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Simultaneous Spectrofluorimetric Determination of Selenium(IV) and (VI) by Flow Injection Analysis

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A simple, sensitive, highly selective, automatic spectrofluorimetric method for the simultaneous determination of selenium(IV) and (VI) as selenite–selenate by flow injection analysis (FIA) has been developed. The method is based on the selective oxidation of the non-fluorescent reagent 2-(α-pyridyl)thioquinaldinamide (PTQA) in acidic solution (1.5–3.0 m H_2SO_4) by Se^{IV} to give an intensely fluorescent oxidation product (λ_{ex} = 350 nm; λ_{em} = 500 nm). Selenium(VI) is reduced on-line to Se^{IV}, in a reduction coil installed in a photo-reactor, which is then treated with PTQA and the fluorescence due to the sum of Se^{IV} and Se^{VI} is measured; Se^{VI} is determined from the difference in fluorescence values. Various analytical parameters, such as effect of acidity, flow rate, sample size, dispersion coefficient, temperature, reagent concentration and interfering species were studied. The photo-reduction conditions were optimized, with an FIA procedure, for Se^{VI} on the basis of its reduction efficiency. The calibration graphs were rectilinear for 0.1–2.4 μg ml^{-1} of Se^{VI} and 10 ng ml^{-1}–2.2 μg ml^{-1} of Se^{IV}, respectively. The method was applied to the determination of Se in several Standard Reference Materials (alloy, sediments and tea), as well as in some environmental waters (tap and surface water), food samples (flour and egg), a biological sample (human hair), soil sample and in synthetic mixtures. Up to 25 samples per hour can be analysed with an RSD ≈ 0.1–2%.

Keywords: *Flow injection; spectrofluorimetry; selenium speciation; 2-(α-pyridyl)thioquinaldinamide; on-line photo-reduction; environmental; biological; soil samples*

Recently, there has been increasing interest in trace determination of selenium because of its dual role, as an essential nutrient at low concentrations (10–40 μg ml^{-1} in serum), or a highly toxic compound (selenosis) at an intake of 5 mg kg^{-1} of Se (in the mammals of a seleniferous region).[1] It is contained in the enzyme glutathione peroxidase (GSHPx), which affords cells protection against oxidative damage.[2] A selenium deficiency in man may also result in cardiomyopathy.[2] The narrow concentration range between the two opposite effects (0.1–4.0 mg kg^{-1} in plants), requires accurate and precise knowledge of the selenium species present in the environment.[3] In the environment, Se levels generally fall in the ranges 0.1–400 ng ml^{-1} in natural waters,[4] 1 ng ml^{-1} in the atmosphere[3] and 0–80 μg g^{-1} in soils.[5] Selenium finds its way into the environment through its widespread use in the glass and electronics industries, as well as from the combustion of fossil fuels and uses in agriculture. Detailed information about the availability and mobility of Se in the environment and its biogeochemical cycle, however, requires the additional knowledge of the different chemical forms and oxidation states in which this element can exist. The inorganic Se species most frequently found in water and soils are selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}).[3] In this regard, a method for speciation is needed because its availability for plant uptake, mobility in soil, and toxicity in biota depends on the oxidation state of the Se.[6] Therefore, its accurate determination at trace levels using simple and rapid methods is of paramount importance.

Analytical techniques for Se speciation have recently been reviewed.[7–9] Automatic flow techniques have hardly been applied to the determination of Se. Until now, only few methods have been described that use flow injection analysis (FIA) and these methods either use FIA combined with HG–AAS[8,10] or FIA with spectrophotometric detection.[11] But these methods suffer from several limitations: (*i*) indirect determination of Se^{VI} because it does not give hydrides, and (*ii*) the matrix affects both hydride generation and reduction yields of Se^{VI} to Se^{IV}. The spectrophotometric methods suffer from sensitivity and selectivity due to various or many interferences. In this respect, interlaboratory comparisons of inorganic Se in biological and environmental samples showed unacceptable differences using these techniques.[12] Recent developments in the automation of instrumentation leads to an improved precision.[13] Recently, photochemical reactions have been applied to the on-line reduction and oxidation of inorganic and organic substances in flow injection methods.[14,15] Measures and Burton[16] studied the photochemical oxidation of an organometallic form of selenium(Se^{2-}) into inorganic Se^{IV} with a high pressure mercury lamp. All of these systems have been used for on-line photochemical oxidation or reduction to enhance the detection of a variety of inorganic and organic compounds with AAS[17] and spectrophotometric techniques.[15]

