Optimization study of suitable pre-reduction agents for selenium analysis in tomato samples by flow injection-hydride generation atomic absorption spectrometry

[1]Limchoowong, N., [2]Techawongstein, S. and [1,*]Chanthai, S.

[1]*Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
[2]*Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand*

Abstract

The pre-reducing agents for Se speciation analysis were subject to optimize under the on-line reduction conditions of flow injection-hydride generation atomic absorption spectrometry using 2 M HCl as a carrier solution and 0.3% (w/v) $NaBH_4$ in 0.2% (w/v) NaOH as a reducing agent. For total Se, the pre-reduction of Se^{6+} to Se^{4+} must be completed when optimizing the pre-reducing conditions using (i) conc. HCl, (ii) 5% (w/v) KBr/conc. HCl, (iii) 15% (w/v) KBr/ dilute HCl and (iv) 10% (w/v) thiourea/conc. HCl, under heating for 30 min to overcome their matrix effects on the calibration curve using either Se^{4+} or Se^{6+} as standard compound. From the results, all of these pre-reducing agents, except that in the presence of thiourea interfered only the calibration curve using Se^{4+}, could be used with both Se species as expressed in terms of their conversion factors of 98-116% and 81-103% for Se^{4+} and Se^{6+}, respectively. The linear range was obtained between 5-25 µg/L with 0.5 µg/L LOD and 2 µg/L LOQ. The optimal pre-reducing agent was applied for real tomato varieties, resulting in almost satisfactory recoveries (75.6-115.9%). The Se contents in these samples were found to be 0.07-0.10 µg/g, 0.43-0.92 µg/g and 0.50-0.99 µg/g for Se^{4+}, Se^{6+} and total Se, respectively.

Introduction

Selenium can be found naturally in four valence states including selenide, Se^{2-}, elemental Se^0, selenite, Se^{4+} and selenate, Se^{6+}. It is essential yet toxic, and exhibits a narrow window between essential and toxic concentrations. Consumption of foods containing less than 0.1µg/g results in deficiency whereas regular consumption of food containing more than 1 µg/g results in toxicity (Clement *et al.*, 2000). The toxicity of different species for Se differs, in which the toxicity of Se^{4+} is stronger than that of and Se^{6+} (Gallignani *et al.*, 2000; Hernandez *et al.*, 2007; Kongsri *et al.*, 2013). Therefore, it is more important to analyze Se speciation than to determine the total concentration of inorganic Se in samples.

Nevertheless, few analytical methods for the selective determination of the Se species include high performance liquid chromatography (HPLC), gas chromatography (GC), which has been coupled to mass spectrometry (MS), inductively coupled plasma mass spectrometry (ICP-MS) or flow injection-hydride generation atomic absorption spectrometry (FI-HGAAS). However, ICP-MS approaches interferences between the dominant isotope of Se and the argon

dimer, Ar_2 (both mass 80) (Jackson and Miller, 1998). Chromatographic techniques gave decomposition of species and peak overlap (Raptis *et al.*, 1983). Even, considering that HG-AAS determines Se^{4+} directly, while Se^{6+} cannot directly be detected but it must be reduced to Se^{4+} prior to measurement. Thus, the pre-reducing step to convert Se^{6+} to Se^{4+} is still needed.

Pre-reducing agent is normally achieved using HCl at various concentrations and temperatures. For instance, the reduction with HCl solution in a closed system at room temperature takes 7 days. If its reduction step works at elevated temperature (90 - 100°C), it is faster. The reaction time usually varies from 20 to 45 min. However, too long heating can lead to the appearance of elemental Se (Raptis *et al.*, 1983; Cobo *et al.*, 1994; Olivas *et al.*, 1994; D'Ulivo, 1997; Pyrzyńska *et al.*, 1998; Pyrznska *et al.*, 1999). The pre-reduction can also be using a warm co-reducing agents i.e. KBr and HBr (Brindle and Ługowska, 1997) or a mixture of KBr and HCl (D'Ulivo, 1989; Brindle and Ługowska, 1997; Rodas, 2013), thiourea with the addition of HCl (Niedzielski *et al.*, 2002) and 20% solution of KI with HCl and thiourea. The later system restricts the interference of transition metals (D'Ulivo, 1997).

