Determination of Selenium in Milk by Graphite Furnace Atomic Absorption Spectrometry

A. VONGKUL^{1,*} and S. DEJMANEE²

¹Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Thung Yai, Nakhon Si Thammarat 80240, Thailand

²Department of Chemistry, School of Science, Walailak University, Thasala, Nakhon Si Thammarat 80161, Thailand

*Corresponding author: E-mail: auy_vk@hotmail.com

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This work has optimized the graphite furnace atomic absorption spectrometry to directly determine the total selenium concentrations in milk samples. The study emphasized the effect of modifiers on the sensitivities and stabilizations of three selenium species (selenomethionine, selenite and selenate) that were spiked in aqueous solution and diluted milk solutions. At the same time, palladium nitrate, rhodium chloride and the combinations of these modifiers with magnesium nitrate were used as chemical modifiers. The use of a combination between 5 μg $\text{Pd}(\text{NO}_3)_2$ and 3 μg $\text{Mg}(\text{NO}_3)_2$ was successful modifier in an equal sensitivities and stabilization for all these species in both aqueous and milk solutions. This modifier can be applied for the direct determination of selenium in milk samples with standard addition method. The Se content in milk samples was found range from 33 to 70 $\mu\text{g L}^{-1}$. The percentage recovery was found within the range between 87.42 to 111.69 %.

Key Words: Selenium, Milk, Chemical modifiers, Graphite furnace atomic absorption spectrometry.

INTRODUCTION

Selenium element is classified as an essential micronutrient for humans, but at trace level, selenium is toxic to human beings. The dual behaviours of selenium as an essential or toxic element are in very narrow range between deficiency and toxic effects, which is required a minimum level at only 0.04 mg L^{-1} and fulfills an optimum beneficial role at about 0.10 mg L^{-1} . On the other hand, at 4 mg L^{-1} and above, it becomes toxic to animals¹. Meanwhile, the recommended dietary allowance *i.e.*, the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97-98 %) individuals in each life-stage² and gender group-of selenium is 55 $\mu\text{g day}^{-1}$. Selenium concentration in cow's milk is varies between 2 to 1270 $\mu\text{g L}^{-1}$ depending on the availability of this element in the food and geographical area³. The contents of selenium in cow's milk samples in different areas were reported by several studies as 22.4 $\mu\text{g L}^{-1}$ in United State⁴, 39-101 $\mu\text{g L}^{-1}$ in Brazil³, < 1.06-110.58 $\mu\text{g kg}^{-1}$ in Italy⁵, $25.73 \pm 5.25 \mu\text{g L}^{-1}$ in India⁶ and 60-131 $\mu\text{g kg}^{-1}$ in Korea⁷.

Among analytical techniques available for determination of selenium, graphite furnace atomic absorption spectrometry (GF-AAS) is a suitable and widely used technique for determination of selenium at trace levels in biological materials due to its high sensitivity, low sample consumption, reduced matrix-related interferences, minimal sample preparation

requirements⁸ and capability for direct determination in real samples^{3,9-11}.

For the determination of selenium with GF-AAS, many chemical modifiers have been indicated to be effective in thermal stabilizing and/or eliminating various interference with selenium, but very few have been shown to study in actual biological fluids¹². Moreover, the significant problems with the use of chemical modifiers were found to be affected by the valence state of selenium^{13, 14} as well as the type of sample matrix^{12,15}. In biological samples, selenium is found mainly as Se(II) bound to protein thiol group. These species behaves differently from inorganic tetravalent selenium, which is a common used for calibration¹⁶. The effect of metal modifiers on analyte stability is on so matrix specific¹⁷, hence the ability of one modifier to stabilize a particular biological matrix cannot be assumed to apply to a second matrix¹⁸. The selenium analytes are stabilized differently in the graphite furnace although the temperature and employed modifier have been optimized carefully. This may lead to erroneous results if selenium species present in samples are different from the calibration standard by a single species.

