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# On-line copper and iron removal and selenium(VI) pre-reduction for the determination of total selenium by flow-injection hydride generation-inductively coupled plasma optical emission spectrometry

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## Abstract

Selenium was determined in samples with high copper and iron contents by hydride generation-inductively coupled plasma optical emission spectrometry (HG-ICP-OES) after flow-injection (FI) on-line copper and iron removal and selenium(VI) reduction. A Dowex 1X-8 anion-exchange microcolumn was used for the separation of selenium from copper and iron as their chloro-complexes. Se(VI) was then reduced on-line by heating a PTFE coiled reactor (150 cm long, 0.7 mm i.d.) in a 100°C water bath. After reduction of Se(VI), a 900-μl sample was injected into the carrier stream containing hydrochloric acid and sodium tetrahydroborate to generate the hydride. A limit of detection of 0.4 μg l$^{-1}$ (RSD 2.3% for 20 μg l$^{-1}$ selenium) was obtained. The application of the method to Geochemical Standard Reference Samples and copper metal reference materials (MBH) demonstrated that results were in good statistical accordance with certified values. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Selenium determination; On-line pre-reduction; On-line removal of Cu and Fe interferences; hydride generation-inductively coupled plasma optical emission spectrometry (HG-ICP-OES)

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## 1. Introduction

Selenium occurs in the earth's crust with an average abundance of 0.09 mg kg$^{-1}$. Although rarely found in the native state, Se is a major constituent of approximately 40 minerals and a minor constituent of many others, mainly sulfides [1]. Selenium is essential for the human organism: it is an integral part of the enzyme glutathione peroxidase, which protects cell membranes from lipid peroxidation and radicals [2,3], and, as a consequence, it plays an important role in immune defence.

Hydride generation (HG) in combination with inductively coupled plasma optical emission spectrometry (ICP-OES) is a commonly used method for the determination of selenium [4,5]. The HG-ICP-OES technique minimizes spectral interferences caused by other matrix components. However, the interference caused by copper and iron is a serious problem for the determination of selenium by HG-ICP-OES [6,7] or hydride generation-atomic absorption spectrometry (HG-AAS) [8,9], since their presence results in the inhibition of selenium hydride formation. Removal of Cu and Fe has been attempted by the addition of masking agents, although the results have not been satisfactory for high concentrations of these interferents [10,11]. This is partly due to difficulties encountered in finding masking agents that are effective in the highly acidic conditions required for the hydride generation. Furthermore, even if such agents were found, high concentrations of Cu or Fe would prevent them from being effective. Wickstrøm et al. [12] reported the elimination of interferences from some transition metals, using masking agents in alkaline media. However, the interference caused by copper could not be totally eliminated when its concentration exceeded 200 mg l$^{-1}$. Procedures for separating selenium from the matrix by means of co-precipitation with lanthanum hydroxide [13], liquid–liquid extraction with APDC–chloroform [14], or its isolation it by means of ion-exchange in batch systems, have also been employed [15,16], but they have the disadvantage of being tedious, labor-intensive and dependent on operator skill.

Flow injection (FI) HG-AAS has been reported to reduce interference effects in the determination of selenium [17,18]. Åström [19] suggested that this improvement was probably due to the fact that the sample residence time in the FI system is very short and well controlled. This is particularly favorable for the fast formation of hydrogen selenide, while the slower interfering reactions are suppressed. However, the utilization of FI is not enough to correct for the interference of high contents of copper or iron.

In the last decade, on-line FI separation techniques have become increasingly popular for the determination of trace elements in different types of samples. The use of ion-exchange resins in continuous flow systems has proved to be very efficient [20–23]. However, in these systems Se(VI) reduction to Se(IV) has been carried out in batch sequence, resulting in an increased analysis time and contamination risk.

Although Stripeikis et al. [24] developed a continuous flow system for the on-line reduction of Se(VI), direct coupling of this methodology to ion-exchange columns is not possible, as the sample flow-rate through the reduction reactor is incompatible with the process occurring in an ionic-exchange column.