The aim of the present study was to develop a more simple FIA system for the simultaneous determination of Se^{IV} and Se^{VI} with 2-(α-pyridyl)thioquinaldinamide (PTQA) using a reduction coil installed in a photo-reactor in the reaction manifold. PTQA has been reported as a spectrofluorimetric reagent,[18] but has not previously been used for the simultaneous determination of Se^{IV} and Se^{VI} in a flow injection system. This paper reports its use in a very sensitive, highly specific automatic spectrofluorimetric method for the simultaneous determination of Se^{IV} and Se^{VI}. The method is based on the selective oxidation of the non-fluorescent reagent, PTQA, in an acidic medium (1.5–3.0 m H_2SO_4) by Se^{IV} to produce an intensely fluorescent product followed by the direct measurement of the fluorescence intensity in aqueous solution at room temperature. Oxidation is very rapid and no extraction is required. With suitable masking the reaction can be made highly selective. The reaction mechanism of the present method is as reported earlier.[18]

Experimental

Apparatus

The manifold for simultaneous determination of Se^{IV} and Se^{VI} was of Teflon tubing (0.8 mm id) and linear dual connectors were used (Fig. 1). It consisted of a four-way pneumatically

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actuated injection valve (Rheodyne, Type 50 Teflon, Cotati, CA, USA), an eight-channel peristaltic pump (Ismatec, Glattburg-Zurich, Switzerland) and a spectrofluorimeter (RF-551, Shimadzu, Japan), equipped with a 12 μl flow-through cell for measurement.

Data processing and collection was performed with an IBM-compatible Personal Computer (PC) by means of software written in Microsoft Q-Basic. The interface unit was an RTL 800/815 multifunction Input/Output board. A Varian AA-300 Atomic Absorption Spectrophotometer equipped with a hydride system at 196.1 nm using an air–acetylene flame was used for comparison of the results. A digital pH-meter (Model-PHM83 AUTOCAL, Radiometer, Copenhagen, Denmark) was used to measure the pH of the solutions.

Photoreduction-reactor

The photoreduction-reactor comprised a high pressure mercury light source (2 cm od, 25 cm long, 125 W, DESAGA) and a quartz coil (40 cm long × 0.8 mm id). The source emits short wavelength light at λ_{max} = 254 nm. The effective irradiation length was 6 cm. The unit is covered with aluminium foil or thick paper in order to increase the light intensity reaching the coil by reflectance and to prevent eye exposure to ultraviolet radiation.

Reagents

All chemicals used were of analytical-reagent grade or the highest purity available. Doubly distilled water and HPLC-grade propan-2-ol, which is non-fluorescent under ultraviolet radiation, were used throughout.

Se^{IV} standard solutions. A 100 ml amount of stock Se^{IV} solution (1 mg ml^{-1}) was prepared by dissolving 333.1 mg of general-reagent grade sodium selenite (Merck, Darmastadt, Germany) in doubly distilled water. The solution was kept in a refrigerator in a polyethylene container for preservation. Working standard solutions were prepared daily by appropriate dilution in 2 m H_2SO_4.

Se^{VI} standard solutions. A 100 ml amount of stock Se^{VI} solution (1 mg ml^{-1}) was prepared by dissolving 467.4 mg of ACS-grade sodium selenate (99%, Aldrich, Steinheim, Germany) in doubly distilled water. The solution was kept in a refrigerator in a polyethylene container. Working standard solutions were prepared daily by appropriate dilution in 2 m H_2SO_4.

Carrier solution. 2.0 m H_2SO_4 (Merck) was used as the carrier solution.

2-(α-Pyridyl)thioquinaldinamide (PTQA), solution (10^{-3} M). The reagent was synthesized according to the method of Porter.[19] The contents, containing 2-aminopyridine (2 mol), quinaldine (1 ml) and sulfur powder (1.5 mol), were mixed and refluxed for 6 h in a 250 ml round bottomed flask fitted with bulb condenser under controlled temperature (140–150 °C) at 1 atm pressure over a sand bath. The reaction mixture was kept overnight. The thio-compound was filtered and crystallized using petroleum ether to give a bright yellow crystalline (needle) solid. The compound recrystallized from ethanol was dried under vacuum (0.1 mg of Hg) for 24 h. The melting point of this synthesized compound (PTQA) was 155 ± 2 °C and the elemental analysis data (C = 72.25, N = 13.35 and H = 4.25%) were very close to the literature values,[19] *e.g.*, melting point (155 ± 1 °C); C (=72.43); N (=13.55) and H (=4.55%). The reagent solution (10^{-3} m) was prepared by dissolving the requisite amount (0.0266 g in 100 ml) of PTQA in HPLC-grade propan-2-ol. A freshly prepared reagent solution (10^{-4} m) was used whenever required.