Several studies^{16,18-22} have dealt with this problem, but the modifiers which equally stabilize all selenium species in different matrixes for direct determination of total selenium by GF-AAS in real samples have been a few studies. Gammelgaard and Jons¹⁹ studied thermal stabilities of trimethyl-selenonium

(TMSe), selenomethionine (SeMet), selenite [Se(IV)] and selenate [Se(VI)] in aqueous and plasma. It was found that the addition of 20 µg of palladium alone is just as effective as the combination of palladium and magnesium nitrate. But, the addition of magnesium nitrate did not improve the stabilization and sensitivity of different selenium compounds. Gammelgaard and Larsen²³ found that using GF-AAS with palladium as chemical modifier, the sensitivities for Se(IV), SeMet and TMSe in aqueous solution were similar. In blood plasma, the GF-AAS sensitivities of Se(IV) and Se(VI) were equal.

For the direct determination of selenium in milk samples, the addition of surfactant agents (Triton X-100 and tertiary amines) were used to prepare the suspension milk samples. While, Ni, Pd and the combination of Rh with Mg(NO₃)₂ and Pd with Mg(NO₃)₂ were used as chemical modifiers^{3,9,24,25}.

The purpose of this work was to compare the effect of palladium nitrate, rhodium chloride and combinations of these modifiers with magnesium nitrate as chemical modifiers on the sensitivity and stabilization of different selenium species (three common valance states; Se(II) as selenomethionine, Se(IV) as selenite and Se(VI) as selenate) in aqueous and milk solutions by using GF-AAS. The attempt was made to determine the modifier which could serve as the best modifier for direct determination of total selenium in milk samples by using only an inorganic tetravalent selenium standard.

EXPERIMENTAL

All reagents were used as analytical grade and ultrapure grade for HNO₃ (Merck, Germany). Deionized water was from a Milli-Q system (Millipore, USA).

Standard solution of selenium species (1000 mg L⁻¹) in 0.5 M HNO₃ were prepared. Se(VI) from Na₂SeO₄ (Fluka) and Se(II) from selenomethionine (ACROS Organic C) and Se(IV) were obtained from SeO₂ (Merck).

Modifier solutions were prepared by appropriate dilution of 10 g L⁻¹ Pd(NO₃)₂ (Perkin-Elmer part no. BO19-0635) and Mg(NO₃)₂·6H₂O (Perkin-Elmer part no. BO19-0634) stock solutions in deionized water, respectively. While, Rh modifier solution was prepared by using RhCl₃ (ACROS -Organic C) and Triton X-100 (Merck, Germany) was employed for modification of the natural viscosity samples.

Milk samples were used liquid milk that purchased from minimart in the same day. All sample containers, glass-ware and autosampler cups were soaked in 10 % nitric acid for 48 h and then rinsed thoroughly with deionized water and dried.

A Perkin-Elmer model AAnalyst 800 atomic absorption spectrometer was connected to a transversely heated graphite atomized equipped with an AS-80 furnace autosampler and longitudinal Zeeman background correction, also from Perkin-Elmer. High purity argon (99.999 %, Thailand) was used as a purge gas at the flow rate 250 mL min⁻¹. A selenium electrode discharge lamp from Perkin-Elmer (Germany) operated at 220 mA was used for all investigation. A 2 nm spectral band width was selected to isolate the 196.0 nm resonance line of Se. All absorbance measurements were performed in peak area mode. The atomization program and instrumental parameters presented in Table-1.

TABLE-1
TEMPERATURE PROGRAMS OF GF-AAS
FOR SELENIUM DETERMINATION

Step	Temp. (°C)	Time (s)		Ar flow rate (mL min ⁻¹)	Stage
		Ramp	Hold		
1	110	1	30	250	Drying
2	130	15	30	250	Drying
3	Variable	10	20	250	Pyrolysis
4	Variable	0	5	0 (read)	Atomization
5	2450	1	3	250	Cleaning

For thermal stabilization, 20 µL of 50 µg L⁻¹ solutions of the three selenium species were analyzed by the addition of 5 µL of chemical modifier, corresponding to the application of 5 µg of Pd or Rh and 3 µg of Mg(NO₃)₂ in to the furnace. All solutions were analyzed in triplicate.

The standard solutions of these selenium species (50 µg L⁻¹) were prepared in only 0.2 % nitric acid for aqueous solutions. While, the spiked milk solutions with these selenium species (50 µg L⁻¹) or diluted milk solutions were carried out at the ratio of 1:4 by volume between low fat milk samples and 7.0 % Triton X-100 in 0.2 % HNO₃ that were occurred homogeneous milk solutions. The effectiveness of four modifiers on thermal behavior was investigated by using several pyrolysis temperatures at 600 to 1500 °C in aqueous solutions and 900 to 1700 °C in diluted milk solutions by using constant atomization temperature for each modifier.

