Ann. Ist. Super. Sanità
Vol. 25, N. 3 (1989), pp. 505-510

DETERMINATION OF SELENIUM IN FOODS BY INDUCTIVELY-COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY AND HYDRIDE GENERATION

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Summary. - *Within the framework of a survey launched by the Istituto Superiore di Sanità to assess the average daily intake of Se with food in the Italian population, a preliminary study was carried out to develop an analytical procedure for the quantification of the element in food products. The method implies the use of a hydride generation system in combination with inductively-coupled plasma atomic emission spectrometry (ICP-AES) and exploits the inherent advantages of this last also for the analysis of non-metals. The overall approach was tested in some Hungarian and Italian foods, adequately lending itself to routine and reliable Se assay.*

Riassunto (Determinazione del selenio negli alimenti mediante spettrometria di emissione atomica con plasma induttivo e generazione di idruri). - *Nell' ambito di uno studio intrapreso dall' Istituto Superiore di Sanità per stabilire l' assunzione media giornaliera di Se col cibo per la popolazione italiana, è stata condotta un' indagine preliminare per sviluppare una procedura analitica che consenta la determinazione dell' elemento nei prodotti alimentari. Il metodo si basa sull' uso di un sistema a generazione di idruri in combinazione con la spettrometria di emissione atomica a plasma induttivo (ICP-AES) e sfrutta i vantaggi intrinseci di quest' ultima anche per l' analisi di non-metalli. L' applicabilità pratica di questa strategia è stata verificata nel caso di alcuni alimenti ungheresi ed italiani ed è stato quindi possibile concludere che essa si presta adeguatamente per l' esecuzione di saggi routinari ed attendibili.*

Introduction

Selenium is a well known growth factor for plants and an essential element for animal organisms, even though it may manifest toxic effects at exceedingly high amounts. Plants can absorb only selenate from soils and the element intake is limited exclusively to acidic soils with high humus and Fe content. Selenium is deemed to play an antioxidant role like vitamin E, i.e. it would protect the cell membrane from the pernicious action of peroxides. The antitumoral effect of Se finds its rationale in its antagonism to both radicals and heavy metals like Cd, Hg and Pb [1].

Toxic consequences of Se intake were observed among the rural population cultivating selenoferrous soils, as in China and Venezuela. Hair loss, nail deformation and tooth decay are the main symptoms as a consequence of selenosis. Endemic geobiochemical diseases as Kashin-Beck and Keshan typical of East Siberia and certain parts of China cause health impairments such as cardiomyopathy and osteoarthropathy. These ailments are related to a too low Se intake [2]. The biogeological significance and the related analytical assay of Se were reviewed exhaustively by Raptis *et al.* in a survey containing 480 references [3]. More recently, a WHO monograph was published which summarizes the present knowledge on the health and environmental roles of Se [1].

From an analytical point of view this element greatly challenges the experimentalist, although a considerable progress has been made since the adoption of hydride generation in combination with modern atomic spectrometric methods. In general, the analytical applications of gaseous hydride generation were already described in the early 1950's [4]. However, only in 1969 this technique was exploited in atomic absorption spectrometry (AAS). Holak collected the products of the Marsh-reaction in a liquid nitrogen trap with subsequent determination in air-acetylene flame after evaporation [5]. Fernandez in turn proposed the introduction of $NaBH_4$ in aqueous solution as the reductant [6]. In the 1970's, Robbins and Caruso [7] as well as Godden and Thomersen [8] reviewed the methodological developments in hydride generation-AAS. In a comprehensive paper, Nakahara attempted a survey of the literature on hydride generation-AAS [9]. Later on, Thompson *et al.* described a continuos hydride generation

system which can be interfaced to an inductively-coupled plasma atomic emission spectrometry (ICP-AES) instrument for the determination of hydride forming elements [10, 11].