Other solutions. Solutions of a large number of inorganic ions and complexing agents were prepared from their AnalaR grade or equivalent grade water soluble salts. In the case of insoluble substances, special dissolution methods were adopted.[20]

Stock solutions and environmental samples were kept in a refrigerator in poly(propylene) bottles.

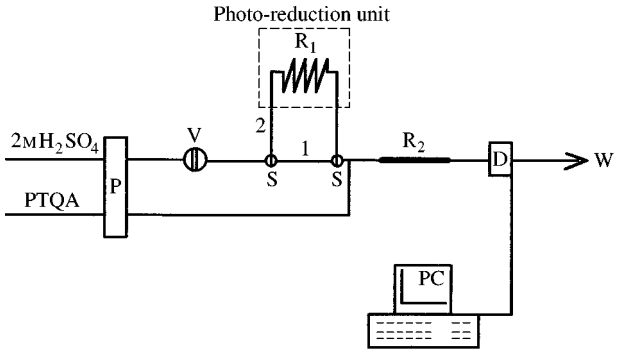

Fig. 1 Schematic representation of FI manifold employed for the simultaneous determination of Se^{IV} and Se^{VI}. P, Pump; V, valve; S, selector valve; R_1, photoreduction coil; R_2, single bead string reactor (SBSR); D, detector; W, waste; and PC, personal computer.

Preparation of the Samples

Food samples (rice flour and egg) were purchased from a local supermarket. These samples were homogenized thoroughly. Soil samples was collected from local agricultural field sites and homogenized in a mortar. Human hair (3–5 cm long from male) was cut from the occipitonuchal region of the head. They were cleaned by stirring with acetone, rinsing with tap water, stirring in a detergent solution (which had no detectable Se), rinsing with tap water, doubly distilled water and finally with acetone. They were then dried at 45 °C and cut into small pieces for analyses (sampled person did not use Se-containing shampoo).

Procedure

The standards (0.01–2.2 μg ml^{-1} Se^{IV} or 0.1–2.4 μg ml^{-1} Se^{VI}) and samples were injected into a carrier stream by means of the peristaltic pump, P (Fig. 1). Then the sample was measured by different ways using a selector valve. The sample stream was firstly directed through path 1, treated with a 10–30-fold molar excess of the PTQA reagent solution and passed directly into the measuring cell of a spectrofluorimeter where the fluorescence intensity due to Se^{IV} was measured at 500 nm with excitation at 350 nm. Then the sample stream was passed through path 2 to photo-reduction coil (R_1) by using a second selector valve where Se^{VI} was reduced to Se^{IV}. The sample stream was then treated with the PTQA reagent at the end of the coil and the overall mixture was passed to the same cell of the spectrofluorimeter where the fluorescence intensity due to total Se was measured; Se^{VI} was determined from the difference in fluorescence intensity values. The reaction is very rapid and the fluorescence intensity remains stable for 24 h. The PTQA reagent does not show any fluorescence in the absence of Se^{IV}.

The concentrations of Se^{IV} and Se^{VI} were evaluated from the peak heights of the signal by using the calibration curves prepared with standard solutions.

Results and Discussion

Optimization of the Flow Injection System

Preliminary tests were carried out with the aid of different flow assemblies to select the optimal manifold configuration. The assembly in Fig. 1 was selected as the one producing the best compromise between peak height and the shape of the peak.

In order to optimize the proposed flow injection manifold, the influence of the hydrodynamic and chemical parameters on the magnitude of the peak height, the shape of the peak and reproducibility of the results were studied. The univariate method was adopted for the optimization of the system. Table 1 shows results of optimization of working conditions for 0.5 μg ml^{-1} of Se^{IV} and 1.0 μg ml^{-1} of Se^{VI}.