RESULTS AND DISCUSSION

Effect of modifiers on stabilizations and sensitivities of selenium species: In the absence of any chemical modifier and nitric acid, equal sensitivities of different selenium species could not be observed²². The effect of Pd, Rh and combination of these modifiers with magnesium nitrate on the thermal stabilization and sensitivity of selenium species in aqueous and diluted milk solutions were studied.

In aqueous solutions, the addition of Pd (Fig. 1) and Pd with Mg(NO₃)₂ (Fig. 3) modifiers were shown that pyrolysis curves for SeMet, Se(IV) and Se(VI) species gave a similar pattern. The results obtained from the addition of Rh (Fig. 2) and Rh with Mg(NO₃)₂ (Fig. 4) modifiers that were indicated the same pattern of pyrolysis curves for these species with a equal stabilization of these selenium species. However, small differences in sensitivities do occur for Se(IV) and Se(VI) for Rh and Rh with Mg(NO₃)₂ modifiers.

For milk solutions in the presence of Pd (Fig. 1) and Pd with Mg(NO₃)₂ (Fig. 3) modifiers, the pyrolysis curves were almost stabilized to the same pattern. The stabilization of 1900 °C, with adding 1 ng of each selenium species in graphite tube) Se(IV) and Se(VI) were almost equal while SeMet is rather low at all temperatures studies with small differences of sensitivities.

The addition of these selenium species in milk solutions were stabilized to the same extent by addition of Rh modifier (Fig. 2). The stabilization of Se(IV) and Se(VI) were equal while SeMet was much lower as compared to other selenium species. In Fig. 4 shown that the pyrolysis curves of these species were almost similar stabilized when using Rh with Mg(NO₃)₂ modifier. The stabilization of Se(IV) and Se(VI) were equal while SeMet was much lower as compared to other selenium species.

Fig. 1. Pyrolysis curves of selenium species by using Pd modifier in aqueous and diluted milk solutions (atomization fixed at 2000 °C, with adding 1 ng of each selenium species in graphite tube)

Fig. 2. Pyrolysis curves of selenium species by using Rh modifier in aqueous and diluted milk solutions (atomization fixed at 1900 °C, with adding 1 ng of each selenium species in graphite tube)

Fig. 3. Pyrolysis curves of Se species by using Pd with $\text{Mg}(\text{NO}_3)_2$ modifier in aqueous and diluted milk solutions (atomization fixed at 2000 °C, with adding 1 ng of each selenium species in graphite tube)

Fig. 4. Pyrolysis curves of selenium species by using Rh with and $\text{Mg}(\text{NO}_3)_2$ modifier in aqueous and diluted milk solutions (atomization fixed at

Even after careful optimization of thermal stabilization with different modifiers, it was possible to find modifier which was able to equally stabilize all selenium species in aqueous and milk solutions. At constant absorbance signals of pyrolysis curves, a randomized complete block design (RCBD) was applied. Analysis of variance (ANOVA) and the Scheffe's test at 5 % confidence level of significance were used to compare the mean of absorbance signals between different modifiers and selenium species. The result found that no statistical difference occurred when using the chemical modifier combination of Pd with $\text{Mg}(\text{NO}_3)_2$ in both aqueous and milk solutions. From this results, it may conclude that the addition of magnesium to the Pd-Mg modifier increases the speed of diffusion by causing palladium to form smaller droplets/conglomerates on thermal pre-treatment (*i.e.* a less closely packed matrix lattice), with a consequent sharpening of the absorbance peak. Furthermore, the addition of $\text{Mg}(\text{NO}_3)_2$ as an oxidizing agent and the high concentration of HNO_3 present may delay such a reduction and facilitate the formation of mixed oxides for stabilization of high volatile selenium compounds. The decrease in the permissible thermal pre-treatment temperatures on the addition of magnesium to the modifier is most likely due to the lower decomposition temperature of MgO in the furnace relative to PdO ¹⁸ with a corresponding loss of adsorbed SeO_2 . These results are in good agreement with that described results for palladium, either as the Pd-Mg modifier. They have proven to be very successful and reliable to prevent the loss of selenium from all compounds, due to a pyrolysis temperature of at least 1000 °C. Furthermore, a direct comparison of various platinum-group metals Pd was found to be the most effective modifier⁸.