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*Corresponding author.
Email: *sakcha2@kku.ac.th*
Tel: +66 4300-9700 ext. 12371; Fax: +66 43202373

In this study, the optimal pre-reducing agents to convert Se^{4+} to Se^{6+} for trace analysis of an inorganic Se species and total Se were investigated using an autoclave digestion and determination by FI-HGAAS. The effect of the selected pre-reducing systems on the calibration curves of both Se^{4+} and Se^{6+} was studied in detail. Application of optimal pre-reducing agent of the Se species was then carried out with five varieties of tomato samples.

Materials and Methods

Chemicals

All reagents were of the highest purity available or at least of analytical reagent grade. Deionized water (resistivity 18.2 MΩ cm) was used to prepare all solutions in the work. A 1000 mg/mL Se^{4+} stock solution was prepared from SeO_2 (Carlo Erba, France) in HCl (Carlo Erba, France). A 1000 mg/mL Se^{6+} stock solution was prepared from Na_2SeO_4 (Sigma Aldrich, USA) stored in a refrigerator at 4ºC. Lower concentrations were prepared on the day by diluting the stock solution with 0.1 M HCl. A 0.3% (w/v) of $NaBH_4$ was prepared daily by dissolving 0.75 g of $NaBH_4$ (Lab Chem, France) in 0.2% (m/v) NaOH (Carlo Erba, France) and diluting quantitatively to 250 mL with the same solution. The carrier solution was 2.0 M HCl.

Pre-reducing agents included only conc. HCl or the solution containing KBr (Asia Pacific specialty, Australia) or thiourea (BDH laboratory supplies, England) in the presence of either conc. HCl or dilute HCl were employed to reduce Se^{6+} to Se^{4+}. For digestion of tomato sample, 65% (w/v) HNO_3 (Lab Scan Asia, Thailand) was used.

Instruments

A Perkin-Elmer AAnalyst 100 atomic absorption spectrometer (Connecticut, USA) was used as detector. It was equipped with a selenium hollow cathode lamp (Victoria, Australia) set at 196.0 nm wavelength, 16 mA lamp current and 2.00 nm slit width. PTFE tubing was used for 500 µL loop for sample and solutions. Argon flow of 50-55 mL/min was lined at the gas-liquid separator to carry the hydride gas to AAS. A Perkin-Elmer FIAS 100 flow injection hydride generation system (Connecticut, USA) with a heated quartz tube atomizer (900°C) was used for hydride generation and coupled to AAS.

Sample digestion

The tomatoes analyzed were obtained from the cultivar areas of Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kean University. All samples were soaked and washed clean with tap water followed by deionized water. The tomato samples were homogenized. The homogenized fresh matter was transferred into PTFE centrifuge tube and freeze-dried (SCANVAC centrifuge for vacuum concentrator freeze-dry) at -20°C. Once the samples were powdered and dried, they were kept at -20°C prior to digestion. Approximately 0.5 g of the sample were weighed accurately and dissolved in 10 mL conc. HNO_3. The sample mixture was digested under high temperature and pressure conditions using an autoclave at 121°C, 15 psi for 60 min. After cooling, each solution was made up to 25 mL final volume with 0.1 M HCl. The digested clear sample solutions were further treated for pre-reduction step of Se^{6+} to Se^{4+} prior to measurement by FI-HGAAS as described below.

Pre-reduction of Se^{6+} to Se^{4+}

Concerning the pre-reduction of Se^{6+}, various reducing agents were commonly used including some potassium halide salts and HCl, and some organic compounds such as ascorbic acid and thiourea, in the presence of dilute or conc. HCl, and the effect of heat was also investigated. Although the optimum reducing agent can be applied to real sample, the matrix effect on trace analysis of selenium species still appears from their reducing agent. Thus, the analytical matrices on the absorbance of Se by adding each of the optimal pre-reducing agents into the standard solutions of both common Se^{4+} and pre-reduced Se^{6+} used as calibration curves, compared with the normal calibration curve of Se^{4+} as a control were investigated under the same optimum conditions of the hydride generation. The results obtained from each of their calibration curves of both Se species with or without the corresponding pre-reducing agents can be expressed as a percentage conversion of the Se oxidation state.

Recovery study

To test recovery study of real sample in order to restrict other matrix effects of the plant materials, each of the optimal pre-reducing agents was chosen and applied by spiking each standard solution of Se^{4+} and Se^{6+} at 10 µg/L, and both Se species at 20 µg/L into the tomato Seeda (S05) as a model sample. However, only conc. HCl was ultimately selected as the most suitable pre-reducing agent for five varieties of the tomato samples used in this study including Phuang Thong 80 (S01), Mo Kho 40 (S02), Mani Thapthim (S03), Mani Siam (S04) and Seeda (S05).

