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UV-photolysis assisted digestion of food samples for the determination of selenium by electrothermal atomic absorption spectrometry (ETAAS)

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Abstract

An UV-oxidation procedure has been developed to completely digest biological/food samples for the determination of trace levels of selenium. A combined use of UV photolysis and hydrogen peroxide in the presence of an oxidant (HNO_3), results in the complete oxidation of the organic matter. This method is simpler and requires fewer reagents when compared with other sample pre-treatment procedures. The clear solution obtained was analysed for the selenium content by graphite furnace atomic absorption spectrometry. Unreduced palladium-nitrate modifier was used in all cases. However, in case of samples that are known to contain sulfur (like mushroom, Brazil nut, etc.), reduced palladium was used as modifier. The method was verified using three standard reference materials i.e., Whole egg powder-8415, Tuna fish-IAEA 350, Oyster tissue-1566a, and the results were in agreement at 95% confidence level. By using standards addition as calibration method, accurate results were obtained for the certified reference material, the precision for the CRM's were in the range of 3.5–8%. The characteristic mass of selenium is assessed as 10 pg. The detection limits were in the range of 35–40 ng/g.

Subsequently the developed method was used for the determination of selenium in pumpkin seed, beetroot, Brazil nut, mustard seeds, curry leaves (Murraya koenigi), and wheat flour.

Keywords: Selenium; UV-photolysis; Electrothermal atomic absorption spectrometry; Reduced palladium

1. Introduction

Selenium is an essential element for living beings as it is involved in many enzymatic processes. It is one of the indispensable trace elements in humans, mainly due to its antioxidant action that removes damaging peroxides and hydro peroxides generated by free radicals (Cai, 2000). Further, selenium has beneficial roles, especially in protecting against cardiovascular diseases, inhibiting tumor growth, maintaining normal thyroid function and stimulating the immune system against viral infections (Rayman, 2000). The daily amount of Se intake recommended for human beings is in the range of 55–75 μg (Dietary Reference Intakes by Food and Nutrition Board, 2000). On the other hand, at levels only three to five times above the essential levels it is toxic. Thus it has the narrowest biological tolerance range of all the elements. With such narrow concentration range for dietary suitability it is essential that reliable and accurate methods should be available for the determination of selenium concentration in food. Commonly used instrumental methods include either atomic absorption spectrometry (Oliveira, Neto, Nóbrega, Correia, & Oliveira, 2005; Rosa, Moraes, Neto, Nóbrega, & Nogueira, 2002; Smrkolj, Pograjc, Hlastan-Ribic, & Stibilj, 2005), inductively coupled plasma atomic emission spectrometry (Velitchkova, Pentcheva, & Daskalova, 2004).

Compared to other techniques, graphite furnace atomic absorption is a suitable and widely used technique for the determination of this element at trace levels due to its

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selectivity, simplicity, high sensitivity and is relatively cheap to purchase and operate. However, direct determination of Se in biological materials with high organic content is always troublesome (Capelo et al., 2006; Cava-Montesinos, Cervera, Pastor, & de la Guardia, 2004). Sample introduction is the critical step in the determination of total selenium in biological samples (Manuel, Luisa, Manuel, Enrique Sanchez, & Sanz-Medel, 1996; Robberecht, Van Grieken, Van Den Bosch, Deelstra, & Vanden Berghe, 1982; Verlinden, 1982; Welz & Marianne, 1984). In open digestion, it has been recommended to use strong oxidizing acid mixtures such as $HNO_3 + H_2SO_4$ or $HNO_3 + HClO_4 + H_2SO_4$ with final digestion temperature reaching ~310 °C (Manuel et al., 1996; Robberecht et al., 1982; Verlinden, 1982; Welz & Marianne, 1984). These conditions were necessary in order to avoid losses of some volatile selenium compounds and to perform the breakdown of some acid resistant organoselenium compounds such as the trimethylselenium ion and selenomethionine, which are the major species of selenium found in foods (Smrkolj & Stibilj, 2004).