The optimum length of the photo-reduction coil (R_1) was established by using a 1.0 μg ml^{-1} Se^{VI} solution, the single bead string reactor (SBSR) (R_2), for better mixing and lower dispersion, being of length 100 cm and having an acidity of 1.5–3.0 m H_2SO_4. Photo-reduction coil (R_1) lengths of 15, 30, 40, 60 and 80 cm were tested, keeping the power of the lamp and its distance from reaction coil constant. For any combination of the above parameters the efficiency of the reduction was determined by comparison of the plateau achieved with that corresponding to a 1.0 μg ml^{-1} Se^{IV} solution processed in the same way. A coil length of 40 cm was chosen, because the reduction was almost complete, reproducibility was good and back-pressure relatively low. Different wavelengths of the UV radiation were also tested. For each wavelength, the efficiency of the reduction was determined. The effective wavelength of photo-reduction of the Se^{VI} was λ_{max} = 254 nm. Different lamp powers were also tested but no significant effect on reduction was observed. Different distances of the reaction coil from the lamp were tested keeping the length of the coil and wavelength constant. A length of 6 cm was selected because maximum conversion efficiency was achieved.

A length 100 cm for the SBSR reactor (R_2), a sample size of 100 μl, an overall flow rate of 0.4 ml min^{-1} and a reagent flow rate of 0.3 ml min^{-1} were selected, these being a compromise between the sampling rate and the height of the peak.

Of the various acids (sulfuric, hydrochloric, nitric and phosphoric) studied, sulfuric acid was found to be best acid for the system. Different concentrations of sulfuric acid were tested in the range shown in Table 1. The fluorescence intensity was at maximum and constant when the solution (1.0 μg ml^{-1}) contained 1.5–3.0 m H_2SO_4 (Fig. 2) at room temperature, which was the optimum acidity range. The photoreduction efficiency for 1.0 μg ml^{-1} of Se^{VI} in this acidity range was also tested. More than 97% of the Se^{VI} can be reduced to Se^{IV} in this acidity range. For all subsequent measurements 2.0 m H_2SO_4 was used as carrier for this manifold.

The effect of propan-2-ol on the fluorescence was studied and no adverse effect was observed over a wide range of propan-2-ol concentrations. A 10^{-4} m solution of PTQA in propan-2-ol was sufficient to prevent any precipitation or turbidity or bubbling and to allow accurate measurements for this manifold. Other common organic solvents, *e.g.*, chloroform, benzene, tetrachloromethane and ethanol, were also tried but no fluorescence was observed in the organic phase, with the exception of ethanol.

The reaction is rapid. A constant maximum fluorescence intensity was obtained just after the dilution to volume and remained strictly unaltered for 24 h. Different concentrations of PTQA solution were tested in the ranges shown in Table 1. A reagent concentration of 2×10^{-4} m was selected as optimum for this manifold. The fluorescence intensity of the Se^{IV}–PTQA system within the prescribed acidity range was maximum and constant for Se^{IV} to reagent molar ratios in the range 1 : 10–1 : 30 when the Se concentration was 1.0 μg ml^{-1} (Fig. 3). At different Se^{IV} concentrations (0.1 and 0.5 μg ml^{-1}), the effect of varying the reagent concentration was similar.

Evaluation of the Method

The reproducibility of the proposed procedure and sample throughput were determined by repeated injection of a sample containing 0.5 μg ml^{-1} Se^{IV} and 1.0 μg ml^{-1} Se^{VI}. The RSD (n = 5) was 0.1–2% for 0.01–2.2 μg ml^{-1} Se^{IV} and 0.1–2.4 μg ml^{-1} Se^{VI} indicating that this method is highly precise and reproducible. The calibration graphs obtained from the peak heights were rectilinear for 10 ng ml^{-1} to 2.2 μg ml^{-1} of Se^{IV} and 0.1–2.4 μg ml^{-1} of Se^{VI}, respectively. The detection limits, defined as three times the baseline noise, were 1 ng ml^{-1} for Se^{IV} and 10 ng ml^{-1} for Se^{VI}. The sample throughput was 25 measurements per hour. The dispersion coefficients were estimated with a 0.5 μg ml^{-1} Se^{IV} and 1.0 μg ml^{-1} Se^{VI} standard solutions as described earlier.[21] Important features of the proposed method for simultaneous determination of Se^{IV} and Se^{VI} are summarised in Table 2.