Results and Discussion

Analytical figures of merit

The calibration curve was constructed between 0.5 µg/L and 50.0 µg/L and the linearity was maintained up to 100.0 µg/L with a correlation coefficient (r^2) greater than 0.999. Limits of detection (LOD) and quantification (LOQ) calculated at three and ten times standard deviation of an absorbance signal of 10 reagent blanks divided by the standard calibration slope of Se^{4+} were found to be 0.5 µg/L and 2 µg/L, respectively. This LOD was lower than those which previously reported by Li *et al.* (1998) and Sigrist *et al.* (2012) to be 3 µg/Land 2.0-7.0 µg/L, respectively. The precision was less than 10% RSD (*n* = 3). The precisions of the calibration curve of Se^{4+} standard solution between 5 µg/Land 25 µg/L (*n* = 10) were demonstrated within the acceptable ranges as RSD of 8.8% and 10.2% for an inter-day and an intra-day analysis. The results gave rather higher precision for each of the calibration curve for Se speciation analysis.

The selected optimal pre-reduction agents

Without HCl and heating, no signal was produced by FI-HGAAS. The results of using these following pre-reducing agents, four optimal pre-reducing agents were chosen for further studies as shown in Figure 1.

(i) *Conc. HCl*: About 1 mL of the digested sample, 6 mL of conc. HCl were added, heated in a water bath at 70°C for 30 min, cooled, diluted to 25 mL with deionized water and analyzed by FI-HGAAS.

(ii) *5% (w/v) KBr/conc. HCl*: About 1 mL of the digested sample, 5 mL of 25% (w/v) KBr and 2 mL of conc. HCl were added. The mixture was heated in a water bath at 70°C for 30 min, cooled, diluted to 25 mL with deionized water and analyzed by FI-HGAAS.

(iii) *15% (w/v) KBr/dilute HCl*: About 1 mL of the digested sample, 15 mL of 25% (w/v) KBr and 2 mL of 6 mol L^{-1} HCl were added. The mixture was heated in a water bath at 70°C for 30 min, cooled, diluted to 25 mL with deionized water and analyzed by FI-HGAAS.

(iv) *10% (w/v) thiourea/conc. HCl*: About 1 mL of the digested sample, 10 mL of 25% (w/v) thiourea and 2 mL of conc. HCl were added. The mixture was heated in a water bath at 70°C for 30 min, cooled, diluted to 25 mL with deionized water and analyzed by FI-HGAAS.

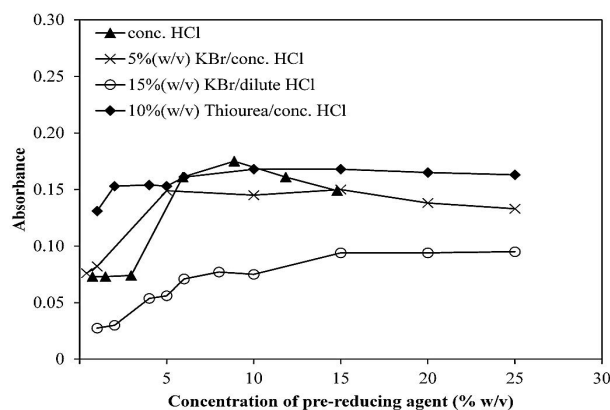In this case, KI is not applied, since it tends

Figure 1. The effect of various pre-reducing agents for Se^{6+} solution on the absorbance of Se^{4+} under hydride generation
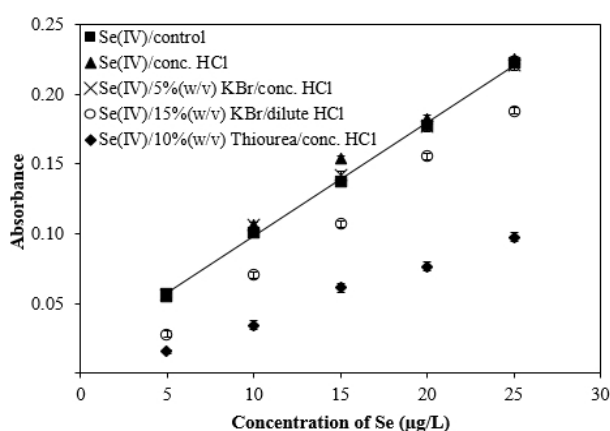

