

การตรวจวัดซีลีเนียม(IV) ที่จำเพาะเจาะจงในตัวอย่างผลผลิตทางการเกษตร โดยใช้แคดเมียมซัลไฟด์นาโนโพรบ

SPECIFIC DETECTION OF SELENIUM(IV) IN AGRICULTURAL SAMPLE BY CdS-Cys-DAB FLUORESCENT NANOPROBES

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บทคัดย่อ

ในการวิจัยนี้มีเป้าหมายในการพัฒนานาโนโพรบฟลูออเรสเซนต์ CdS-Cys-DAB ที่มีความจำเพาะในการตรวจวัดซีลีเนียม(IV) ในตัวอย่างหัวหอม นาโนโพรบประกอบด้วยอนุภาคนาโนแคดเมียมซัลไฟด์ที่เคลือบด้วยกรดอะมิโนซิสเทอีนเป็นแกน และเคลือบด้วยชั้นของ 3,3'-diaminobenzidine (DAB) ซึ่งเป็นโมเลกุลที่ทำให้เกิดฟลูออเรสเซนต์กับซีลีเนียม แสงฟลูออเรสเซนต์ที่คายออกมาจากซีลีเนียม(IV) ที่จับกับนาโนโพรบจะถูกตรวจวัดที่ความยาวคลื่น 390 นาโนเมตร โดยใช้ความยาวคลื่นในการกระตุ้นที่ 280 นาโนเมตร ซึ่งเมื่อทำการศึกษการตรวจสอบการใช้ได้ของนาโนโพรบ พบว่าเมื่อนำไปทดสอบความเข้มของฟลูออเรสเซนต์ที่คายออกมาจากซีลีเนียมที่จับกับนาโนโพรบจะเป็นสัดส่วนโดยตรงแบบเชิงเส้นกับปริมาณของซีลีเนียม(IV) ในช่วงความเข้มข้น 1.00-5.00 ppm โดยมีสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.9925 ค่าขีดจำกัดต่ำสุดของการตรวจวัด (Limit of Detection, LOD) และค่าขีดจำกัดต่ำสุดของการวิเคราะห์ (Limit of Quantitation, LOQ) อยู่ที่ 0.12 ppm และ 0.34 ppm ตามลำดับ จากนั้นนำนาโนโพรบไปประยุกต์ใช้ในการตรวจวิเคราะห์ปริมาณซีลีเนียม(IV) ในตัวอย่างหัวหอม พบว่ามีปริมาณซีลีเนียม(IV) ในปริมาณ 1.42 ppm จากผลที่ได้ นาโนโพรบที่สังเคราะห์ขึ้นสามารถที่จะนำไปประยุกต์ใช้ในการตรวจวิเคราะห์ปริมาณซีลีเนียม(IV) ในตัวอย่างทางการเกษตรอื่น ๆ ต่อไป

คำสำคัญ: นาโนโพรบ ซีลีเนียม หัวหอม

Abstract

This research aims to develop a selective CdS-Cys-DAB fluorescent nanoprobe for selective determination of selenium(IV) in sample such as onion. The nanoprobe consists of a cysteine-capped cadmium sulfide core and a coating layer containing selenium(IV)-induced fluorescent molecules, 3,3'-diaminobenzidine (DAB). The fluorescence intensity emitted from the selenium-bound nanoprobe was

measured at 390 nm with an excitation wavelength at 280 nm. Validation of the nanoprobe was investigated. The fluorescence intensity of nanoprobe shows linearity that proportional to selenium(IV) quantity in the range of 1.00 - 5.00 ppm with a correlation coefficient of 0.9925. The detection limit (LOD) and quantification limit (LOQ) of this nanoprobe were 0.12 and 0.34 ppm respectively. The results of the interfering study of some metal ions on the nanoprobe response showed a high selectivity of the CdS-Cys-DAB nanoprobe towards Se(IV) ions. The nanoprobe was subsequently applied to determine selenium(IV) content in onion sample. It was found that the concentration of as low as 1.42 ppm of selenium(IV) was obtained. Based on the results, the synthesized nanoprobe should be successfully applied for further analysis of selenium(IV) content in other agricultural sample

Keywords: Nanoprobes, Selenium, Onions

Introduction

Selenium (Se) plays an important role in human and animal nutrition. It is an essential component of a number of enzymes like glutathione peroxidase (GPX) [1-2]. Insufficient selenium intake cause several health problems including cardiac failure. Although selenium is required for health in a certain concentration, at high selenium doses can be toxic to human health [3]. The intoxication depends on selenium concentration and the chemical forms of this element. The recommended daily dietary intakes allowance for selenium in European countries is in the range of 30 to 70 μg [4].