This paper reports a FI system for the generation of selenium hydride, in which copper and iron interferences are removed on-line using a microcolumn of a Dowex 1X-8 ion-exchange resin (chloride form), with the subsequent on-line pre-reduction of Se in a continuous-flow system.

## 2. Experimental

### 2.1. Reagents

A 0.6% (w/v) sodium borohydride solution (Aldrich Chemical Co. > 98%) was prepared in 0.5% (w/v) sodium hydroxide solution and was filtered through Whatman No. 42 filter paper to remove undissolved solids. This solution was prepared daily.

Standard solutions of selenium were prepared by dissolution of suitable amounts of the oxide (99.999% pure, Johnson Matthey) in small volumes of nitric acid, followed by dilution with

ultrapure water. The anion exchange resin was Dowex 1X8 200-400 dry-mesh, hydrogen form, 8% cross-linkage (Aldrich Chemical Co.).

Ultrapure water (18 MΩ cm$^{-1}$) was obtained from a EASYpure RF (Barnstedt, Iowa, USA).

All the reagents were of analytical-reagent grade and the presence of selenium was not detected in the working range.

## 2.2. Apparatus

The measurements were performed with a sequential ICP spectrometer [Baird ICP 2070 (Bedford, MA, USA)]. The ICP operating conditions are listed in Table 1. The FI system used is shown in Fig. 1. A Minipuls 3 peristaltic pump [Gilson (Villiers-Le-Bell, France)] was used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, four-way rotary valve. The hydride unit used was a (PS Analytical Ltd.) hydride generator and the flow rate of the reagents was controlled by a Watson−Marlow 303X peristaltic pump. The 900-μl sample loop and the coiled reactor (150 cm long, 0.7 mm i.d.) were

Table 1
ICP instrumental parameters

| | |
|---|---|
| RF generator power (kW) | 1.0 |
| Frequency of RF generator (MHz) | 40.68 |
| Plasma gas flow rate (l min$^{-1}$) | 8.5 |
| Auxiliary gas flow rate (l min$^{-1}$) | 1 |
| Carrier gas flow rate (ml min$^{-1}$) | 90 |
| Observation height (above load coil) (mm) | 15 |
| Analytical line: Se (nm) | 196.026 |

prepared using PTFE tubing. A microbore glass column (100 mm long 3 mm i.d.) fitted with porous 25 μm glass beads was used as the resin holder. Tygon-type pump tubing (Ismatec, Cole−Parmer, Vernon Hills, IL, USA) was employed to propel the sample, reagent and eluent. The Se 196.026-nm spectral line was used.

