl ⁻ , NO ₃ ⁻ , F ⁻ , CHCOO ⁻ , CO ₃ ²⁻ , K ⁺	2080
Ba ²⁺ , SO ₄ ²⁻ , CN ⁻ , SCN ⁻ , Tartarate	1090
PO ₄ ³⁻ , Al ³⁺ , Cd ²⁺ , NO ₂ ^{-a)}	810
Cu ^{2+b)} , Ni ²⁺ , Co ²⁺ , Ca ²⁺	86 ^{b)}
Zn ²⁺ , Pb ²⁺ , SO ₃ ²⁻ , NO ₃ ⁻ , Cr ³⁺ , As ⁵⁺	55
Fe ²⁺ , S ²⁻	40

^{a)} Can be masked up to 810 $\mu\text{g ml}^{-1}$ by the addition of 3 ml of 2 % sulfamic acid. ^{b)} Can be masked up to 86 $\mu\text{g ml}^{-1}$ by the addition of 3 ml of 5% EDTA.

Table 3. *Effect of Foreign Species on the Determination of 1.2 $\mu\text{g ml}^{-1}$ of Selenium(IV) in the DPH/4-AAP (4/1) Method*

Species	Tolerance limit [$\mu\text{g ml}^{-1}$]
EDTA	4070
Na ⁺ , Mg ²⁺ , Tartarate, oxalate, Mn ²⁺	1030
F ⁻ , PO ₄ ³⁻ , Hg ²⁺ , SO ₄ ²⁻ , CO ₃ ²⁻ , K ⁺ , CH ₃ COO ⁻ , S ²⁻ , Al ³⁺ , Fe ²⁺ , SO ₃ ²⁻ , Cd ²⁺ , CN ⁻ , SCN ⁻ , Fe ^{3+a)}	100
NO ₂ ^{-a)} , Cu ^{2+b)} , Zn ²⁺ , Ca ²⁺ , Cl ⁻	86
Pb ²⁺ , Co ²⁺ , Te ²⁺ , Ni ²⁺ , I ^{-c)} , NO ₃ ⁻	29

^{a)} Can be masked up to 100 $\mu\text{g ml}^{-1}$ by the addition of 2 ml of orthophosphoric acid. ^{b)} Can be masked up to 86 $\mu\text{g ml}^{-1}$ by the addition of 2 ml of 5% EDTA. ^{c)} Can be masked up to 29 $\mu\text{g ml}^{-1}$ by the addition of 1 ml of 1% HgCl₂.

The proposed methods were applied to the determination of selenium(IV) in natural water, polluted water, plant material, a soil sample, and synthetic mixtures. The

results of our analyses were compared with those of the reported method [3] and are summarized in *Table 4*. Recovery results of the selenium in synthetic mixtures are shown in *Table 5*.

Table 4. Determination Selenium(IV) in Environmental Samples

Sample	Selenium added [μg]	Proposed method		Reported method [3]	
		Selenium found ^{a)} [μg]	Mean recovery [%]	Selenium found [μg]	Mean recovery [%]
Polluted water ^{b)}	–	0.28		0.24	–
	–	0.37		0.37	–
	–	0.42		0.40	–
	–	0.51		0.50	–
Natural water ^{b)}	0.4	0.39	97.5	0.38	95.0
	1.0	0.98	98.0	0.98	98.0
	1.6	1.59	99.3	1.58	98.8
Fresh cabbage ^{b)}	–	1.0	–	0.98	–
	–	1.2	–	1.2	–
Soil ^{c)}	0.4	0.39	97.5	0.38	95.0
	1.0	0.99	99	0.97	97.0
	1.4	1.39	99.2	1.36	97.1
	1.6	1.58	98.8	1.58	98.8

^{a)} $n = 5$. ^{b)} 4-AAP/NEDA (**1/2**) method. ^{c)} DPH/4-AAP (**4/1**) method.

Table 5. Determination^{a)} of Selenium(IV) in Synthetic Mixture Corresponding to Se–Fe, Se–Hg and Se–Pb Alloys.

Se taken [$\mu\text{g ml}^{-1}$]	Fe added [$\mu\text{g ml}^{-1}$]	Hg added [$\mu\text{g ml}^{-1}$]	Pb added [$\mu\text{g ml}^{-1}$]	Se found ^{b)} [$\mu\text{g ml}^{-1}$]
1.0	1.1	–	–	1.0
2.0	2.2	–	–	1.99
1.0	–	3.0	–	0.98
2.0	–	6.0	–	1.99
1.0	–	–	3.0	0.99
2.0	–	–	6.0	2.08

^{a)} DPH/4-AAP (**4/1**) method. ^{b)} $n = 3$

Conclusions. – The proposed methods for the spectrophotometric determination of selenium(IV) in various environmental samples are facile, rapid, and sensitive. The coupling reagents employed in the present methods, *i.e.*, **1**, **2**, and **4**, are fairly soluble in H_2O , very cheap, and commercially available. The color derivatives **3** and **5** formed by oxidative coupling are quite stable when compared to that of the reported method [3]. The methods do not require heating or extraction and are devoid of interference effects. Statistical analysis of the results indicates that the methods yield exact values.