Besides the well-known advantages of ICP-AES, in the case of Se there is a serious drawback in that the conventional nebulization system used for the analysis of solutions has a relatively poor efficiency. Much more expediently, the analyte can be introduced into the plasma with nearly 100% efficiency by using hydride generation. Matrix effects are thus minimized and the energy available for the excitation of Se is sensibly increased, yielding a 1-2 orders of magnitude improvement in the detection power [8]. Within this general framework, Bax *et al.* investigated the role of $NaBH_4$ in order to elucidate the mechanism of hydride evolution. The authors [12] found that, whilst the main reaction path is:

$$3BH_4^- + 4H_2\,SeO_3 + 3H^+ = 4H_2Se + 3H_3BO_3 + 3H_20$$

the borohydride ions are hydrolyzed in a competitive reaction producing gaseous hydrogen,

$$BH_4^- + 3H_2O + H^+ = 4H_2 + H_3BO_3.$$

Only selenite ions can be reduced by borohydride as selenate reduction is probably inhibited by kinetic effects. Therefore, Se(VI) species must be brought to the Se(IV) valence by using HCl or HB prior to hydride generation [13]. Welz and Schubert-Jacobs studied the interference of transition metals and pointed out that Ni(II) reduces to metallic Ni and catalyzes the hydride decomposition in the reaction chamber while Cu(II) interferes with the analysis by forming insoluble CuSe [14]. This parasitic reaction can be minimized by high Cl^- concentration [14] or by adding complexing agents to the analyte solution [15]. Hershey and Keliher studied the possible inteferences exerted by approximately 50 elements coming to the conclusion that the undesired effects can be reduced by an appropriate choice of the hydride generation system and by an appropriate adjustment of the acid strength [16]. Stable analytical signals can be obtained by effective gas-liquid separation and proper interfacing of hydride generator and plasma torch [17]. Both sequential and simultaneous determinations with ICP-AES require the optimization of plasma gas flows, observation height and conditions of hydride generation [17, 18]. The dissociation of H_2 Se takes place at 700-900 °C [19] in the presence of H_2 and O_2 traces revealing the participation of OH radicals in the dissociation process [12]. Baucells *et al.* critically compared hydride-ETA-AAS, hydride-ICP-AES and GFAAS for the determination of Se in serum, showing that the detection power and precision were similar in the three cases [20]. Speciation of various Se forms can be performed by separation prior to hydride generation. Fodor and Barnes e.g. separated selenite and selenate from urine through ion-exchange and could thus quantify them by hydride generation and ICP-AES [21].

This being the state of the art, a hydride generation-based method with ICP-AES detection was developed for the determination of Se in acid-digested food samples in order to further extend the applicability of the technique and hence contribute to the assessment of the daily intake of this element.

Experimental

Reagents

H_2SO_4, HNO_3, $HClO_4$ and HCl (Aristar, BDH Chemicals Ltd., UK) and 95% purity $NaBH_4$ (Carlo Erba, Italy) were used throughout the study.

In turn, calibration standards were prepared from a Se stock solution (Spectrosol R, BDH Chemicals Ltd., UK) containing 1.00 mg/ml Se in the form of H_2SeO_3 in 1 N HNO_3. Doubly-distilled water was used for the preparation of all solutions.

Digestion procedure

Two different approaches were followed. In the first case, 1.00 g fresh sample was placed in an open flask to which a reflux device was fitted. Predigestion was performed by treating the sample with 5 ml of a concentrated HNO_3-$HClO_4$-H_2SO_4 mixture at a 24+24+1 ratio for 2 h at room temperature. Five additional ml of this acid mixture were added to the predigested sample and the flask was afterwards heated on a sandbath for 6 h until fuming of nitrous vapors. As an alternative, 1.00 g fresh sample was digested with 70% HNO_3 in capped teflon vessels placed within an MDS.81D microwave oven. This system allows specimens to be completely digested at about 180 °C and 10 kPa for no more than 25 min. Not only is the time required for the treatment considerable shortened, but also the blank signal is significantly lowered owing to the low amount of acid necessary to perform the digestion [22]. The instrumentation employed is listed in Table 1. The turntable was rotated continuously. After cooling, the pressure inside the vessels was vented in the hood.