## 2.3. Procedure

### 2.3.1. Dissolution of copper metal reference materials

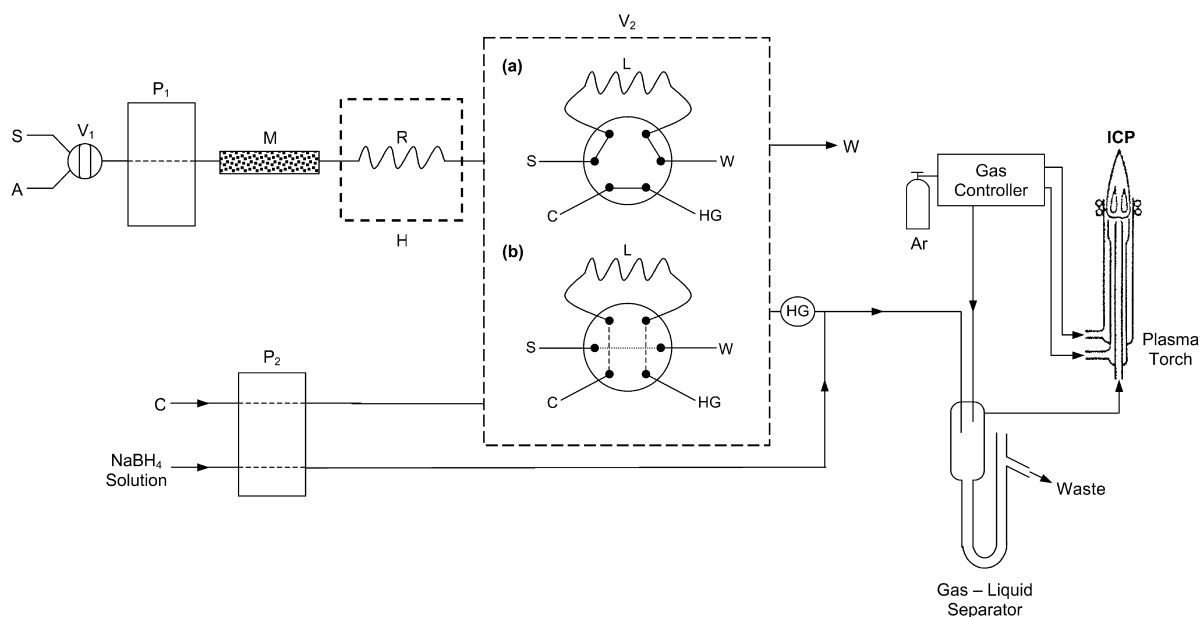A sample of 0.125 g of copper metal was digested with approximately 10 ml of a nitric and



Fig. 1. Schematic diagram of the instrumental set-up. S, sample (flow rate 1.5 ml min$^{-1}$); A, HNO$_3$ 2 M (flow rate 1.5 ml min$^{-1}$); C, HCl 7.0 M (flow rate: 9 ml min$^{-1}$); NaBH$_4$ 0.6% w/v (flow rate 3 ml min$^{-1}$); M, microcolumn; R, coiled reactor; H, boiling water bath; L, injection loop; W, waste; P$_1$, P$_2$, peristaltic pumps; V$_1$, two-way valve; and V$_2$, load-injection valve. Valve positions: (a) sample loading; and (b) injection.

hydrochloric acid (1:3) mixture, the resulting solution was heated to near dryness and taken up with 30.0 ml of 12 mol l$^{-1}$ hydrochloric acid and made up to 50 ml with distilled water. The solution was then ready for analysis.

### 2.3.2. Dissolution of geological samples (rocks and soil)

A 0.5-g portion of the sample was boiled in a covered Teflon beaker with 6 ml of HCl and 2 ml of HNO$_3$. After cooling, 5 ml of HClO$_4$ and 10 ml of HF were added, and the solution was kept at room temperature for several hours and evaporated until white fumes of HClO$_4$ appeared. This final step was be repeated if necessary until the sample was totally dissolved. The residue was taken up with 12 ml of 12 mol l$^{-1}$ hydrochloric acid and made up to 20 ml with distilled water. The solution was then ready for analysis.

### 2.3.3. Selenium determination with the FI system

The FI system shown in Fig. 1 was used for removal of Cu and Fe and reduction of Se. The microcolumn (M), filled with the anionic resin, was conditioned for 15 s with a 2-mol l$^{-1}$ HCl solution at a flow rate of 1.5 ml min$^{-1}$. For Se determination in the sample, 7.0 mol l$^{-1}$ HCl at 1.5 ml min$^{-1}$ was pumped through the reactor (R) placed in a boiling water bath (H), where Se(VI) was reduced to Se(IV). The reduced sample was charged into the 900-μl loop (L), with the V$_2$ valve in load position (a), and injected into the 7.0 mol l$^{-1}$ HCl carrier solution (C) through the V$_2$ valve (b). The carrier flow rate was 9 ml min$^{-1}$. After three injections, the two-way valve (V$_1$) was switched to line A and the interferents were eluted using 2 mol l$^{-1}$ HNO$_3$, which was pumped for 30 s through the microcolumn at a flow rate of 1.5 ml min$^{-1}$. The microcolumn was then regenerated by washing with 2 mol l$^{-1}$ HCl at a flow rate of 1.5 ml min$^{-1}$ for 30 s. After the regeneration period, the microcolumn was switched back in-line and the sampling procedure was repeated after washing the sample line with distilled water. Standard solutions, requiring no iron or copper isolation, were pumped through the injection valve, by-passing the microcolumn and reactor. The HG manifold ran continuously

throughout the procedure, independently of the matrix isolation unit operation. The operating conditions were established and the determination was subsequently carried out.