The main drawback of the above procedure can be summarized as follows (i) Both perchloric acid and sulfuric acid interfere in ETAAS determinations (Welz & Sperling, 1999) (ii) the presence of perchloric acid at elevated temperatures may create some risk for the operator. Plant samples contain high amounts of siliceous compounds and therefore a hydrofluoric step is needed during the digestion procedure to assure its destruction in order to liberate the selenium (Smrkolj & Stibilj, 2004). Furthermore, the digestion of plant samples is rendered difficult by their high fiber content (Diaz-Alacorn, Navarro-Alarcon, Lopez-Garcia de la Serrana, & Lopez-Martinez, 1994). In direct determination, the presence of fat compounds may affect the quality of sampling by changing the amount of analyte inside the pipette of auto sampler of spectrometer. As a consequence the mass of analyte inside the atomizer can vary randomly, and so can the reproducibility and/or accuracy of measurements (Quinaia & Nobrega, 2000). These difficulties pertaining to mineralization of biological samples are rectified to a large extent by the use of microwave chemistry for sample decomposition (Jassie & Kingston, 1988). However a common hindrance to closed vessel microwave methods when preparing biological samples is the sample size restriction. The ability to digest larger sample sizes is important, when determining elements at or near the detection limit. Reaction of acids with organic sample matrix results in a build-up of pressure due to the evolution of decomposition gases and can cause some problems if safety precautions are not strictly followed. Alternately in the case of analysis of biological materials for Se, arrangements in monitoring increased pressure (AOAC method, 1990) have been used for complete digestion. However, though very effective, this AOAC method requires ~20 h for a single digestion, and hence time consuming.

A preferred approach for the breakdown of metal–organic complexes with subsequent mineralization of organic matter, involves treatment of the samples with ultraviolet radiation (Golimowski & Golimowska, 1996; Takayanagi & Wong, 1983; Velitchkova et al., 2004). UV-digestion is a clean sample preparation method, as it does not require addition of large amount of reagents. The UV-photolysis digestion method has been applied for the destruction and mineralization of organic compounds in samples such as sea water (Achterberg & Van den Berg, 1994), honey (Buldini, Cavalli, Mevoli, & Sharma, 2001) and urine (Philippeit & Angerer, 2001). To the knowledge of the authors no complete digestion of solid samples of biological origin for the determination of traces of selenium has been carried out till now.

This paper reports on the effectiveness of UV-photolysis based digestion of food samples prior to selenium determination using ETAAS. Experiments on UV digestion efficiency and recovery of Se have been conducted using various certified reference materials, (Oyster tissue, Tuna fish homogenate, Whole egg powder) and subsequently a number of common food items were digested similarly. These were analysed for their Se content.

2. Experimental

2.1. Instrumentation

All the measurements were carried out using Zeenit 65 model (Analytik Jena AG, Jena, Germany) transversely heated graphite furnace atomic absorption spectrometer equipped with an MPE 60 auto sampler. The spectrometer was provided with both Zeeman (transverse variable magnetic field) and deuterium hollow cathode lamp based background correction. Normally traces of Fe present in food materials absorb continuum radiation and thus suffer over correction in deuterium background correction mode. Hence the Zeeman background connection was used throughout the present work. The light source was a hollow cathode lamp (GBC, Australia) operating at 7 mA current at 196 nm resonance line. The furnace temperature program used is given in Table 1.

Pyrolitic coated graphite platforms (Analytik Jena, Part No. 407-152.011) were used. Atomic signals were measured in the peak area mode. High purity argon (99.99% pure, BOC, India) was used as the purge gas. All the AAS measurements were carried out using the stabilized temperature platform furnace (STPF) (Welz & Sperling, 1999) conditions.

