Original Article

Determination of trace selenium content in soil based on methylene blue reduction

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Abstract

Currently used methods for selenium quantification include X-ray fluorescence spectrometry and inductively coupled plasma analysis. However, both methods require expensive equipment, specialized knowledge, making them unsuitable for simple on-site quantification. Therefore, in this study, we developed a simple and rapid method for selenium content determination based on methylene blue reduction. We investigated the feasibility of selenium quantification using a superabsorbent polymer supporting methylene blue. When a selenium solution was added to the polymer, a correlation was observed between the fading time of methylene blue and selenium concentration in the range of 0-10 ppm. These results suggest that selenium content in soil eluate can be quantified using the proposed method. Furthermore, this method enables the determination of selenium content in both aqueous solutions and soil samples. Additionally, it does not depend on temperature, making it suitable for quantifying selenium under various temperature conditions.

Key words

methylene blue, selenium, super absorbing polymer, color detection, temperature dependence

1. Introduction

Selenium in soils is released into the environment through mining, beneficiation, smelting, and refining operations. Environmental selenium exists in various oxidation states, including selenates, selenites, and selenides. Large amounts of selenate and selenite are present in specific soil pollutants. Both selenite and selenate ions dissolve in water and move readily through groundwater. Selenite can also be oxidized in alkaline soils to form soluble selenate, which is readily absorbed by plants (He et al., 2018). Although selenium is an essential element in the human body, excessive selenium causes endocrine alterations in the synthesis of thyroid hormones (THs) or increase the risk of Type 2 diabetes (T2D) (Schomburg, 2020). In contrast, a lack of selenium leads to conditions such as cardiomyopathy and arrhythmia (Ito et al., 2018). Selenium pollution has been confirmed in countries worldwide. For example, selenium pollution has been reported in reservoirs in California and at smelting plant sites in Belgium (Suwaki and Okazaki, 2005).

To address these concern, standard values for selenium concentrations in soil have been established under the Japanese Soil Contamination Countermeasures Act Additionally, methods for determining selenium concentrations have been standardized by the International Organization for Standardization (ISO, 2013). Major techniques for measuring selenium concentrations include X-ray fluorescence spectrometry (XRF) and inductively coupled plasma (ICP) analysis. However, both require expensive equipment and specialized knowledge, making them impractical for simple on-site quantification. In contrast, colorimetry allows intuitive assessment of pollution without requiring specialized knowledge. According to the ISO-17379-2, 3,3’-diaminobenzidine (DAB) spectrophotometry is defined as the colorimetric determination of selenium content in a solution. In this method, selenium is separated by coprecipitation with iron hydroxide, complexed with DAB, extracted with benzene, and the absorbance is measured. DAB spectrophotometry has advantages over XRF and ICP analyses, as it facilitates determining the presence or absence of selenium without requiring specialized knowledge (Iwasaki et al., 1961). However, DAB spectrophotometry is influenced by pH and temperature, which limits its applicability for simple on-site quantification. Additionally, quantifying selenium concentrations using this method requires expensive equipment, such as a spectrophotometer (Munemori et al., 1978, Shayessteh et al., 2013). Another method involves reducing selenate to selenite with potassium bromide and quantifying selenium (IV) via solvent extraction using the fluorescent reagent 2,3-diaminonaphthalene. This method enables the selective quantification of selenium even in the presence of coexisting components. However, this method requires a heating step, there remains a need to quantify selenium concentrations without relying on expensive equipment, such as fluorimetry (Vladimir et al., 2021).

In this study, the feasibility of quantifying selenite and selenate contents in aqueous solutions and soil eluates was investigated using a superabsorbent polymer supporting methylene blue. This method is based on the reduction of methylene blue. Hydroxyethyl cellulose (HEC) gel, a nonionic hydrophilic polymer, was examined as a superabsorbent polymer for absorbing selenium from the tested solutions. Furthermore, the temperature dependence of this method and its application in determining selenite and selenate contents in soil eluates were examined. Our findings establish a novel and effective method for determining selenium content in soil based on methylene blue reduction, and this method can be applied under a wide range of temperature conditions. Unlike conventional quantitative methods, the proposed method is expected to enable quantitative determination without specialized knowledge.