Several methods to determine selenium content have been developed for many years such as spectrophotometric method, spectrofluorometric method, neutron activation analysis method, electro chemical techniques and atomic absorption spectrometry method. There are several spectrophotometric methods for the determination of selenium, some methods show low sensitivity in selenium detection in trace level as $\mu\text{g L}^{-1}$ or lower. Owing to the low limit of detection, selectivity, sensitivity and minimum sample quantity, the electro thermal atomic absorption spectrometry (ETAAS) is more widely used to determine selenium in different matrices [5]. However, the ETAAS still need to solve the spectral interference problems by Zeeman background correction [6]. As known that ETAAS used high cost with experience controller.

In the recent years, owing to the simplicity and selectivity, fluorescence based probes becomes more popular in the detection of heavy metal ions [7]. Common molecules used in the fluorescence sensor studies are organic dyes [8-9]. However, most organic fluorophores suffer from poor photostability and photo-bleaching. Compared with classical organic fluorophores, fluorescent nanoprobe possess serious merits such as excellent luminescence, continuous excitation spectrum, greater photostability, size-tunable and narrow emission bands [10]. Herein, we prepared cadmium sulfide (CdS) nanoprobe sensor for selenium detection. CdS nanoprobe based was interesting over the past years [11-12] it has been widely used as luminescent probes, sensor, optical filters, solar cells, electrochemical devices and microelectronics [13-16].

Objectives

To develop water soluble CdS-Cys-DAB fluorescent nanoprobe and study on the effects of ions interference for detect selenium(IV) in onion sample.

Chemicals and Apparatus

Cadmium chloride (CdCl_2), sodium sulfide (Na_2S), L-cysteine, 3,3'-diaminobenzidine (DAB), N-hydroxysuccinimide (NHS), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and selenium(IV) standard were purchased from Sigma-Aldrich, United States. Absolute ethanol and sodium hydroxide were purchased from Merck, Germany. All the chemicals were HPLC and AR grade. Deionized-distilled water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$ resistivity) produced from Milli-Q water purification system (Massachusetts, USA) was used for preparation of all solutions.

Fluorescence spectrometer (JASCO FP-8200) was employed to measure the fluorescence intensity of standard and sample solution. The quality of nanoprobe was evaluated by the scanning electron microscope (SEM) (LEO 1450 VP) with energy dispersive spectroscopy X-rays (EDX), transmission electron microscope (TEM) (Philips Tecnai 20). An inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer's NexION 300Q) was used to validate method and compared with synthetic nanoprobe

Methods

1. Synthesis of cysteine-capped CdS nanoparticles

The cysteine-capped CdS nanoparticles was synthesized by modified from Devi et al. [17]. The 0.5 mmol portion of cysteine and 0.5 mmol of $\text{CdCl}_2 \cdot 10\text{H}_2\text{O}$ were dissolved in 250 ml of double distilled deionized water in a round bottom flask. The pH of the mixture solution was then adjusted with 0.1 NaOH to obtain the final pH value of 6.0. The solution was purged with N_2 for 30 mins and continuously stirred with magnetic stirrer. Then 0.5 mmol portion of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ was added dropwise to 20 ml of double distilled deionized water with vigorous stirring. The N_2 gas was purged to the system for 120 mins. Then, absolute ethanol was added to gain the cysteine-capped CdS (CdS-Cys) nanoparticles. The functionalized CdS-Cys nanoparticles was washed and redispersed to remove the contaminants. The luminescent CdS-Cys nanoparticles was dissolved again in double distilled deionized water.