UV-photolysis was carried out in quartz test tubes fitted with PTFE stoppers that were subjected to UV-photolysis in a Metrohm Heirsau, Switzerland 705 UV digester equipped with 500 W high-pressure mercury lamp. UV-radiation is mirrored into the samples by means of a mirror surface that surrounds the quartz vessels component, in order to enhance UV-intensity. The lamp cooling fan and the fluid cooling oil placed outside the mirroring surface permit the temperature control of the sample compartment.

Table 1
Temperature programme for the determination of Se in photolyzed digests

Step no.	Furnace temp. (°C)	Ramp (°C/s)	Hold (s)	Argon flow (ml/min)
1	90	2	10	300
2	120	5	20	300
3	200	10	30	300
4	800/900[a]	10	15	300
5	2100	1	3	0
6	2300	2	3	300

[a] Reduced palladium modifier.

Distilled and deionised water with a specific resistance of 18 MΩ cm greater was obtained through a Milli-Q (Millipore, USA) water purification system; Nitric acid and hydrogen peroxide (Suprapur grade, E-Merck, Germany) were used. Selenium stock solution (1000 μg/L) was prepared by dissolving anhydrous sodium selenite (Anal R, Aldrich) in deionised water. Working standards were prepared on the day of use by appropriate dilution of the stock solution.

Quartz digestion vessels were cleaned by soaking for at least 24 h in 1 M HNO3 followed by rinsing with deionised water. The following 10.0 ± 0.2 g/L stock solution were used, magnesium nitrate solution (Merck No. B-593213431) and palladium nitrate solution (Merck No. B-936989710).

2.2. Sample preparation

Food and vegetable samples were purchased from local market or obtained from local sources. Brazil nut samples were obtained from Brazil.

Flour samples – rice, wheat and ragi – were used as obtained. The curry leaves, mushrooms and beet root vegetable matter was washed thoroughly with DI water to remove any adhering soil particles, dried in an hot air oven at 40 °C overnight to remove moisture. These were ground in a planetary ball mill (Fritsch, Pulversiette, Idar oberstein, Germany) and sieved through a 200-mesh sieve and particles finer than 200 meshes were collected. Tea granules and pulses procured from the market were ground in the ball mill and sieved to obtain finer than 200 mesh particles.

Nuts and seeds (Brazil nut, ground nut, mustard and pumpkin) containing oil were ground to a fine paste and analysed whole.

2.3. Sample digestion using UV-photolysis

A portion of the sample (250 mg) was weighed and transferred into the quartz vial of the UV-digester. Before digestion, samples were left at room temperature for predigestion with 7 ml of concentrated nitric acid to prevent foaming during digestion. Then 200 μl of H_2O_2 (30%) was added to the sample and was subjected to UV-photolysis at 65 ± 5 °C for 1 h. The quartz vials were covered with lids that nonetheless allowed any vapors formed to escape to prevent any pressure build-up. During this period, at 15 min interval 100 μl aliquot of hydrogen peroxide was added and this cycle was repeated giving a total of four additions of H_2O_2. This procedure aims at ensuring that oxidizing conditions are maintained throughout the digestion. Clear, colorless digests were obtained in most of the cases, except in certain cases like curry leaves and wheat flour, where some silica precipitated. Later the lids were removed and the volume of the solution was reduced to 1.5 ml by evaporation; samples were cooled, diluted to 5 ml with water and analysed by ETAAS. Five microliters of standard and 10 μl of Pd modifier were injected into the furnace. The concentration of the analyte was obtained by standard addition method for sample and standards.

Table 2
The furnace programmer used to reduce the palladium modifier

Step no.	Furnace temp (°C)	Ramp (°C/s)	Hold (s)	Argon flow (μL/min)
1	75	5	–	300
2	90	35	–	300
3	500	10	50	300

2.4. Reduced palladium modifier

Pyrolysed samples of Brazil nuts and mushrooms were analysed using reduced palladium modifier. The reduction of the modifier was accomplished by introducing 2000 ng of Pd in a 20 μl drop and reducing it at 500 °C using the temperature programme in Table 2. Thereafter, the analysis proceeded according to the previously optimized furnace programme as given in Table 1.