The performance and reproducibility of the proposed method are also shown in Tables 3–6. The reliability of the proposed procedure was also assessed by analysing Certified Reference Materials. The results for total Se were in good agreement with certified values (Table 3). The method was also tested by analysing several synthetic mixtures containing standard Se^{IV} and Se^{VI} (Table 4). The reliability of the proposed procedure was also tested by performing recovery studies. The average

Table 1 Selected chemical and FIA parameters obtained with the optimization experiments

Parameter	Studied range	Selected value
Size of sample loop/μl	30–180	100
Overall flow rate/ ml min^{-1}	0.20–1.0	0.40
Reagent flow rate/ ml min^{-1}	0.05–0.60	0.30
Length of the photo-reaction coil, R_1/cm	15–80	40
Length of the SBSR reactor, R_2/cm	20–180	100
pH	0.1–1.3	0.40–0.75 (preferably 0.6)
Concentration of reagent (*m*)—		
H_2SO_4	0.50–4.5	1.50–3.0 (preferably 2.0)
PTQA	3×10^{-5}–6×10^{-4}	2×10^{-4}

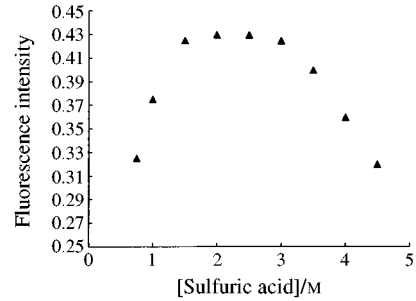

Fig. 2 Effect of acidity on the fluorescence intensity of the Se^{IV}–PTQA system.

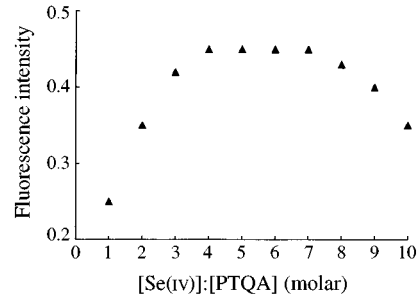

Fig. 3 Effect of reagent on the fluorescence intensity of the Se^{IV}–PTQA system.

percentage recovery obtained for the addition of Se^{IV} and Se^{VI} spikes to some environmental water and flour samples was quantitative as shown in Table 5. The results of food, hair and soil analyses by the present method was in excellent agreement with those obtained by HG-AAS (Table 6). The precision and accuracy of the method are satisfactory.

Table 2 Analytical features of the proposed method

Parameter	Se^{IV}	Se^{VI}
Acidity/m	1.50–3.0	1.50–3.0
Fluorescence stability/h	24	24
Temperature/°C	25	25
Reagent (fold molar excess)	1 : 10–1 : 30	1 : 10–1 : 30
Linear range/μg ml^{-1}	0.01–2.2	0.1–2.4
Detection limit/ng ml^{-1}	1	10
Dispersion coefficient	1.65	1.70
Reproducibility (% RSD)	0.1–2	0.1–2
Sample throughput/samples h^{-1}	28	22

Table 3 Recoveries of total Se for Certified Reference Materials

	Se/μg g^{-1}	
Type	Certified value	Found ± *s**
Marine Sediment (NRC-PACS 1)	1.09 ± 0.11	1.06 ± 0.08
Estuarine Sediment (CEC-CRM 277)	2.04 ± 0.18	1.95 ± 0.10
Tea (NRC-CRM C85-05)	0.041 ± 0.004	0.043 ± 0.004
Selenium eutectic alloy (%)	2.60 ± 0.10	2.58 ± 0.15

* $n = 5$.

Table 4 Simultaneous determination of Se^{IV} and Se^{VI} in synthetic mixtures of standard Se^{IV} and Se^{VI}

Added/μg ml^{-1}			Found*/μg ml^{-1}			Relative error (%)		
Se^{IV}	Se^{VI}	Total†	Se^{IV}	Total†	Se^{VI}‡	Se^{IV}	Total†	Se^{VI}
0.20	0.20	0.40	0.20	0.40	0.20	+1.0	+1.3	+1.5
0.20	0.50	0.70	0.20	0.70	0.50	−0.50	−0.20	−0.20
0.50	0.20	0.70	0.50	0.70	0.20	−0.20	+0.30	+1.5
0.40	0.60	1.0	0.40	1.0	0.60	0.0	0.0	0.0
0.50	1.0	1.5	0.50	1.5	1.0	0.0	+1.0	+1.0
1.0	0.50	1.5	1.0	1.5	0.50	0.0	−0.20	−0.40
0.0	1.0	1.0	0.01	1.0	1.0	+1.0	0.0	−1.0
1.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0

* $n = 5$. † $Se^{IV} + Se^{VI}$. ‡ Calculated by subtraction of Se^{IV} from total Se.