Complete digestion of samples was achieved with both procedures. The resulting solutions were acidified with 5 M HCl and heated at 80 °C for 1.5 h. Final volumes were brought to 10 ml. The $NaBH_4$ solution was stabilized with NaOH and filtered; three drops of Silicon (Merck, FRG) antifoaming agent were added to decrease the risk of pulsed flow and some drops of decanol were used to eliminate foaming from the gas-liquid separator. Table 2 summarizes the main working parameters.

Apparatus

The ICP-AES system employed is a combination of a sequential and simultaneous spectrometer. The main instrumental parameters are listed in Table 2. As regards the hydride generation device, this allowed a steady state

Table 1. - *Working conditions for the hydride generation IPC-AES system*

ICP-AES	
Spectrometer sequential and simultaneous	Jobin-Yvon 32+38 VHR, (France)
HF Generator	DURR-JY 3832, 56 MHz
Torch	INSA
Monochromator	HR 1000 M, f = 1 m, Czerny-Turner mounting, 3600 groove/mm holographic plane grating, linear dispersion 0.27 nm/mm (first order), theoretical resolution 504,000, spectral range 170-450 nm
Polychromator	HR 1000 M, f = 0.5 M, Paschen-Runge mounting, equipped with a 3600 grooves/mm holographic concave grating, linear dispersion 0.55 nm/mm (first order), spectral range 170-410 nm
Computer	Apple II GS, Pascal 2-5
Hydride generation	P.S. Analytical Ltd. (UK), continuous, cross flow
Microwave oven	CEM Corp., MDS. 81D (USA), magnetron power 0-600 W, adjustment in 1% steps, frequency of 2450 MHz
Peristaltic pump	Watson-Marlow Ltd. (UK), PVC tube, d = 5 mm

Table 2. - *Instrumentation and working conditions*

ICP-AES	
Plasma gas	21 l/min argon
Auxiliary gas	0.2 l/min argon
Sample gas	0.7 l/min argon
Applied power	2.2 kW
Observation height	15 mm
Slit width	40 μm
Integration time	1 s
Wavelength	Se(I) 196.09 nm
Hydride generation	
Sample solution acidity	5 M HCl
flow rate	9.4 ml/min
NaBH concentration	1.0% in 0;1 M NaOH
flow rate	4.7 ml /min
Microwave digestion program	Power (%) Time (min)
	1. 300 10
	2. 360 5
	3. 240 5

signal to be produced, which is mandatory for the subsequent spectrometric determination. A block diagram of the system is shown in Fig. 1. The hydride evolved in the T-piece is carried by argon gas flow to the plasma torch after separation from the liquid phase. Calibration was made up in the 0-50 ng/ml concentration range.