2. Materials and methods

2.1 Principle of the proposed method

Methylene blue (MB) is blue in its oxidized state; however, its reduction caused by sulfide ions (S^{2-}) makes the reduced state of methylene blue (HMB) appear colorless.

$$2MB + S^{2-} + 2H_2O \rightarrow 2HMB + 2OH^- + S$$

Excess S^{2-} ions and sulfur (S) combine to form polysulfides:

$$S + S^{2-} \rightarrow [S - S]^{2-}$$

When selenite is present in the system, it is reduced by S^{2-} ions to elemental Se. Elemental Se further combines with S^{2-} ions to form selenosulfides:

$$SeO_3^{2-} + 2S^{2-} + 3H_2O \rightarrow Se + 6OH^- + 2S$$
$$Se + S^{2-} \rightarrow [Se - S]^{2-}$$

The resultant polysulfides and selenosulfides react with MB, reducing it to HMB.

$$2MB + [S - S]^{2-} + 2H_2O \rightarrow 2HMB + 2OH^- + 2S$$
$$2MB + [Se - S]^{2-} + 2H_2O \rightarrow 2HMB + 2OH^- + Se + S$$

Since selenium is generated at the end of the reaction, it acts as a catalyst for the reduction reaction of methylene blue (Songsasen et al., 2002). When the concentration of sulfide ions is fixed, the generation rate of selenium depends on the selenium concentrations in the tested solutions. As selenium concentrations correlate with the fading time of methylene blue, selenium concentrations in the tested samples can be determined based on the fading time of methylene blue.

The time dependence can be formulated as a first-order reaction using Equations (1) and (2) as follows:

$$\frac{dC}{dt} = -kC \quad (1)$$

$$C = C0e^{-kt} \quad (2)$$

where C is the concentration of the selenate or selenite ions, C_0 is the initial concentration, k is the kinetic constant, and t is the reaction time.

2.2 Preparation of solutions

All reagents were supplied by FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) unless otherwise stated. Standard solutions of selenite and selenate (10 ppm Se) were prepared by dissolving sodium selenite and sodium selenate in distilled water and were diluted to concentrations ranging from 0.0001 to 1.0 ppm using distilled water. A sodium sulfide solution was prepared by dissolving 0.504 g of sodium sulfide and 0.480 g of sodium sulfite in distilled water and diluting the mixture to a total volume of 10 mL. A conditioner solution was prepared by mixing 0.02415 g of iron (III) chloride hexahydrate, 0.1380 g of ethylenediaminetetraacetic acid disodium dihydrate (Kishida Chemical Co. Ltd.), and 0.25 mL of triethanolamine, followed by dissolving the mixture in distilled water. The final volume of the solution was adjusted to 50 mL. A methylene blue solution was prepared by dissolving 0.05 g of methylene blue trihydrate in distilled water and making up the final volume to 100 mL.

2.3 Preparation of HEC gel

HEC gel (Kono, 2015) was prepared by dissolving HEC in a 1.5 mol/L aqueous NaOH solution. Subsequently, ethylene glycol diglycidyl ether was added to the solution at 25 °C with stirring at 300 rpm using a Teflon impeller. After 10 minutes, the crosslinking reaction was performed at 60 °C while stirring at 300 rpm for 3 hours. The reaction mixture was washed twice with a 1:1 (v/v) solution of deionized water and ethanol, followed by dialysis against distilled water using a dialysis membrane. The dialyzed hydrogel was then freeze-dried to obtain a white granular HEC hydrogel. The selenium-detecting agent was prepared by mixing 25 mL of methylene blue solution and 5 g of HEC gel, followed by drying.

2.4 Preparation of simulated selenium-contaminated soil and procedure for dissolution and measurement of selenium concentrations in the contaminated soil

Selenium-contaminated soil samples were prepared by adding the standard sodium selenite solution to soil to achieve selenium concentrations of 0, 50, 100, and 200 mg Se/kg. Subsequently, 2 g of each contaminated soil sample was added to 20 g of water in a centrifuge tube, which was shaken at 600 rpm for 6 h using a shaker (SR-1; AS One). After shaking, the solution was centrifuged at 3,000 × g for 20 min, and the supernatant was filtered using a syringe filter (13 mm, 0.45 µm). The filtrate was used as the soil eluate containing selenium, and selenium concentrations were measured using XRF. The selenium concentrations in the eluates increased with increasing sodium selenite concentrations in the soil, yielding values of 0, 1.1, 2.0, and 5.3 ppm for soil containing 0, 50, 100, and 200 mg Se/kg, respectively. These soil eluates were used to quantify selenium concentrations in the soil.