2. Synthesis of CdS-Cys-DAB nanoprobe

The CdS-Cys-DAB nanoprobe was synthesized by modified from Song et al [18]. The 2.5 ml of 100 mg/ml NHS, 2.5 ml of 100 mg/ml EDC, and 2.0 ml of 0.013 mg/ml CdS-Cys nanoparticles solution (from 1) were mixed together and leaved for 30 mins. The products was washed with pH 7.4 buffer (PBS). Then, 100 μL of 2.14 mg/ml DAB solution was added to the products and stirred for 270 mins. The CdS-Cys-DAB nanoprobe was precipitated by addition of ethanol and centrifuged at 10,000 rpm for 10 mins. The purified precipitate was finally re-dispersed in 100 ml deionized water and stored at 4°C for further application. The probe was checked its size and surface characteristic by SEM and TEM.

3. Determination of selenium in onion samples using CdS-Cys-DAB nanoprobe

Onion samples were brought from Foodland Supermarket in Bangkok, Thailand. All of the samples were peeled and washed with deionized water to remove impurities and chopped into small pieces. Then 50 g of samples were digested with 50 ml HNO₃ (65%) and 15 ml H₂O₂ (30%) on a hotplate for 120 mins. After digestion, the residues samples were filtered out and the solution samples were collected and stored at 4°C.

Selenium(IV) in a sample solutions were determined using the developed nanoprobe. A 1.0 ml aliquot of CdS-Cys-DAB was add to 1.0 ml of sample solutions and various Se(IV) concentrations solution (final concentration, 1-5 ppm). Then the pH was adjusted to 6 (0.1M NaOH) and vortexed for 5 mins. The mixture solution was filtered using 50 nm porous membrane and the intensity of fluorescence emitted was then measured by using fluorescence spectrometer. The resulting intensity was compared with the standard calibration curve to obtain analyte's concentration.

Results

1. Fabrication of the CdS-Cys-DAB nanoprobe

An important step in making the selective fluorescent nanoprobe for selenium is to choose the appropriate selenium-induced fluorescence molecule which generates fluorescence signal when bound with selenium contained in the sample. DAB was previously used to detect selenium [19]. That study has proven that DAB showed the high selectively in reaction with selenium ion. Thus, in this work, DAB was chosen as a selenium probe molecule to make the nanoprobe.

After the matrix and probe molecules were selected, the immobilization of the probe molecules onto the CdS-Cys nanoparticle surface is the essential step to make the nanoprobe. A DAB molecule has two pairs of amine groups on different sides, which makes immobilization of DAB to the nanoparticle surface possible. Based on the knowledge of chemical reactions, carboxyl groups can easily react with amine groups. Thus, bringing carboxyl groups onto the CdS-Cys nanoparticle surface will be the next key step for fabrication of designed nanoprobe.

To initiate the reaction of carboxyl groups on the nanoparticle surface with amine groups on DAB molecules, the activation of carboxyl groups on the nanoparticles is necessary. EDC was chosen for this activation since EDC can form o-acylisourea intermediates with the carboxyl groups on the surface of the nanoparticles. Afterward, NHS was applied to increase coupling efficiency of carboxyl groups with amine groups by forming active ester intermediates, which are more stable than o-acylisourea intermediates. Then DAB reacted with the intermediates and replaced the EDC and NHS. When the ratio of nanoparticles to DAB molecules was controlled properly, one amine pair group on a DAB molecule can link to the nanoparticle surface. Thus, the selenium nanoprobe was formed (Figure 1A). The other amine pair group on the DAB molecule can then bind to the target selenium and produce fluorescent complex (Figure 1B).

