

Determination of Selenium in Water Samples by Using a Methylene Blue Kinetic Catalytic Spectrophotometric Method

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ABSTRACT

A kinetic catalytic spectrophotometric method, which was very sensitive and effective, was developed for the determination of selenium in water samples. It based on the catalytic effect of selenium on the reaction of methylene blue with sodium sulfide. A change in the absorbance of methylene blue with times at various concentrations of selenium were monitored, giving the “end point” (time) for each concentration of selenium. A plot of end point versus selenium concentration constituted a calibration graph, which was linear in a range of 2.5-30 ppb of selenium, with the correlation coefficient of 0.9992. The method was applied to determine the amount of selenium in the water sample containing 15 ppb of selenium, giving 91.84% recovery and the relative standard deviation of 2.27%. Compared with the hydride generation atomic absorption spectrometry method, this method is more sensitive for the determination of selenium in the water sample when the concentration of selenium is lower than 100 ppb.

Key words: selenium, methylene blue, spectrophotometric method, water sample

INTRODUCTION

Selenium is essential to life. It shares many properties of sulfur and arsenic. Its compounds are covalent existing in several allotropic forms including Se₈. The oxide dissolves in diluted bases to give selenites such as Na₂SeO₃. Although it is an essential nutrient in small amounts, selenium and its compounds are toxic at slightly high levels. Elemental selenium is widely used in electronic semiconductors, as it conducts electricity in the light, and hexavalent selenium occurs widely as selenate in natural waters. The acute oral dose LD₅₀ of sodium selenite in rats is 7 mg/kg, and that of sodium selenate is 4 mg/kg, with its the principal action affecting the nervous system (Crosby, 1998).

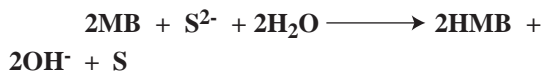
Selenium has been determined by many methods but the most common method is hydride generation atomic absorption spectrometry (Cassella *et al.*, 2002; Bujdos *et al.*, 1999). The hydride generation atomic absorption spectrometry (HGAAS) method is sensitive but the experimental equipment is not readily available in many laboratories. However, spectrophotometer which is much cheaper and easier to operate, is readily accessible in most laboratories.

There are several spectrophotometric methods for the determination of selenium (Afsar *et al.*, 1989) but most of them have limited sensitivity and often high detection limit (ppm to sub-ppb) which can not be used to detect selenium in the samples containing selenium in the level of ppb. However, kinetic catalytic spectrophotometric

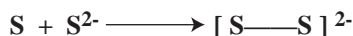
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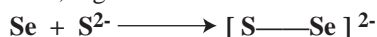
methods have yielded better detection limits for selenium determinations than simple spectrophotometric methods (Shiundu and Wade, 1991; Mottola and Perez-Bendito, 1992). The method based on catalytic effect on a reduction of methylene blue (MB) by sodium sulfide (West and Ramakrishna, 1968) :



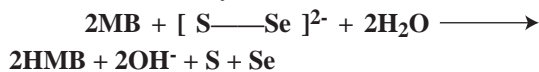
In the reduction, the colour of methylene blue is blue; after the reduction of methylene blue by sodium sulfide, the reaction gives HMB which is colourless. In the presence of excess sulfide, sulfur will combine with sulfide ions, and give the polysulfides :



Similarly, when selenium combines with sulfide ions, it gives selenosulfides :



Then selenosulfides react with methylene blue in a similar way to the sulfide ions:



However, the selenosulfide ion reacts with methylene blue more quickly than sulfide ion and selenium is generated at the end of the reaction.

Gokmen and Abdelqader (1994) used the kinetic catalytic spectrophotometric method for the determination of selenium in urine samples (84.9 % recovery). A plot of t^{-1} (reciprocal of time at the end point) versus various concentrations of selenium was used as the calibration graph. In this study, we tried to simplify the catalytic method by using a plot of time (end point) versus various concentrations of selenium as the calibration graph which made method more convenient to use in laboratories. The catalytic method was also tried to be used in analyzing a very low concentration of selenium in water samples that could not be determined by using the HGAAS methods because of high detection limit.