Effect of dilution factor and the use of 7.0 % Triton X-100 in 0.2 % HNO_3 : The possibility of milk introduction in the graphite without any dilution was unsuitable due to poor repeatability caused by both carbon residues in graphite tube and fat residues in autosampler capillary tube³. The dilution factors were selected as a compromise between required sensitivity and minimum organic matter dispensed inside the graphite tube²⁶ which can improve the homogeneity of milk solution. For the direct determination, low fat milk and fat milk samples were diluted with 7 % Triton X-100 in 0.2 % HNO_3 that was served the higher sensitivity and good homogeneous milk solution at the ratios of 1:9 and 1:24 by volume, respectively. The fat milk samples employed more volumes of diluents than the low fat milk samples due to the fat compounds that were contained in fat milk samples.

For the selected modifier, the limit of detection (calculated as $3\sigma_{\text{blank}}/\text{slope}$, $n = 7$) experimentally determined for only 0.2 % HNO_3 and 7 % Triton X-100 in 0.2 % HNO_3 that were 0.97 and $1.03 \mu\text{g L}^{-1}$. Therefore, the limit of detection of the dilution of 1:9 low fat milk sample, prepared in 0.2 % HNO_3 and then adding with 7 % Triton X-100, was $1.57 \mu\text{g L}^{-1}$. For all analyses, the detection limit of selenium varied slightly depending on sample matrix.

Precision and accuracy studies were carried out by employing addition-recovery experiments for milk samples spiked with $50 \mu\text{g L}^{-1}$ $\text{Se}(\text{IV})$ standard solution. It is interesting to point out that the relative standard deviations were lower than 5 %. The recovery values were fairly good within the range of 87.42 to 111.69 % for all selenium spiked in milk samples.

Matrix interferences: The influence of matrix interferences on the selenium determination in milk samples were evaluated by comparing the slope of the calibration graphs (the comparison of the slope of calibration curve for Se(IV) standard prepared in only 0.2 % HNO₃ and 7 % Triton X-100 in 0.2 % HNO₃ and the calibration curve obtaining by standard addition method in both diluted low fat and fat milk samples). The slopes of the two calibration curves in only 0.2 % HNO₃ and 7 % Triton X-100 in 0.2 % HNO₃ were 0.0020 and 0.0018. Whereas, the slopes of the two standard addition curves of diluted low fat and fat milk samples were 0.0013 and 0.0012, respectively. The four slopes were compared in pairs by *t*-test for the 95 % confidence level²⁷. According to the *t*-test no statistical difference occurred between the calibration curves of Se(IV) standard. But, the difference between two calibration curves of Se(IV) standard and standard addition curves of two milk samples was significant. The slopes of the calibration curves in different matrixes are significantly different. This indicates that a standard addition method is needed in the given the matrix for direct determination of selenium in milk samples.

Application: The experiment was performed using optimum modifier, Pd-Mg(NO₃)₂, to direct determination of selenium by GF-AAS with standard addition method. The study was used only Se(IV) standard for the determination of total selenium in milk samples. The results were given in Table-2. The reported values for selenium in milk samples were found to be the same normal range as those reported on previous studies for direct determination of selenium in milk samples. In Thailand²⁸, the mean of total selenium content of cow's milk samples were 6.4 ± 2.4 µg/100 g.

TABLE-2
SELENIUM CONTENTS IN DIFFERENT MILK SAMPLES

Milk samples	Se found (µg L ⁻¹)	Recoveries (%)
Low fat milk II	48.48 ± 0.52	87.42
Low fat milk III	38.41 ± 1.67	111.69
Plain milk	75.00 ± 2.08	105.56
Whole milk	52.78 ± 1.20	94.82

Conclusion

The main purposed of this work that showed the effect of chemical modifiers on thermal behavior of selenium in aqueous solution and real sample solution (milk solution). It has been shown that the presence of the modifiers combination of 5 µg Pd with 3 µg Mg(NO₃)₂ can be used successfully for equal stabilization and sensitivity of different selenium species in both the aqueous and the diluted milk solutions. The main advantage of the proposed method is it applicability for direct determination of total selenium in milk samples without traditional procedures of sample preparation and the validate

method was studied with addition-recovery in real liquid milk samples.