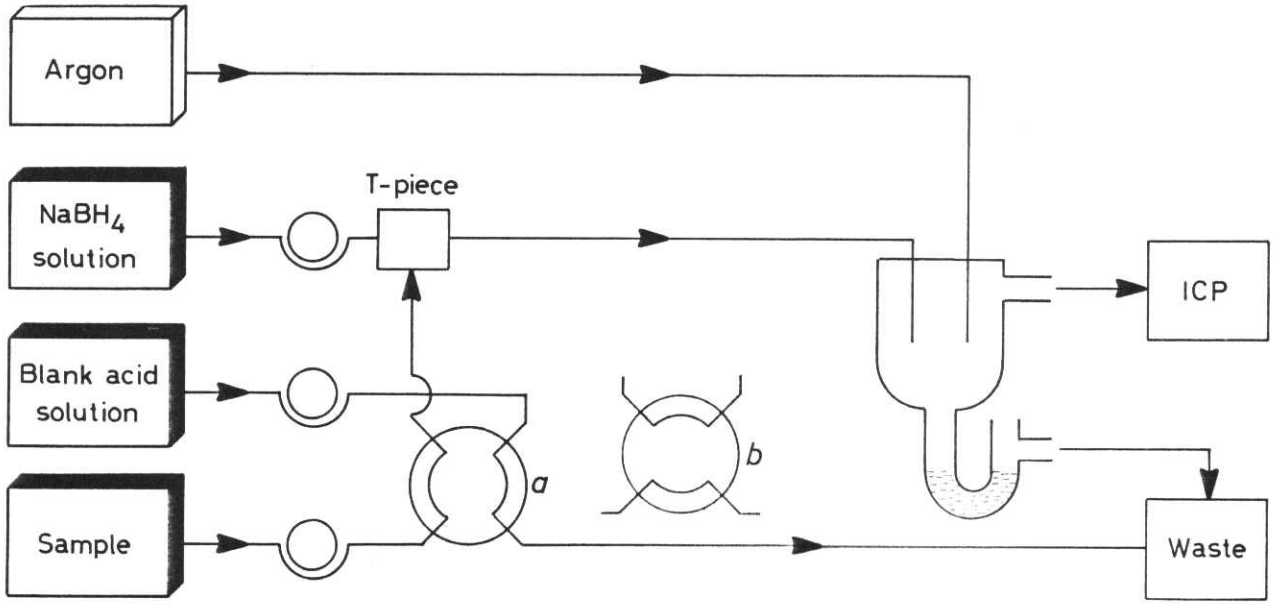

Fig. 1. - Diagram of the hydride generator. Valve position: a) introduction of sample; b) introduction of blank acid solution.

Results and discussion

The capability of this combined strategy to assess the Se content of food was tested in a few cases, which certainly do not claim to be representative of the average Se content in food for both countries, but are certainly indicative of the suitability of the technique developed.

Precision of measurements based on 6 replicates was found to be 5.3% at the 10 µg/l level. Typical analysis range was between 2-20 µg/l, with a detection limit of 0.7 µg/l (3 σ criterion); 98% of 20 ng of Se present in a solution were recovered using the first procedure. The results calculated on a fresh weight basis are summarized in Table 3.

The hydride generation-ICP-AES combination provides an excellent means for the analysis of Se in food. The matrix effect is virtually eliminated by introduction of gaseous hydride into the plasma. The transition metal interferences are also negligible by utilizing HCl at relatively high concentrations (5N). Sample digestion by HNO_3-$HClO_4$-H_2SO_4 mixture proved satisfactory, as stated in the Analytical Methods Committee report [23]. The microwave digestion method in turn needs to be further studied because lipidic substances show a gradual tendency to degrade. Predigestion in particular seems to be necessary prior to microwave dissolution to allow a more efficient attack of the sample. 10-40% of Se is reported to be volatilized in cooking processes [24]. This observation should be carefully considered when estimating this element in prepared foods.

Finally, the above described methodology is currently being applied in a pilot study aimed at establishing the average Se intake with diet in Italy. This ongoing activity, which greatly relies on the availability of an analytical approach both innovative and dependable, has already generated some preliminary results. These would point to daily individual intake of the element that is well within the recommended range of 50-200 µg/day.

Although this outcome is to be confirmed on a much broader statistical, basis, it witnesses the keen need from a nutritional point of view for sound and comprehensive undertakings which in turn demand reliable instrumental techniques and thus allow subsequent regulatory action to be consistently carried out.

Table 3. - *Selenium content of selected foods from Italy and Hungary*

Sample *(a)*	Se (µg/g)
Potato (H)	0.04
Cabbage (I)	0.10
Pork liver (H)	0.27
Egg (H)	0.41
Sour cream (H)	0.36
Bread (I)	0.08
Canned fish (I)	0.90
Pasta with mussels (I)	0.65
Fruit cocktail (I)	0.03

(a) the country of origin of the sample is presented in brackets

Review submitted on invitation by the Editorial Board of the *Annali*.
Accepted for publication: 30 May 1989.

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