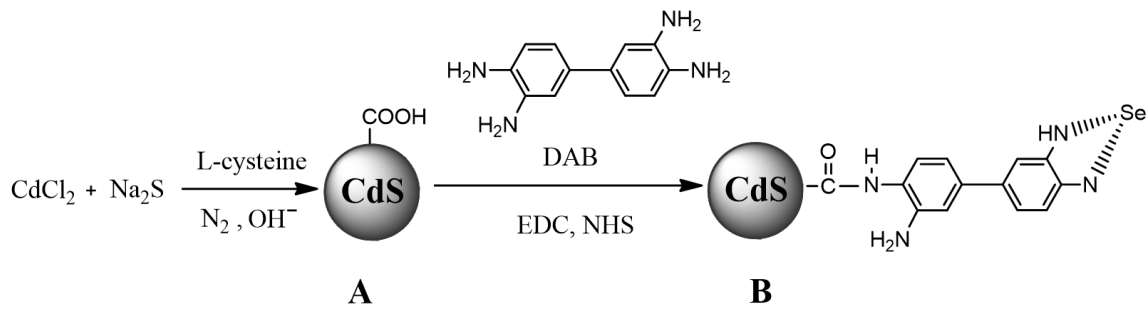


Figure 1. Schematic diagram modified from Song et al. [18] shows fabrication of the CdS-Cys-DAB nanoprobes for determination of selenium(IV). (A) CdS-Cys nanoparticle. (B) DAB modified on CdS-Cys nanoparticle for selenium turned-on fluorescence signal when Se(IV) was present.

2. Characterization of the nanoprobes

The morphology of the nanoprobes was characterized using a scanning electron microscope (SEM). First, the SEM and TEM images of the CdS-Cys nanoparticles were taken. The results showed that the average size of the CdS-Cys nanoparticles was 20 nm in diameter (Figure 2A) and observed shell layer of cysteine capped on CdS core nanoparticles (Figure 2B). After immobilization of DAB molecules on the nanoparticle, the size and shape of nanoprobes were significant changed and DAB modified layer was fixed on core surface (Figure 2C). The average particle size was found to be 50 nm. The fluorescent spectra of the nanoprobes are shown in Figure 3. A strong emission peak was observed at 390 nm (Figure 3A) compared with the emission peak of CdS-Cys nanoparticles (Figure 3B), when the excitation wavelength was 280 nm.

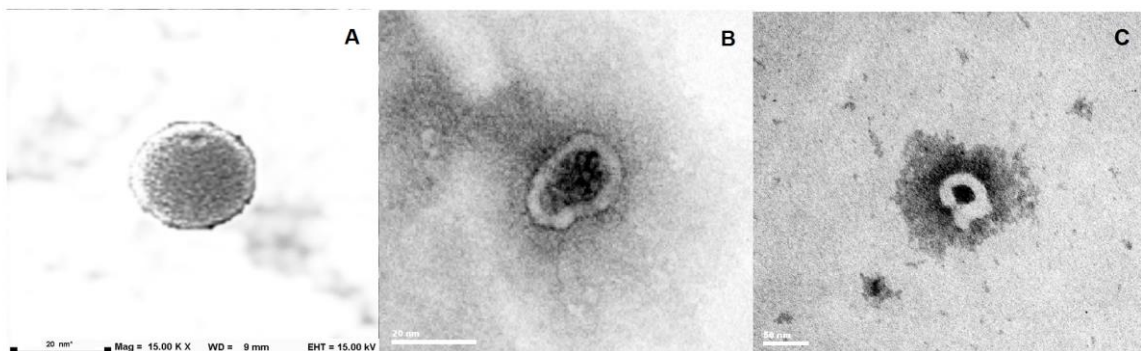


Figure 2. SEM and TEM images of CdS-Cys nanoparticles (A and B). TEM images of CdS-Cys-DAB functionalized nanoprobes (C).