## 3. Results and discussion

### 3.1. Interference removal conditions

Fig. 2 shows the results for the determination of selenium in some synthetic samples with a high iron or copper content by HG-ICP-OES under different operating conditions. Although an increase in the hydrochloric acid concentration decreases the interference effect of these elements [25], it can be concluded that in many samples where the selenium content is at trace levels, and where iron or copper is found as a major interfering component, a separation technique must be used.

In this work, we used a microcolumn filled with an anionic resin (Dowex 1X-8), which retains iron and copper (as anionic chloro-complexes) and not selenium when the concentration of the HCl solution is 7.0 mol l$^{-1}$. When the concentration is less than 5 mol l$^{-1}$, Fe and Cu are not quantitatively retained due to the instability of their chloro-complexes; furthermore, it has been demonstrated that Se(VI) reduction is not quantitative if the acid concentration fall below 6.5 mol l$^{-1}$. For acid concentration above 7.5 mol l$^{-1}$, a slight decrease in the signal is observed, due to the dilution of the hydride by evolved hydrogen. On the other hand, acid concentrations above 7 mol l$^{-1}$ have shown to cause plasma instability that may worsen the precision, although the method for the separation of interferents and selenium works with HCl concentrations up to 9 mol l$^{-1}$. A 7-mol l$^{-1}$ HCl concentration was chosen as a compromise between the Se(VI) reduction optimum conditions and the quantitative retention of the interfering ions.

In order to avoid degradation of the separation resin, the Se(VI)/Se(IV) reduction reaction was performed after the separation of the interfering element.
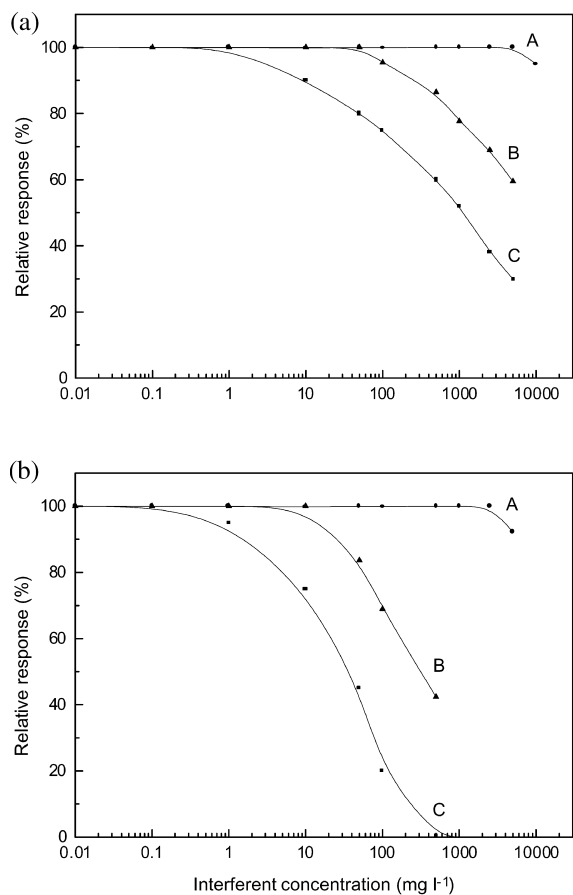
A column of 100 mm length proved suitable for