Figure 2. The calibration curves for standard solution of Se^{4+} in the presence or in the absence (control) of the four pre-reducing agents
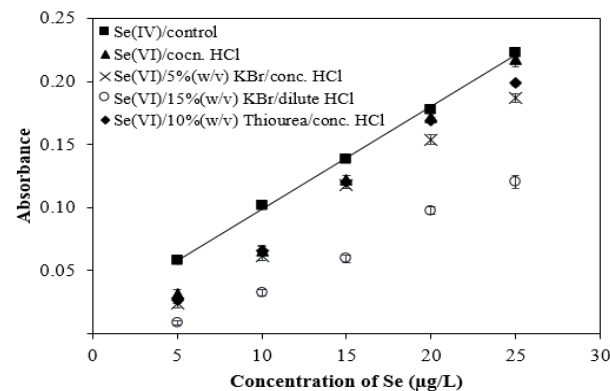

Figure 3. The calibration curves for both standard solution of Se^{4+} in the absence (control) of the pre-reducing agents and that of Se^{6+} in the presence of the four pre-reducing agents

to produce some elemental Se. While thiourea is cooperated as a reductant, Se-thiourea complexes decompose and also form elemental Se (Marcucci *et al.*, 2001). Concerning the iodide effect (D'Ulivo *et al.*, 2000), it is interesting to note that both iodide and thiourea are able to reduce Se^{4+} to its element. However, the effect of reducing a power of thiourea decreases when its concentration increases. In the

Table 1. Comparing between conversion (%)[a] and recovery (%)[b] using two kinds of the pre-reducing agents with respect to their calibration curves in the presence of each of the pre-reducing agents used

Calibration curve/ Pre-reducing agent	Se^{4+}/reducing agent				Se^{6+}/reducing agent			
	Conc. HCl		5%(w/v) KBr/ conc. HCl		Conc. HCl		5%(w/v) KBr/ conc. HCl	
	Conversion	Recovery	Conversion	Recovery	Conversion	Recovery	Conversion	Recovery
Se^{4+}/control[c]	109.9	98.78	97.74	86.83	103.3	84.39	81.28	83.17
Se^{4+}/reducing agent	97.83	97.59	98.06	89.00	91.20	83.37	81.19	85.25
Se^{6+}/reducing agent	106.8	85.26	115.9	85.78	101.0	72.84	99.64	82.18

[a]Conversion refers to a converting of the standard solution of Se^{6+} to be Se^{4+}
[b]Recovery refers to the sample spiked with each of the standard solution of 10 µg/L Se^{4+} or Se^{6+}
[c]standard solution of Se^{4+} without the pre-reducing agent

Table 2. Comparative studies of recovery of Se^{4+}, Se^{6+} and total Se in Seeda tomato as a model sample using the pre-reduction agents after spiking the selenium species

Pre-reducing agent	Recovery (%)		
	Se^{4+a}	Se^{6+a}	Se^{4+}/Se^{6+b}
conc. HCl	98.78 ± 3.22	84.39 ± 6.76	96.34 ± 6.32
5% (w/v) KBr/conc. HCl	86.83 ± 4.08	83.17 ± 2.00	81.83 ± 4.36
15% (w/v) KBr/dilute HCl	103.9 ± 4.51	75.61 ± 2.28	83.05 ± 2.60
10% (w/v) Thiourea/conc. HCl	76.59 ± 4.76	115.9 ± 4.05	83.90 ± 5.13

[a]sample spiked with each of 10 µg/L Se^{4+} or Se^{6+}
[b]sample spiked with 20 µg/L of both Se species

case of more serious interfering problem, the use of masking agents is required. Thiourea seems to produce poor results and in some cases their interference effects become worse (D'Ulivo *et al.*, 2002). While an ascorbic acid is used to study kinetics effects of the conversion of selenium species, Se^{4+} decreases due to its conversion to Se^{6+} and Se^{0} (Pettine *et al.*, 2013), which is not suitable for hydride generation.

The effect of the pre-reducing agents on their calibration curves

The calibration curves of both Se^{4+} and Se^{6+} standard solution in the presence of the pre-reducing agents compared with that of the Se^{4+} standard solution in the absence of the pre-reducing agents as a control was constructed. The obtained equations were as following: for Se^{4+}/control: $Y = 0.0082X + 0.0167$); Se^{4+} with conc. HCl: $Y = 0.0083X + 0.0196$; $Se4+$ with 5% (w/v) KBr/conc. HCl: $Y = 0.0080X + 0.0201$; Se^{4+} with 15% (w/v) KBr/dilute HCl: $Y = 0.0082X - 0.0124$; Se^{4+} with 10% (w/v) thiourea/conc. HCl: $Y = 0.0042X - 0.0054$). These curves

did not show much different slope for Se^{4+} standard solution, at which denoted the absence of any matrix effects for Se^{4+} analysis. On the other hand, only that of Se^{4+} standard solution with 10% (w/v) thiourea/conc. HCl was affected due to strong matrix effect as shown in Figure 2.