2.5 Procedure for measuring selenium concentrations in aqueous selenium solutions or soil eluates containing selenium using the selenium-detecting agent

For each experiment, 2.6 mL of the selenium standard solution or soil eluates containing selenium was added to a 50 mL centrifuge tube, followed by the addition of 0.4 mL of formaldehyde, 1.0 mL of conditioner solution, and 0.2 mL of sodium sulfide solution at 30-s intervals. This solution was poured into a centrifuge tube containing 0.2 g of the color detection reagent, and the discoloration of the reagent was photographed. RGB values of the captured images were measured using ImageJ software. Fading time was calculated from the RGB values. Measurements were conducted at 10 °C, 25 °C, and 40 °C, with images captured every 10 s to assess the dependency of the reaction on temperature and reaction time. The relationship between selenium concentration and fading time at each temperature was confirmed using the calculated RGB values.

2.6 RGB determination

Selenium content in the analyzed solutions was quantified based on color fading due to the reduction reaction of methylene blue. The time at which the total RGB value exceeded 255 was defined as the fading time.

3. Results and discussion

3.1 Determination of selenite and selenate

A semilogarithmic graph illustrates the relationship between selenium concentration in the analyzed solutions and the fading time of methylene blue (Figure 1). When selenite was added, the color of the color detection reagent changed from a dark blue to colorless. higher selenium concentration resulted in shorter fading time (Figure 1). The logarithmic correlation coefficient was 0.985.

In Japan, the standard value for selenium is 0.001 ppm. A significant difference in fading time was observed before and after reaching this standard, allowing for the determination of the concentration of selenite even when methylene blue was supported on the superabsorbent polymer. However, when this method was applied to the determination of selenate concentrations, it was confirmed that there was no correlation between selenate concentrations and fading time. The fading time ranged from 65-80 s for all selenate concentrations. Similarly, while determining selenite concentrations, the fading time of methylene blue ranged from 60-70 s at the selenium concentration of 0 ppm. Thus, it can be inferred that in the case of samples containing selenium in selenate form, only polysulfides cause discoloration. In the case of selenate, it is more mobile and less adsorbable than selenite (Snyder and Um, 2014). In addition, it is inferred that selenate is not retained in the polymer due to its high solubility in aqueous solution, and therefore no catalytic action occurred. Arikan et al. reported that the addition of cationic surfactant cetyltrimethylammonium bromide not only enabled the differentiation of selenate and selenite but also improved analytical sensitivity in the low selenium concentration range (Arikan et al., 1996). However, Arikan's method requires precise time management for every quantification step and is complicated to perform, making it unsuitable for simple quantification. To enable the quantification of selenate, it was necessary to develop a method to suppress the oxidation rate of methylene blue, such as adjusting the amount of formaldehyde added, based on the technique used in this experiment. Based on these findings, subsequent experiments were conducted using selenite only.

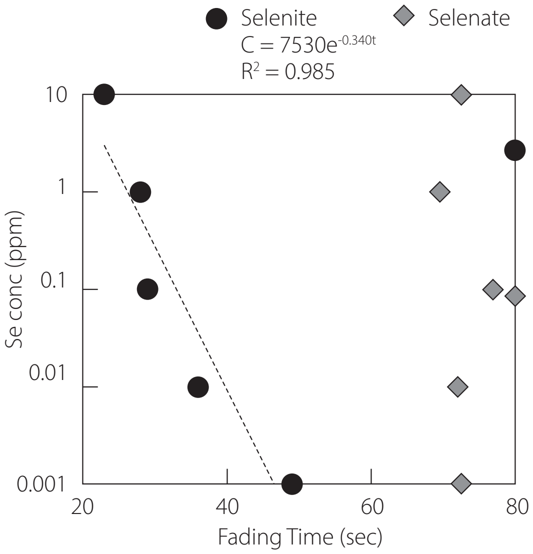

Figure 1: Calibration graph for the determination of selenite and selenate from the fading time

Note: Plots are experimental values and lines are the one-order reaction equations.

3.2 Temperature dependence

The discoloration of the selenium-detecting agent is influenced by its temperature variations (Figure 2). The degree of discoloration remained largely unchanged across all tested temperatures. In contrast, at a selenium concentration of 1.0 ppm, the color faded faster when the detection agent was placed in 40 °C environments.

Additionally, approximation lines were created for the semilogarithmic graph of the relationship between selenium concentration and fading time, as shown in Figure 3. The results revealed that the reaction rate constants were 0.271, 0.327, and 0.241 s^{-1} at 10, 25, and 40 °C, respectively. Figure 4 presents an Arrhenius plot constructed using the reaction