MATERIALS AND METHODS

Reagents

All reagents used were analytical reagent grade and their solutions made up in deionized water. Formaldehyde solution (assay 37%) was purchased from BDH (Poole, England).

Preparation of solutions

- Preparation of selenium standard solution

Selenium atomic absorption stock solution (1000 ppm Merck, Darmstadt, Germany) was used to prepare Se(IV) (10 ppm) by diluting the stock solution with 1% HNO_3 (Malinckrodt, Kentucky, USA). The selenium standard solution (10 ppm) was diluted to lower concentration (2.5-30 ppb) by 1% HNO_3 .

- Preparation of conditioner solution

Conditioner solution was prepared by mixing 0.69 g EDTA (Merck, Darmstadt, Germany), 0.0145 g FeCl_3 (Merck, Darmstadt, Germany) and 1.25 ml triethanolamine $[(\text{HOCH}_2\text{CH}_2)_3\text{N}]$ (Merck, Darmstadt, Germany) together and dissolving the mixture in deionized water, then diluting to 250 ml.

- Preparation of methylene blue solution (0.05% MB)

Methylene blue solution was prepared by 0.05 g of dissolving methylene blue (Merck, Darmstadt, Germany) in deionized water, then diluting to 100 ml.

- Preparation of sodium sulfide solution

Sodium sulfide solution was prepared by dissolving 5.04 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (Ajax Chemical, Auburn, Australia), 4.80 g of Na_2SO_3 (Merck, Darmstadt, Germany) and 1.60 g NaOH (Carlo Erba, Val de Reuil, France) in water, then diluting to 100 ml.

- Solution of the interfering ions

Potassium solutions were prepared from KCl (Carlo Erba, Val de Reuil, France)

Calcium solutions were prepared from CaCl_2 (Carlo Erba, Val de Reuil, France)

Magnesium solutions were prepared from MgCl_2 (Carlo Erba, Val de Reuil, France)

Zinc solutions were prepared from ZnCl_2 (Ajax Chemical, Auburn, Australia)

Iron Solutions were prepared from FeCl_3 (BDH, Poole, England)

Apparatus

A double beam UV-Visible Spectrophotometer (JASCO model 7800) was used to record absorbance versus wavelength and absorbance versus time.

Experimental procedure

Each experiment was carried by adding 6.5 ml standard selenium (or samples), 1.0 ml formaldehyde, 2.5 ml conditioner solution, 0.5 ml sodium sulfide solution and 1.0 ml methylene blue solution into a beaker (The time interval for each addition was 30 seconds). Recording absorbance at wavelength 668 nm which is the absorption maxima of methylene blue (Figure 1) versus time spectra at room temperature is shown in Figure 2. The time (t) for completion of the reaction between methylene blue and sulfide were determined from the intersection of the two tangents.

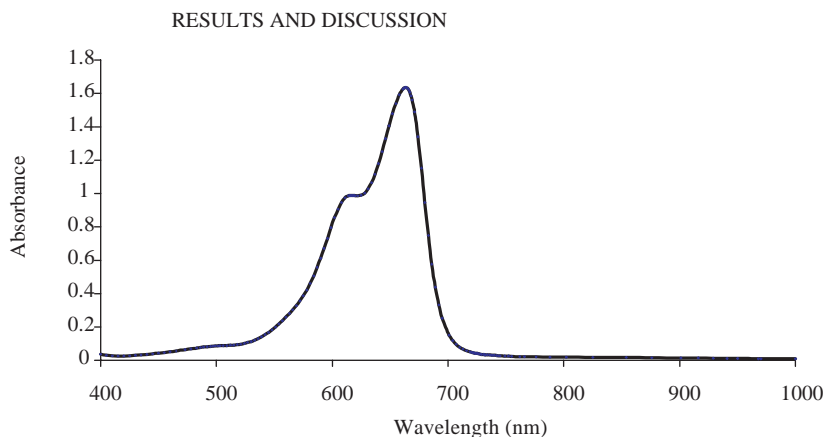


Figure 1 Absorption spectrum of methylene blue.