In the same manner for Se^{6+} standard solution with the pre-reducing agents, it was found as following; Se^{6+} with conc. HCl: $Y = 0.0095X - 0.0209$; Se^{6+} with 5% (w/v) KBr/conc. HCl: $Y = 0.0071X - 0.012$; Se^{6+} with 15% (w/v) KBr/dilute HCl: $Y = 0.0058X - 0.0232$; Se^{6+} with 10% (w/v) thiourea/conc. HCl: $Y = 0.0089X - 0.0175$. From these results (Figure 3), their slopes of the four pre-reduction systems of Se^{6+} also gave not much difference in the matrix effect (Gallignani *et al.*, 2000; Niedzielski *et al.*, 2002), if used as standard working solution which could be tested for the efficiency of the reduction/conversion system. All of these pre-reducing agents can be used to completely convert Se^{6+} to Se^{4+} for the inorganic Se species determination.

However, comparing between the percentage

conversion with their recovery of Se^{4+} and Se^{6+} was tabulated in details about these two selected pre-reducing systems, as determined by their different calibration curves (Table 1). Two optimal pre-reducing agents including conc. HCl and 5% (w/v) KBr/conc. HCl were chosen. Both conversion (97.7-116%) and recovery (85.3-98.8%) are noted in similar trends when using Se^{4+}/control and Se^{4+} in the presence of the pre-reducing agents as their calibration curves. Satisfied trends in both conversion (81.2-103%) and its recovery (72.8-85.3%) were also found for Se^{6+} as well. Consequently, both of them can be used as the calibration curve for the determination of inorganic Se species and total Se in tomato sample.

Accuracy

Recovery studies of Se^{4+}, Se^{6+} and both Se species using selected four pre-reduction systems were shown in Table 2. It was found that all the reducing systems gave rather high recoveries for these Se species (76.6-104%, 75.6-116% and 81.8-96.3% for Se^{4+}, Se^{6+} and Se^{4+}/Se^{6+}, respectively) spiked in Seeda tomato as a model sample. From the results, the conc. HCl was chosen as the optimal pre-reducing agent to apply with five varieties of tomatoes (S01-S05). This procedure is a lot simpler than other conventional methods using another pre-reducing agent. The recoveries obtained from these samples spiked with the Se species followed by the pre-reduction with conc. HCl. The average recoveries of the Se^{4+}, Se^{6+} and total Se in these tomato varieties were ranged from 99.6-109%, 76.8-86.6% and 78.3-89.2%, respectively.

For sample digestion, the main problem may arise from the peculiar chemical resistance of some organoselenium compounds (selenoamino acid, selenonium salts) to be transformed into inorganic Se salts, selenite or selenate forms. Tomatoes have rather high organic selenium (Ahmed, 2010). Although the sample digestion was conducted with an autoclave unit, it seemed to give a completely clear solution, the residues of organic matter disappeared. The residues of organic matter could interfere the hydride generation. The concentration of Se^{6+} was calculated as the difference between total Se and Se^{4+}. These samples gave rather high contents of Se ranging from 0.07-0.10 μg/g, 0.43-0.92 μg/g and 0.50-0.99 μg/g for Se^{4+}, Se^{6+}, and total Se, respectively. Concerning the inorganic Se species in the tomato samples, the hexavalent Se is found in higher contents than the tetravalent one.

Conclusion

The presence of matrix effects and the efficiency of the optimal pre-reduction systems consisting of conc. HCl, 5% (w/v) KBr/conc. HCl, 15% (w/v) KBr/ dilute HCl and 10% (w/v) thiourea/conc. HCl under heating for 30 min for Se speciation analysis were clarified. It was necessary to reduce Se^{6+} to be Se^{4+} prior to on-line hydride generation. The developed method was applied for inorganic Se analysis in the spiked sample solution of tomato after acid digestion using autoclave unit. The pre-reduction step made possibly the completeness of the inorganic Se species determination. The use of a mixture of the pre-reducing agents permitted the quantitative reduction of Se^{6+} to Se^{4+} under mild reaction conditions. This sensitive and highly selective method with good linearity and reproducibility was to ascertain for such an ultra-trace analysis of Se.

Acknowledgements

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