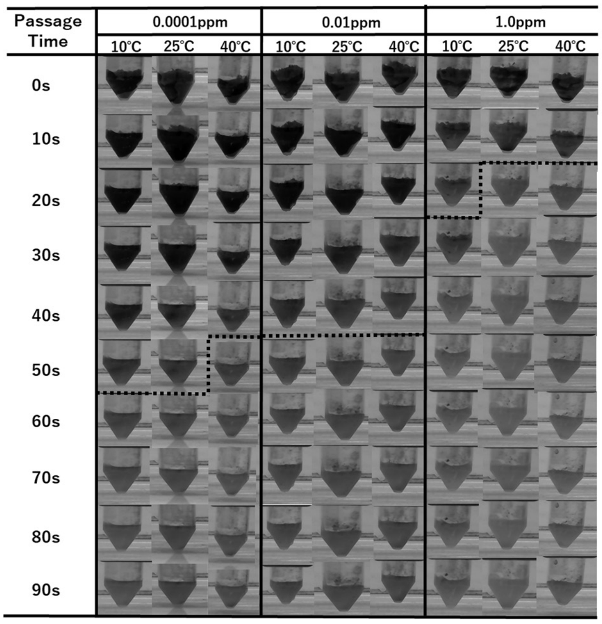

Figure 2: Fading of selenium detection agent under different temperature

Note: The doted lines means the fading time calculated from the results shown in Figure 3.

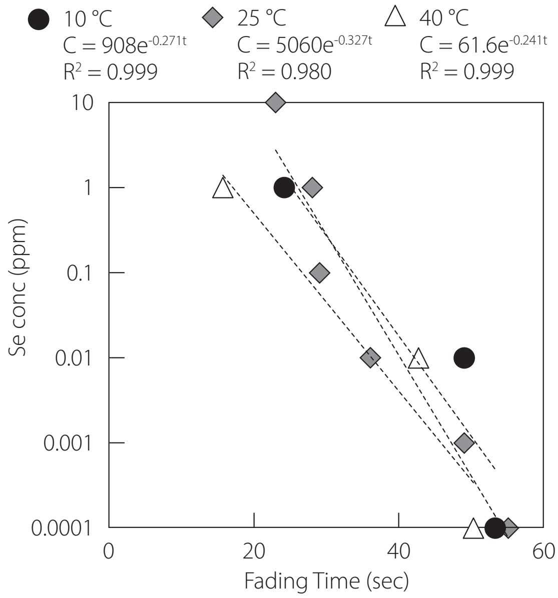

Figure 3: Calibration graph for the determination of selenium from the fading time by temperature

Note: The first-order reaction constants under each temperature were 0.271 s^{-1} (at 10 ℃), 0.327 s^{-1} (at 25 ℃), and 0.241 s^{-1} (at 40 ℃).

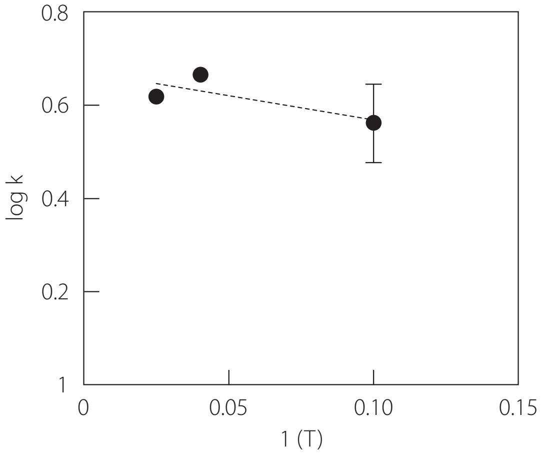

Figure 4: Arrhenius plot for the selenium quantification reaction

Note: The formulated carves means the Arrhenius equation deriving the activation energy, E = 8.730 kJ/mol, R^2 = 0.621.

rate constants. The approximation formula was calculated using Excel (Microsoft Excel for Microsoft 365 MSO, Version. 2407, Build 16.0.17830.20166, 64-bit), and the activation energy was estimated to be 8.730 kJ/mol. However, as the correlation coefficient of determination was 0.621, the relationship between temperature and reaction rate did not follow the Arrhenius equation. Thus, the reaction rate constant did not change with temperature (Figure 4).

These finding indicate that selenium quantification based on the methylene blue reduction reaction is independent of temperature. Furthermore, selenium content can be determined by assessing whether the fading time is approximately 60 s, regardless of temperature.