The interference of several ions which may occur in environmental samples was studied by using a solution containing a mixture of Se^{IV} and Se^{VI} at concentrations of 0.5 μg ml^{-1} and 1.0 μg ml^{-1}, respectively, adding various concentrations of interfering ions up to the amounts where the relative error reached a value of about 5%. The errors were calculated by comparing the peak height to that obtained after the injection of an aqueous solution of Se^{IV} and Se^{VI} containing no interfering ions, as a reference. The results are summarized in Table 7. During the interference studies, if a precipitate was formed, it was removed by centrifugation. Positive interference from permanganate or hydrogen peroxide was eliminated by boiling the solution with sodium azide, a reducing agent which had no reducing effect on either Se^{IV} or Se^{VI}.

Applications

The proposed method was used to determine the total selenium content in a number of certified reference materials (sediments, tea and alloy) (Table 3). The method was also successfully applied to the simultaneous determination of the Se^{IV} and Se^{VI} content in a number of synthetic mixtures of Se^{IV} and Se^{VI} (Table 4). The method was also extended to the simultaneous determination of Se^{IV} and Se^{VI} in a number of environmental waters. The samples were spiked with one of the two concentrations of Se^{IV} and Se^{VI} and recoveries determined (Table 5). The results of the analyses of real samples (soil, human hair and food) by our procedure were in excellent agreement with those obtained by HG-AAS (Table 6).

Determination of Total Selenium in Certified Reference Materials

Sediment (1–2 g) or tea (2–5 g) or alloy (0.05–0.1 g) was placed in a 50 ml beaker and digested using a procedure described by Cutter.[22] The beaker with the testing material and concentrated nitric acid was covered with a watch glass and heated gently for 3 h. Perchloric acid was added and the mixture was heated until

Table 6 Determination of total Se in real samples

	Total Se/μg g^{-1}		
Sample	AAS	Proposed method*	Relative error (%)
Soil (surface)	0.375	0.380	+1.3
Hair (human)	nd†	nd	—
Egg (yolk)	0.170	0.167	−1.7
Flour (rice)	nd	nd	—

* $n = 5$. † nd: not detected.

Table 5 Analysis of spiked environmental water and flour samples

Sample	Se^{IV} added/ ng ml^{-1}	Se^{VI} added/ ng ml^{-1}	Reduced through photo-reactor	Total Se added*/ ng ml^{-1}	Total Se found*/ ng ml^{-1}	Se^{VI} found†/ ng ml^{-1}	Recovery ± *s* (%)
Tap water	100	100	No	200	100	—	100 ± 0.3
	100	100	Yes	200	199	99	99.5 ± 0.5
Lake water—							
Sample 1	200	80	No	280	201	—	100.5 ± 0.4
	200	80	Yes	280	278	79	99.5 ± 0.6
Sample 2	500	50	No	550	499	—	99.8 ± 0.2
	500	50	Yes	550	545	45	99.0 ± 0.8
Flour (rice)	300	60	No	360	301.5	—	100.5 ± 0.5
	300	60	Yes	360	358.0	58	99.5 ± 0.6

* Determined as Se^{IV}. † Calculated by subtracting the Se^{VI} from the total Se.

only a slight amount of moisture remained. A third 3 h nitric acid heating was performed and the sample was again carefully evaporated. Concentrated HCl was added under heating to ensure that all the Se was present as Se^{IV}. The contents of the beaker were filtered through a Whatman No. 40 filter paper into a 25 ml calibrated flask. The solution was then diluted up to the mark with 2.0 m H_2SO_4. The total Se content was determined as described under Procedure using tartrate as masking agent. In the case of the tea sample, interference from permanganate was removed by adding sodium azide and boiling the solution before measurement. The results for total Se were in good agreement with certified values. The results of total Se by on-line photo-reduction were also in excellent agreement with those obtained by HCl reduction. The results are shown in Table 3.