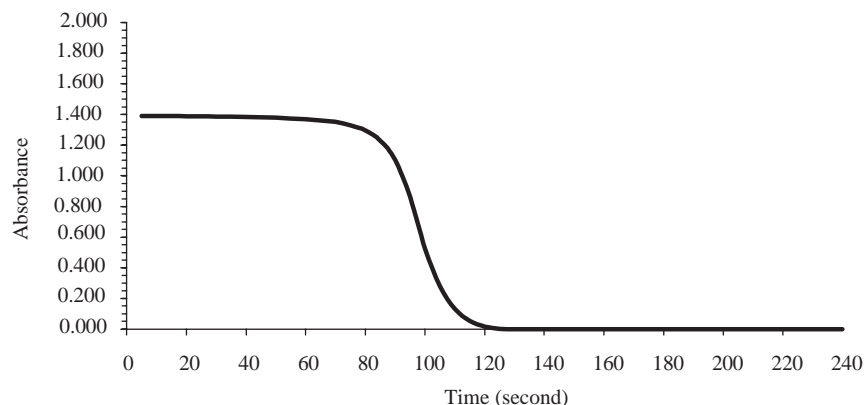


Figure 2 Absorbance versus time spectrum for the mixture of standard selenium solution and methylene blue at 668 nm.

RESULTS AND DISCUSSION

Optimization of experimental parameters

In these experiments, the concentration of methylene blue, formaldehyde and conditioner solution were kept constant. Only the effect of time interval before recording the absorbance and concentration of sodium sulfide solution were investigated in order to increase the sensitivity of the catalytic method. The reason for fixing the volume of 0.05% methylene blue at 1.0 ml was that, we would like to use the method for the determination of selenium at very low concentration of water samples which could not be determined by the HGAAS method. High concentration of methylene blue in the analyzing solution could affect the accuracy of the method. For iron(III), it was reported that in the presence of $\text{Na}_2\text{H}_2\text{EDTA}$ and sulfide, a faintly cherry-red coloured complex formed on the addition of iron(III) resulting in the removing dissolved oxygen (West and Ramakrishna, 1968). This meant that iron(III) could enhance the reduction of methylene blue by selenosulfide. As Gokmen and Abdelqader's works (1994), ferric chloride was also used as part of the conditioner solution together with $\text{Na}_2\text{H}_2\text{EDTA}$ and triethanolamine. Triethanolamine and formaldehyde do not have

any effect on the catalytic reaction but may help in maintaining the higher oxidation state of iron by suppressing the reducing power of sodium sulfide on iron(III) (West and Ramakrishna, 1968). EDTA was a general masking agent to eliminate several interfering ions by complexing them and preventing their reactions with the sulfide ion.

- Effect of time interval before spectrum recording

In the experiment, all other parameters were kept constant except the time interval after mixing the solution and recording a spectrum. Figure 3 shows the adsorption spectra of selenium solutions where the time interval are 20, 40, and 60 seconds. The end point was found to change significantly with the time interval, hence fixing the time interval before recording the spectrum was very important in determining the amount of selenium in the samples. The time interval for 20 seconds was chosen for all experiments with the following reasons; firstly, for increasing in an accuracy in determining the end point from the spectrum and, secondly, for time saving.