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REFERENCES

1. H.W. Lakin, in ed.: E.L. Kothny, Trace Elements in the Environment: Advances in Chemistry, American Chemical Society, Washington, DC, Vol. 123, Ch. 6 (1973).
2. R. Abdulah, K. Miyazaki, M. Nakazawa and H. Koyama, *Int. J. Food Sci. Nutr.*, **56**, 463 (2005).
3. P.C. Aleixo and J.A. Nóbrega, *Food Chem.*, **83**, 457 (2003).
4. I.J. Lean, H.F. Troutt, H. Boermans, G. Moller, G. Webster and M. Tracy, *Cornell Vet.*, **80**, 41 (1990).
5. P. Licata, D. Trombetta, M. Cristani, F. Giofrè, D. Martino, M. Calò and F. Naccari, *Environ. Int.*, **30**, 1 (2004).
6. G. Vivekanandan, V. Krishnasamy, K. Ayyudurai and G.S. Swaminathan, *J. Environ. Biol.*, **14**, 261 (1993).
7. Y. Choi, J. Kim, H-S. Lee, C. Kim, I.K. Hwang, H.K. Park and C-H. Park, *J. Food Compos. Anal.*, **22**, 117 (2009).
8. B. Welz and M. Sperling, Applications of AAS in Body Fluids and Tissue: Atomic Absorption Spectrometry, Wiley-VCH, Weinheim, edn 3, p. 592 (1999).
9. D. Wagley, G. Schmiedel, E. Mainka and H.J. Ache, *At. Spectrosc.*, **10**, 106 (1989).
10. Y. Liu, B. Gong, Z. Li, Y. Xu and T. Lin, *Talanta*, **43**, 985 (1996).
11. W.K. Chen, C.C. Yen, B.L. Wei, C.C. Hu, J.J. Yu, C. Chung and S.C. Kuo, *Spectrochim. Acta B*, **53**, 131 (1998).
12. B. Radziuk and Y. Thomassen, *J. Anal. At. Spectrom.*, **7**, 397 (1992).
13. B. Welz, G. Schlemmer and U. Voellkopf, *Spectrochim. Acta B*, **39**, 501 (1984).
14. A. Cedergren, I. Lindberg, E. Lundberg, D.C. Baxter and W. Frech, *Anal. Chim. Acta*, **180**, 373 (1986).
15. C. Garcia-Olalla, L.C. Robles, M.T. Alemany and A.J. Aller, *Anal. Chim. Acta*, **247**, 19 (1991).
16. E. Bulska and K. Pyrzynska, *Spectrochim. Acta B*, **52**, 1283 (1997).
17. G.R. Carnrick, D.C. Manning and W. Slavin, *Analyst*, **108**, 1297 (1983).
18. M. Deaker and W. Maher, *J. Anal. At. Spectrom.*, **10**, 423 (1995).
19. B. Gammelgaard and O. Jøns, *J. Anal. At. Spectrom.*, **12**, 465 (1997).
20. H. Docekalová, B. Docekal, J. Komárek and I. Novotný, *J. Anal. At. Spectrom.*, **6**, 661 (1991).
21. V. Alexiu and L. Vladescu, *Anal. Sci.*, **21**, 137 (2005).
22. F. Sahin, M. Volkan and O.Y. Ataman, *Anal. Chim. Acta*, **547**, 126 (2005).
23. B. Gammelgaard and E.H. Larsen, *Talanta*, **47**, 503 (1998).
24. E.M. García, M.L. Lorenzo, C.Cabrera, M.C. López and J. Sánchez, *J. Dairy Res.*, **66**, 569 (1999).
25. J. Koops, H. Klomp and D. Westerbeek, *Neth. Milk Dairy*, **43**, 185 (1989).
26. A.P. Oliveira, J.A.G. Neto, J.A.Nóbrega, P.R.M. Correia and P.V. Oliveira, *Food Chem.*, **93**, 355 (2005).
27. S. Theodorolea, N.S. Thomaidis and E. Piperaki, *Anal. Chim. Acta*, **547**, 132 (2005).
28. P.P. Sirichakwal, P. Puwastien, J. Polngam and R. Kongkachuichai, *J. Food Compos. Anal.*, **18**, 47 (2005).