3. Results and discussion

3.1. Mechanism of destruction of organics by UV-photolysis

The spectrum of high pressure Hg vapor lamp is rich in lines in the UV range (40–400 nm), with a pronounced signal at 254 nm arising from the transition of Hg atoms from their lowest excited state ($6\ ^3P_0$) to the ground state ($6\ ^1S_0$). The action of these UV spectra on dissolved organic compounds results in the formation of many intermediate compounds like the excited states of hydrogen peroxide, singlet oxygen and hydroxyl radicals. However, mineralization of solid organics also requires the addition of substances that facilitates the oxidation process. These substances include H_2O_2 and HNO_3. In the presence of these oxidants, UV irradiation has a catalytic character (Golimowski & Golimowska, 1996).

The UV-mineralization processes used in the present experiment make use of the high reactivity of hydroxyl radicals, which were generated from hydrogen peroxide added during the photolysis. When exposed to the action of UV light, H_2O_2 decomposes forming $OH^{\cdot}$ radicals, which initiate the radical chain reactions involving organic substances

contained in the mineralized samples. Nitric acid, in addition to its oxidizing capacity also helps in the dissociation of the metal–organic bond. Nitrates are also a major source of OH radicals as follows

$$NO_3^- + H_2O + h\nu \rightarrow NO_2 + OH^- + OH^{\cdot} \quad (1)$$

During the UV irradiation of nitrate ions, nitrites ions are formed, which on interaction with UV light produces further $OH^{\cdot}$ radicals

$$NO_2^- + H_2O + h\nu \rightarrow NO + OH^- + OH^{\cdot} \quad (2)$$

Thus the presence of nitric acid is essential for the UV photolysis of organics and it certainly reduces the time needed for the decomposition of the organic compounds.

3.2. Optimization of UV-photolysis

The efficacy of the digestion procedure using HNO_3/H_2O_2 was checked by determination of selenium in certified reference material (Whole egg powder-8415, Tuna fish-IAEA 350, Oyster tissue-1566a) and in some real samples. Selenium content was obtained by standard addition method. It was seen that when digestion was carried out using HNO_3 (5 ml) alone in the UV-photolysis, the values obtained were 40% lower than the certified value for a photolysis time of 1 h for a 100 mg sample weight. When H_2O_2 was added periodically, to the pre-digested samples with HNO_3, the values obtained were very close to the certified values. To establish optimal digestion conditions in the UV photolysis, different parameters were modified such as sample mass, volume of HNO_3, H_2O_2, digestion temperature and time. Sample weights in the range of 100–250 mg were used. The weight of the reference materials taken was ~200 mg which is necessary in order to avoid any inhomogeneity. By using different volumes (1–4 ml) of HNO_3 (60% v/v), it was found that for 250 mg of sample 7 ml of HNO_3 and 800 μl of H_2O_2 (added in four steps) was required to obtain a colorless solution, when photolysed for 1 h. The temperature was maintained below 65 °C by chilled water circulation in order to avoid drying up of the sample that could result in loss of Se.

3.3. Chemical modifiers and heating programs

The choice of chemical modifiers is an important experimental parameter in ETAAS analysis. The use of chemical modifiers for Se is essential due to its volatility. Further, during the photolysis, sulfur normally present in food materials gets oxidized to sulfate. It has been reported (Fischer & Rademeyer, 1999) that sulfate causes severe interference in the determination of selenium. This interference arises mainly due to the loss of gaseous SeS (Frech, Lundberg, & Cedergren, 1985). To reduce the interference, palladium nitrate, reduced Palladium (Pd°) and Pd–Mg modifiers were tested. Pyrolysis and atomization curves were carried out in order to determine the optimum pyrolysis and atomization temperatures. All the results were based on peak area measurements. For this study, pyrolysed digests of food materials were spiked with 1 ng Se. It was seen that the atomization temperature was 2100 °C for all the three modifiers, whereas the reduced palladium modifier provided higher signal sensitivity. Pre-reduced palladium (Pd°) provided highest pyrolysis temperature of 900 °C. Typical absorbance and back ground signals of photo digested Brazil nut sample using pre-reduced palladium as modifier is shown in Fig. 1. It should be noted that the levels of magnesium usually found in plant/food materials are around 0.75% m/v and probably this helped in partial selenium stabilization, so that only palladium was sufficient as a chemical modifier.