- Effect of concentration of sodium sulfide in the analyzing solution

In this experiment, all others parameters were kept constant except the concentration of sodium sulfide solution. The volumes of sodium

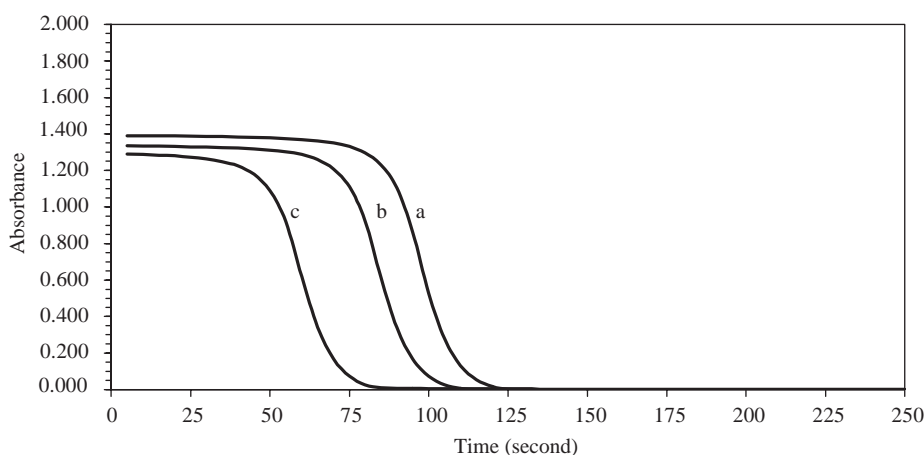


Figure 3 Absorbance versus time spectra for the analyzing solution at 668 nm when time interval before recording the spectra were (a) 20 seconds (b) 40 seconds (c) 60 seconds.

sulfide solution used were 0.5, 1.5 and 2.0 ml. The absorption spectra are shown in Figure 4. We found that the end points were changed significantly with the volume of sodium sulfide solution changed. When the concentration of sodium sulfide were high (1.5 and 2.0 ml), the accuracy in determining the end point reduced. This might be because, at high concentration, sodium sulfide exceedingly stimulated the methylene blue to HMB (colourless) and caused difficulty in determining the end point, therefore volume of sodium sulfide solution at 0.5 ml was chosen for all experiments.

Calibration graph, recovery and accuracy of the method

After studying the effect of concentration of sodium sulfide and the time interval before spectrum recording, conformity to Beer's law over the concentration of selenium was determined. Under the optimized condition, end point changed linearly with selenium concentration over the ranges of 2.5-30 ppb. The linear calibration graph with the correlation coefficient of 0.9992 is shown in Figure 5. For the water sample containing 15 ppb of selenium, it was found that the kinetic catalytic

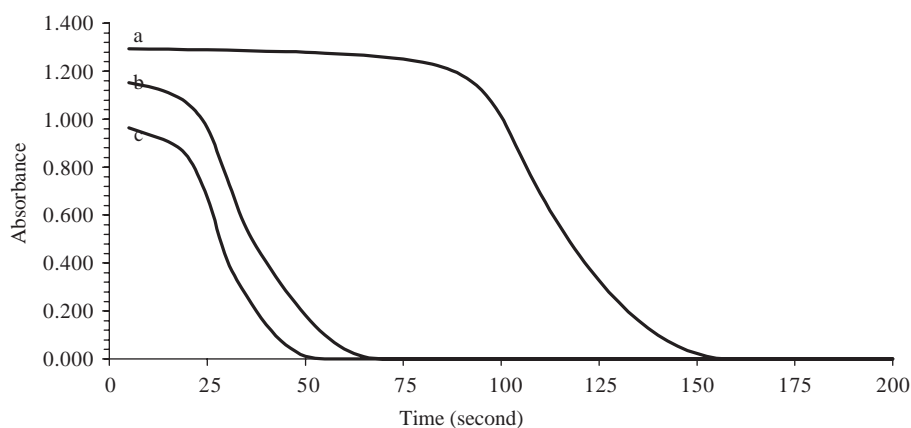


Figure 4 Absorbance versus time spectra for the analyzing solution at 668 nm when volume of sodium sulfide solution were (a) 0.5 ml (b) 1.5 ml (c) 2.0 ml.