3.3 Determination of selenium in simulated selenium-contaminated soil

The selenium detection agent undergoes discoloration upon the addition of soil spiked with 0-200 mg Se/kg (Figure 5). At the eluted selenium concentration of 5.3 ppm, the fading time of selenium detection agent could not be measured,

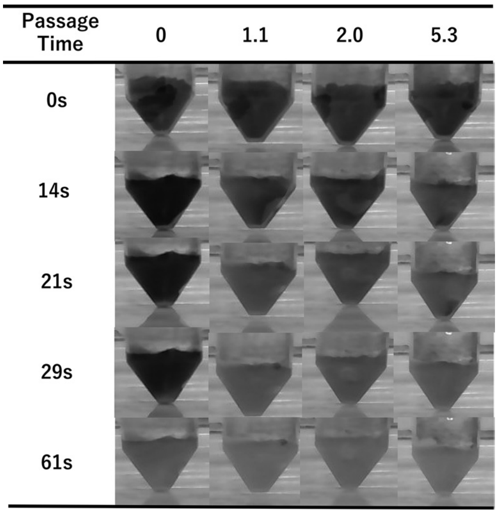

Figure 5: Discoloration of selenium detection agent after addition of selenite elution

Note: The selenite elution is a solution obtained by shaking soil to which sodium selenite solution has been added with water, centrifuging them, and filtering them.

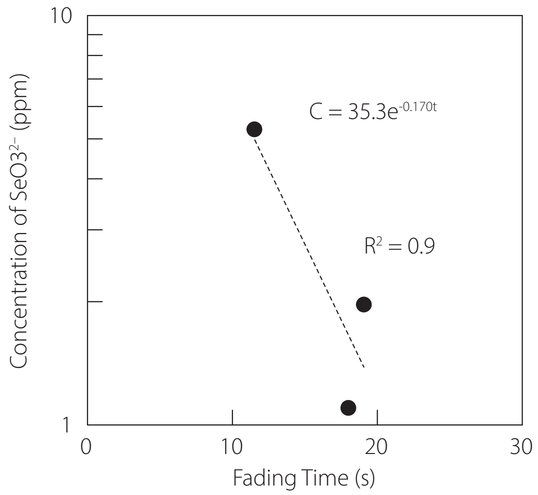

Figure 6: Relation between fading time and selenium concentration of selenium detection agent

Note: The selenium concentration refers to the concentration of selenite dissolved from the soil containing selenite.

as discoloration occurred immediately upon the addition of the selenium solution to the superabsorbent agent. Nonuniform fading occurs when the fading rate exceeds the rate of solution absorption. The discoloration time was significantly longer in the absence of selenite in the soil.

RGB measurements were performed using the colors of the selenium detectors. Figure 6 shows a semilogarithmic graph of the relationship between selenium concentration and fading time. The fading time at 1.1 ppm was shorter than that at 2.0 ppm. However, the correlation coefficient for Figure 6 was 0.931. In addition, there was a significant difference in the fading time around the environmental standard for 0.01 ppm of selenium. Thus, it was possible to determine whether the standard value was exceeded. The shorter fading time at the selenium concentration of 1.1 ppm than at 2.0 ppm can be attributed to the impact of other metals present in the soil. It has been reported that metal ions, such as Cu^{2+}, Fe^{3+}, and Fe^{2+}, can interfere with the reduction reaction of methylene blue (Arikan et al., 1996).

In conventional selenium content analysis, masking agents are used to prevent interference from other metal ions. However, as this study aims to perform a simple on-site analysis method, a complicated process is unsuitable for screening. Therefore, selenium concentration in soil must be measured even in the presence of substances that interfere with the reduction reaction of methylene blue.

Based on these considerations, artificially contaminated soil was spiked with multiple heavy metals, both with and without selenium. The fading time for the soil containing heavy metals other than selenium was 64 s. Selenium concentration in soil leachate was 0 ppm. The fading time for the soil contaminated with heavy metals mixed with selenium was 36 s. Selenium concentration was 0.22 ppm in soil leachate. This result is consistent with the time dependence shown in Figure 6. Thus, this method is suitable for determining whether pollution levels exceeded the recommended environmental standard values.

4. Conclusion

For the simple detection of selenium, a reduction reaction of methylene blue supported on a superabsorbent polymer was used. selenite can be determined from the fading time of methylene blue by adding a selenium standard solution. If selenium is present in its oxidized state as selenite, an environmental standard of 0.01 ppm selenium can be identified. However, selenate is difficult to detect using this method. Moreover, this method exhibited no temperature dependence and enabled selenium quantification in soil regardless of ambient temperature. The fading time of methylene blue was influenced by the presence of other heavy metals during soil selenium analysis. This method allowed for the determination of whether pollution levels exceeded environmental standards.

Future research should focus on improving the quantification method to enhance accuracy. In selenium determination via the reduction of methylene blue, the presence of metallic, oxidizing, and reducing agents affects the fading time. Therefore, strategies for removing these interfering substances should be explored. In addition, the type and proportion of soil contents affect the fade time. Therefore, the effect of different soil types on the quantification should be evaluated.

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