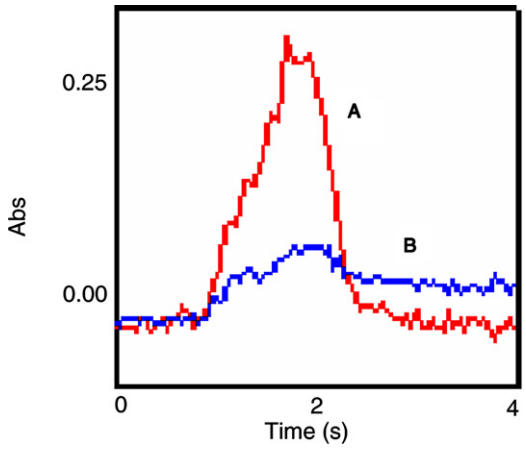

Fig. 1. Atomic absorbance (A) and background (B) transient signal of Se using reduced palladium as modifier of photolyzed Brazil nut sample.

3.4. Optimization of magnetic field

Analytical sensitivity in Zeeman ETAAS can be improved by controlling the magnetic field strength (Heike, Klaus, & Welz, 2003). An increase in magnetic field strength causes an increase in specific absorption, while the total absorption remains the same. This effect is due to a decrease in non-specific absorption. An increase in the field strength improved the separation of σ compounds, which results in a higher specific absorption and finally improved sensitivity. Fig. 2 represents the change in atomic absorption signal when the magnetic field strength changes from 0.6 T to 1.0 T for Se. So in all our measurements a magnetic field strength of 1 T was used to obtain maximum signal sensitivity.

3.5. Performance quality parameters

The detection and quantification limits (LOD and LOQ) were established. For this, blank extracts were performed, by following the overall procedure. The values obtained were 1.2 μg/L for LOD and 4.0 μg/L for LOQ (by considering three and 10 times the standards deviation respectively). The accuracy and the precision of the method were assessed by determination of selenium content in

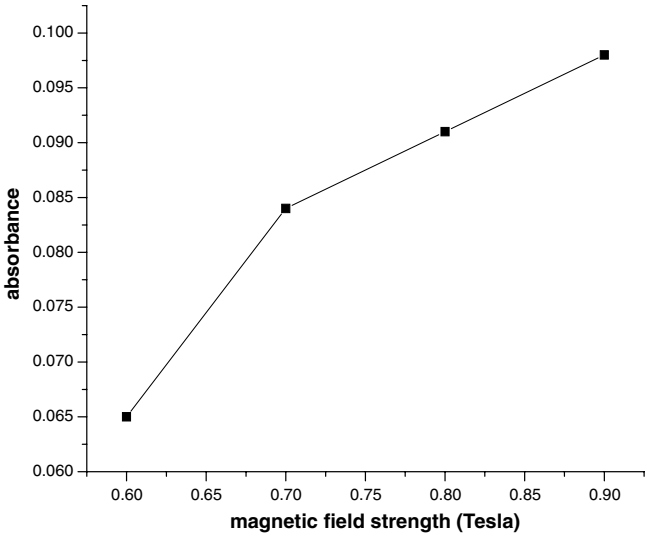

Fig. 2. Atomic absorption (peak area)for 300 pg of Se at different magnetic field strengths.