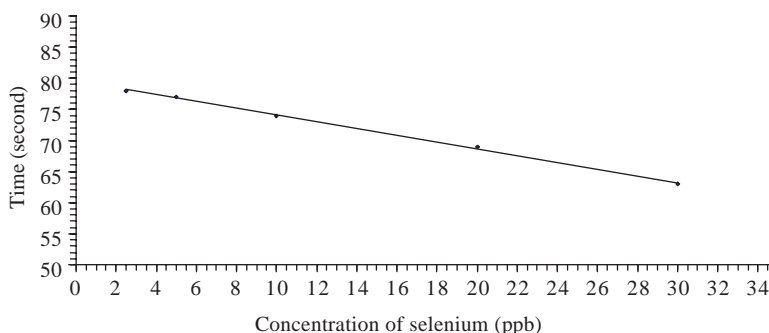


Figure 5 The calibration graph for the determination of selenium in the water sample by using the catalytic method (a plot of end point versus selenium concentration, correlation coefficient(r) = 0.9992)

spectrophotometric method gave 91.84 % recovery with the relative standard deviation of 2.27%. This meant that the catalytic method was very sensitive and effective for the determination of selenium in the samples containing selenium in the level of ppb

Interference studies

The ions chosen for interference studies were K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} and Fe^{3+} which were normally present in high concentration in water samples. Different concentrations of individual interferent ions were added to the samples consisting of 15 ppb selenium. The concentration of the studied ion increased until the error in determination of 15 ppb selenium was over 2SD (SD = standard deviation, confidence limit = 95%). The tolerance

ratio was defined as the ratio of the concentration of the ion causing error over 2SD in the determination of selenium to the concentration of selenium which was calculated for each ion studied (table 1). Of the studied ions, Zn^{2+} had the lowest tolerance ratio, followed by Fe^{3+} , Ca^{2+} , Mg^{2+} , and K^+ , respectively. This implied that the removal of Zn^{2+} and Fe^{3+} from the samples before the determination of selenium by this catalytic method is necessary. However, Ca^{2+} , Mg^{2+} , and K^+ , seemed not to have affect on the determination of selenium by this method.

Comparison of the kinetic catalytic spectrophotometric method with the HGAAS method

Concentration of selenium in five unknown water samples (supplied by the analytical laboratory of Thailand Institute of Scientific and Technological Research) were determined by the catalytic method and the HGAAS method. The results are shown in table 2. The kinetic catalytic method had an advantage in determining of selenium in the water sample at the concentration lower than 0.1 ppm which is the lowest limit of the HGAAS method.

CONCLUSION

The kinetic catalytic spectrophotometric

Table 1 Tolerance ratio for various interfering ions on the determination of 15 ppb Se(IV).

Ions	Tolerance ratio
K^+	7000
Mg^{2+}	3000
Ca^{2+}	2000
Fe^{3+}	5.5
Zn^{2+}	4.5

Table 2 Comparison of the kinetic catalytic spectrophotometric method with the HGAAS method for the determination of selenium in the water samples.

Sample	HGAAS method ^a	Kinetic catalytic spectrophotometric method ^b
1	< 0.1 ppm	100 ppb
2	< 0.1 ppm	70 ppb
3	< 0.1 ppm	50 ppb
4	< 0.1 ppm	25 ppb
5	< 0.1 ppm	70 ppb

a = HGAAS method was determined and reported by the Analytical laboratory of Thailand Institute of Scientific and Technological Research.

b = average value from 4 replicates.

method based on the catalytic effect of selenium on the reaction of methylene blue with sodium sulfide had a high sensitivity and accuracy in determining the concentration of selenium in water samples, with the relative standard deviation of 2.27% at 15.00 ppb standard selenium. This method is easy to use and has an advantage over the hydride generation atomic absorption spectrometric method (HGAAS) in determination of selenium at a very low concentration (< 0.1 ppm).

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