Fig. 2. Effect of interferent concentration on the response of selenium(IV) 20 $\mu$g l$^{-1}$ prior to on-line reduction: (a) in the presence of Fe(III); and (b) in the presence of Cu. A, anion-exchange microcolumn and pre-reduction of Se steps prior to determination; B, without anion-exchange microcolumn prior to pre-reduction and determination of Se in 7 mol l$^{-1}$ HCl; and C, without anion-exchange microcolumn prior to pre-reduction and determination of Se in 3.5 mol l$^{-1}$ HCl.

the samples assayed under our operating conditions (7.0 mol l$^{-1}$ HCl and sample flow rate 1.5 ml min$^{-1}$). However, should this capacity be insufficient, as in the case of a very low selenium/interferent ratio, the microcolumn length can be increased up 150 mm without observable changes in the magnitude of the signal.

### 3.2. Reduction of Se(VI) in a coiled reactor

A PTFE coiled reactor immersed in a boiling water-bath was used in order to reduce Se(VI) to Se(IV). Quantitative Se reduction was achieved with a minimum reactor length of 150 cm with an internal diameter of 0.7 mm when working at a flow rate of 1.5 ml min$^{-1}$ (Fig. 3). It should be mentioned that the sample flow rate employed was the maximum attainable before compaction of the ion-exchange resin.

### 3.3. Optimization of selenium hydride generation with the FI system

Sodium tetrahydroborate concentration was optimized in order to obtain the maximum sensitivity for selenium determination. It was found that the best sensitivity was obtained when the NaBH$_4$ concentration ranged between 0.45 and 0.75% (w/v). For concentrations greater than 0.75%, the sensitivity decreased slightly, and when the concentration was greater than 1.25%, the plasma became unstable and easily extinguished. A concentration of 0.6% (w/v) was subsequently adopted.

The optimum flow rate for sample introduction into the hydride generation system was found to be 9 ml min$^{-1}$. However, when running the sample through the column at this flow rate, resin compaction and subsequent clogging of the column was observed. This problem was solved by using a FI system for the sample introduction.

### 3.4. Performance of the FI system

Using the system described above, Fe and Cu were separated in the ion-exchange column and Se(VI) was then reduced in the coiled reactor at a flow rate of 1.5 ml min$^{-1}$; afterwards, the sample-containing loop was injected into the carrier stream at 9 ml min$^{-1}$. The overall time required for separating interferent ions, pre-reducing Se(VI), charging the injection loop (three times), signal development and conditioning of the microcolum for a new sample was approximately 3 min. Thus, sample throughput was approximately 20 samples h$^{-1}$.

The selenium signal (for samples and standards) was obtained as the average of three 900-$\mu$l injections. An injection volume of 900 $\mu$l was
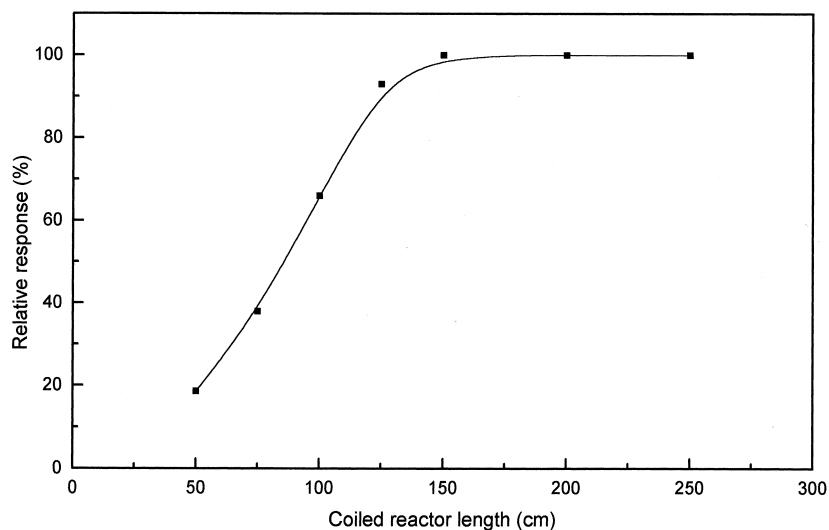
Fig. 3. Influence of coiled reactor length on the reduction of Se(VI) to Se(IV). The Se(VI) concentration was 100 $\mu g \, l^{-1}$.

chosen for this study because it was the minimum volume for which maximum sensitivity was obtained (Fig. 4).