CRMs, Oyster tissue, Whole egg powder and Tuna fish. For selenium quantification the results obtained by using both external curve and standard addition were compared. For this six independent determinations were performed in two non-consecutive days and the results are summerised in Table 3. From the table, it can be concluded that standard addition leads to more accurate results, whereas when external curve is applied low results are obtained in some cases. A paired *t*-test was applied to examine whether the results of CRM's obtained by the present method differed significantly at the 95% confidence level limit. As the calculated values of *t* were less than the critical *t* value of 2.776 (degree of freedom 4), it follows that there is no statistical difference between the results.

3.6. Recovery study

In the present paper, oxidizing free radicals have been utilized for UV assisted decomposition of biological samples. In contrast to oxidation, photochemical reduction of Se to volatile SeH_2 has been shown to take place when the photolyzing aqueous solution fortified with low molecular weigh organic acids, like formic or acetic acid (Guo, Sturgeon, Mester, & Gardner, 2004). This fact indicated that if at any stage during the photolysis low molecular weigh organic acids are produced, Se might be lost. Thus it was imperative to study the recovery behavior of Se.

Table 3
Se content (μg/g) in the CRM's by photolysis – ETAAS

Sample	External	Standard addition (μg/g)	Certified value (μg/g)
Oyster tissue	1.3 ± 0.07	2.05 ± 0.102	2.2 ± 0.24
Whole egg powder	0.8 ± 0.03	1.46 ± 0.087	1.4 ± 0.17
Tuna fish-IAEA	3.4 ± 0.21	5.30 ± 0.45	5.56 ± 0.35

Table 4
Selenium level in commonly consumed foods

Serial no.	Sample	Se (ng/g)	Recovery (%) ($n = 3$)
1	Brazil nut	38000 ± 145	98
2	Mushroom	1340 ± 13	103
3	Curry leaves	585 ± 21	99
4	Pumpkin seeds	418 ± 17	99
5	Mustard seeds	248 ± 15	98
6	Ground nut	246 ± 8	96
7	Dal (pulses)	181 ± 9	100
8	Rice flour	136 ± 4	101
9	Herbal tea	198 ± 18	94
10	Wheat flour	121 ± 5	96
11	Cardamom	80 ± 4	94
12	Ragi flour (*Panicium decompositum*)	84 ± 2	94
13	Beetroot	62 ± 2	98

Hence the samples were spiked with 500 ng of Se before the photolysis and analysed using the described ETAAS procedure. The recoveries of the spikes were in the range of 94–103% (Table 4). The precision of the recovery was between 2% and 5% (RSD).

3.7. Selenium in selected foodstuffs

The optimized and validated photolysis–ETAAS procedure was applied for selenium determination in different food materials and the results are presented in Table 4. Selenium values found were above the LOQ, means that quantification was feasible for all samples analysed. Between batches, precision expressed as relative standard deviation was in the range of 3–12%. Selenium contents found varied in a very wide range, from 62 ng/g in beetroot to 38 μg/g in Brazil nut. Earlier Vonderheide et al. (2002) had reported Se content in Brazil nuts obtained from central region of Brazil as 35 μg/g. Among the vegetables, mushroom was found to posses highest selenium content, but this is not surprising as plants with high content of sulfur containing compounds are a rich source of selenium.

4. Conclusions

The present study indicates that UV photolysis is a reliable, fast and efficient method in mineralization of solid biological samples and also does not result in conditions under which selenium is lost. UV photolysis of solid biological/food materials followed by ETAAS was found to be very effective for the determination of traces of selenium. Hydrogen peroxide provided free $OH^{\cdot}$ radicals, which accelerated the sample decomposition. Nitric acid addition was required to contribute to sample dissolution via $NO^{\cdot}$ radicals and to maintain oxidizing conditions. In case of samples that contain sulfur, reduced palladium is used as modifier to eliminate the resulting sulfate interferences on Se determination by ETAAS.

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