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Experimental Part

General. All chemicals and solvents used were of anal. reagents grade, and doubly dist. H₂O was used to prepare all solns. in the experiments. From a stock soln. containing 100 mg l⁻¹ of selenium dioxide (*E-Merck*, India) in 1 l of doubly dist. H₂O, a working soln. of selenium(IV) was prepared by further dilution. An aq. 1.5% (*w/v*) 4-AAP/NEDA (**1/2**) 1:2 reagent soln. was prepared by dissolving 1 g of **2** (from *Sigma*, USA), 0.5 g of **1** (from *SD Fine Chemicals*, India), and a few drops of conc. HCl in H₂O and filling up to 100 ml the soln. was refrigerated. Solns. (0.5% (*w/v*)) of dopamine hydrochloride (**4**·HCl) and 4-AAP (**1**) (both from *SD Fine Chemicals*, India) were prepared by dissolving 0.5 g of each in 100 ml of H₂O, and anal. grade sulfuric acid was used for the experiments. Absorption spectra: *Hitachi U-2001* spectrophotometer; 1.0-cm pathlength; λ_{\max} in nm.

4-AAP/NEDA (1/2) Method. Stock solns. containing 1–100 μg (0.03–3.5 $\mu\text{g ml}^{-1}$) of selenium(IV) (the volume of the test soln. was restricted to 1 ml) were transferred into 25-ml calibrated flasks, 1.5% **1/2**·2 HCl reagent (2 ml) was added to each flask. Each was allowed to stand for 10 min to complete the reaction. After dilution to 2.5 ml with H₂O, the absorbance at 563 nm was measured against the corresponding reagent (blank) and the calibration curve was constructed.

DPH/4-AAP (4/1) Method. Stock soln. containing 2–60 μg (0.07–2.5 $\mu\text{g ml}^{-1}$) of selenium(IV) were transferred to 25-ml standard flasks; to each flask, conc. H₂SO₄ soln. (4 ml), 0.5% **4**·HCl (2 ml) and 0.5% **1** (4 ml) were added. Each mixture was allowed to stand for 10 min with occasional shaking to complete the reaction. After dilution to 25 ml with H₂O, the absorbance at 495 nm was measured against the corresponding reagent (blank), and the calibration curve was constructed.

Determination of Selenium(IV) in Polluted Water. The distillation procedure for the determination of selenium in polluted water was as follows: To an aliquot (100 ml) of a known sample of polluted water in a distillation flask, KBr (1 g) and conc. H₂SO₄ soln. (10 ml) treated with sat. Br₂/H₂O (0.5 ml) were added, and the soln. was then distilled under vacuum until copious white fumes of SO₃ vapors evolved. This process converted all other forms of selenium to selenium(IV). The distillate was collected in 10 ml of 5% hydroxylamine hydrochloride soln., the soln. filled to a fixed volume, and analyzed by the standard procedure.

Determination of Selenium in Plant Material. Finely chopped fresh cabbage (5 g) was placed in a 250-ml beaker, and a soln. of conc. H₂SO₄/HNO₃ 1:1 (*v/v*) (10 ml) was added. This mixture was heated until the soln. was clear. The soln. was filtered off and concentrated to 5 ml, then cooled and diluted to 50 ml with deionized H₂O. Then the standard procedure was applied to 1 ml of this soln.

Determination of Selenium in Soil Sample. A known weight of selenium was mixed with soil (20 g), fused with Na₂CO₃/KNO₃ 1:1 in a nickel crucible, and extracted with H₂O. The filtrate of the extract was treated with 10M HCl (20 ml) and then heated to expel Cl₂ and nitrogen oxides. The soln. was further diluted with H₂O to give a suitable concentration of selenium. An aliquot of the stock soln. was passed through the cation exchange resin to remove the iron present in soil. The selenium contents in the eluate was determined as described in the standard procedure.

Determination of Selenium in Synthetic Mixtures. Synthetic mixtures corresponding to the alloys of Se–Fe (52% Fe), Se–Hg (71% Hg), and Se–Pb (75% Pb) were prepared, and the selenium content was determined by the aforesaid standard procedure.

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