### 3.5. Method validation

Recovery studies were carried out on synthetic samples in order to test the proposed method for determining selenium. The percentage recovery ranged from 98.5 to 99.9%. The precision of the system was 2.3% relative standard deviation (R.S.D.), based on 10 injections of an aqueous 20 $\mu g \, l^{-1}$ selenium standard, and the detection limit found was 0.4 $\mu g \, l^{-1}$. The system was calibrated using aqueous standards (5–150 $\mu g \, l^{-1}$); the cali-



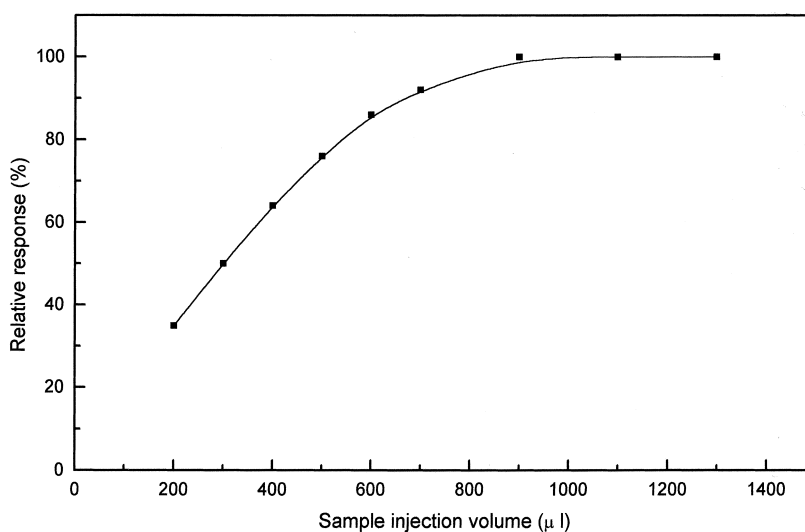Fig. 4. Effect of sample injection volume on the response of 20 $\mu g \, l^{-1}$ of selenium(IV).

Table 2
Results of the analysis of copper and iron reference materials[a]

| Sample | Certified value Se ($\mu$g g$^{-1}$) | Se found ($\mu$g g$^{-1}$) |
|---|---|---|
| MBH-39 DK 3601 | 90[b] | 90.5 ± 1.3 |
| MBH-39 DK 3604 | 15[b] | 14.8 ± 1.5 |
| GSR-6 (rock) | 0.099 ± 0.028 | 0.093 ± 0.01 |
| GSR-7 (soil) | 0.32 ± 0.09 | 0.38 ± 0.02 |

[a] 95% confidence limits, $n = 6$.
[b] Recommended values.

bration graph was linear up to at least 150 $\mu$g l$^{-1}$ Se, and a correlation coefficient of 0.9998 was obtained.

Matrix isolation and the reduction system were tested by performing analyses of two copper reference materials, MBH 39 DK 3601 and 39 DK 3604, and two Geochemical Standard Reference Samples, GSR-6 (Rock) and GSR-7 (Soil). The results are shown in Table 2.

## 4. Conclusions

From the results obtained, it can be concluded that it is possible to determine selenium precisely and accurately in samples with a high copper or iron concentration by using HG-ICP-OES. The FI method allows the on-line pre-reduction of selenium and the removal of copper and iron by means of a rapid and simple on-line ion-exchange procedure, as demonstrated by the analysis of two different copper metal and geological reference materials.

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