Simultaneous Determination of Se^{IV} and Se^{VI} in Synthetic Mixtures

Synthetic mixtures of standard Se^{IV} and Se^{VI} of different concentrations were prepared with 2.0 m H_2SO_4. The Se^{IV} and Se^{VI} contents were determined spectrofluorimetrically as described under Procedure. The precision for the determination of Se^{IV} and Se^{VI} was measured by analysing (n = 5) the samples listed in Table 4. The relative errors for all samples were < 2%.

Table 7 Effect of interfering ions on the determination of 0.5 μg ml^{-1} Se^{IV} and 1.0 μg ml^{-1} Se^{VI}, respectively

	Maximum permissible concentration*/ μg ml^{-1}	
Interfering ion	Se^{IV}	Se^{VI}
Ammonium	100	100
Bromide	100	100
EDTA	100	100
Fluoride	100	100
Persulfate	100	100
Silicate	100	100
Tartrate	5000	5000
Aluminium	100	100
Antimony(v)	50	50
Arsenic(iii)	50	50
Beryllium	50	50
Calcium	50	50
Cerium(iii, iv)	50	50
Chromium(iii)	100	100
Cobalt(ii)	100	100
Copper(ii)	100	100
Iron(ii, iii)	50	50
Lanthanum	100	100
Magnesium	50	50
Manganese(ii)	100	100
Mercury(ii)	50	50
Molybdenium(vi)	100	100
Nickel(ii)	100	100
Potassium	2000	2000
Silver(i)	50	50
Tin(ii, iv)	50	50
Tungsten(vi)	50	50
Uranium(vi)	50	50
Vanadium(v)	50	50
$Zinc^{+}$	100	100

* For acetate, alkali metals, sodium azide, carbonate, citrate, chloride, dichromate, fluoride, iodide, nitrate, oxalate, perchlorate, phosphate and sulfate the maximum permissible concentration is 1000 μg ml^{-1}. A 5% error criterion is adopted for all the interferents.

Analysis of Spiked Environmental Water and Flour Samples

The proposed method was applied to the determination of Se^{IV} and Se^{VI} added to some environmental water and flour samples. A preliminary study showed Se^{IV} and Se^{VI} to be below the limits of detection in the samples. The samples were spiked with one of two concentrations of Se^{IV} and Se^{VI} with 2.0 m H_2SO_4 and the recoveries determined (the standard additions technique was used and in the case of the flour sample, the standard was added before digestion). The recoveries in all cases were high (between 99.0 and 100.5%) and are shown in Table 5.

Determination of Total Selenium in Real Samples

An air-dried homogenised soil sample (5–10 g) was weighed accurately and placed in a 100 ml beaker. The sample was digested and reduced following the method recommended by Cutter.[22] The content of the beaker was filtered through a Whatman No. 40 filter paper into a 25 ml calibrated flask. It was then diluted up to the mark with 2.0 m H_2SO_4.

Human hair (2–5 g) or egg yolk (5–10 g) or rice flour (5–10g) was placed in a 100 ml beaker. Following the procedure recommended by Bratakos *et al.*,[23] the sample was digested with a mixture of nitric and perchloric acids and the Se^{VI} species were reduced with HCl. The contents of the flask were filtered through a Whatman No. 40 filter paper into a 25 ml calibrated flask. The solution was then diluted up to the mark with 2.0 m H_2SO_4.

Suitable aliquots of the above samples were transferred into a 10 ml calibrated flask and the total Se contents were determined as described under Procedure using tartrate as masking agent. The results of soil, hair, egg and flour analyses by the FIA method were found to be in good agreement with those obtained by HG-AAS. The results are shown in Table 6.

The very low value of total selenium for the hair sample is probably due to the type, quality and quantity of the foods consumed by Greeks.[24] The low value of total selenium for the rice flour sample is probably due to low selenium in soils. Bratakos *et al.*[23] also reported such low selenium contents in foods produced in Greece. Occurrence of such low selenium contents have been reported in the soils of some countries.[5,25]

Conclusions

The use of a photo-reduction-reactor with an FIA system has been shown to be effective in the speciation of selenium. Automation of the system has resulted in much shorter analysis times, with greater reduction efficiency than using conventional heated digestion methods. The proposed FIA method using PTQA is not only one of the most sensitive methods for the simultaneous determination of Se^{IV} and Se^{VI} but is also excellent in terms of selectivity and simplicity. It offers also a very efficient procedure for speciation analysis. Therefore, this method will be successfully applied to the monitoring trace amounts of selenium species in environmental, biological and soil samples.

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