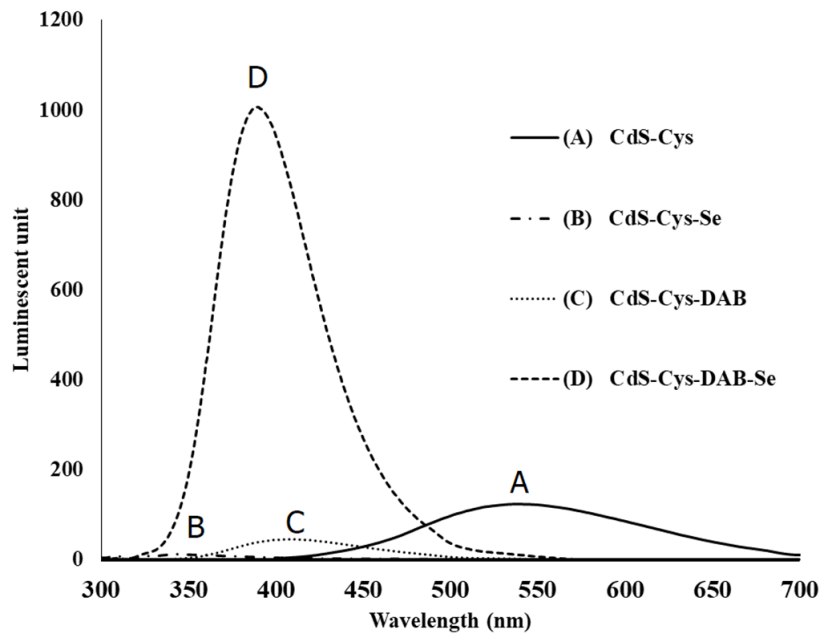


Figure 3. (A) Fluorescence emission spectrum of CdS-Cys nanoparticles, (B) CdS-Cys nanoparticles in the presence of Se(IV), (C) CdS-Cys-DAB nanoprobe and (D) CdS-Cys-DAB nanoprobe in the presence of Se(IV). The excitation wavelength was set at 280 nm.

3. Selectivity of the nanoprobe on selenium

To evaluate the selectivity of CdS-Cys-DAB nanoprobe toward various metal ions, the fluorescence spectra of nanoprobe were measured in the presence of several metal cations such as Ag(I), Cd(II), Co(II), Cu(II), Fe(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II) and Zn(II). The effect of metal ions on the fluorescence of nanoprobe in solution was shown in Figure 4. It can be clearly seen that the fluorescence signal of the nanoprobe for selenium was 14 times higher than others.

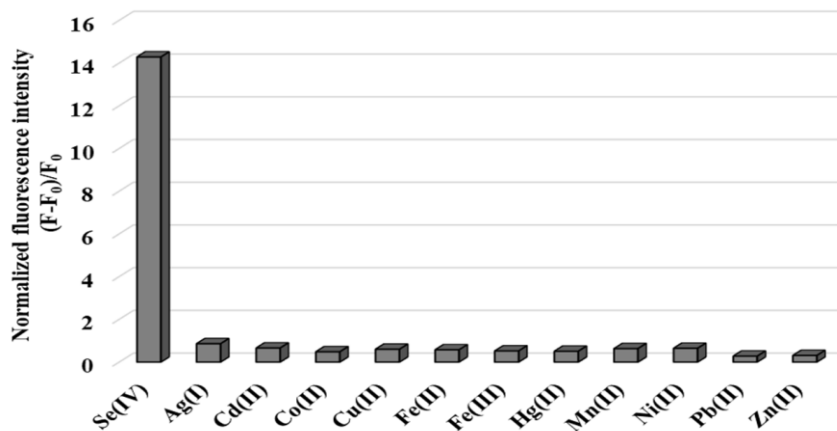


Figure 4. Comparison of the responses of the nanoprobe for common metal ions to the selenium ion.

4. Analytical performance of CdS-Cys-DAB nanoprobe

The fluorescence emission at 390 nm with an excitation of 280 nm was chosen for determining selenium(IV) by nanoprobe in an aqueous solution. The fluorescence intensity was proportional to the Se(IV) concentration. The linear range of this detection was 1.0 ppm to 5.0 ppm (Figure 5) with a calibration equation of $y = 0.1014x - 0.0737$, $R^2 = 0.9925$. The detection limit (LOD) and the quality limit (LOQ) was 0.12 ppm and 0.38 ppm. Respectively.

The relative standard deviation was 3.2% (five replication measurements). Both the linear range and the detection limit are similar to ICP-MS method on selenium detection. The application of this nanoprobe for in situ detection of selenium is in process. The analytical performance of CdS-Cys-DAB nanoprobe compared with ICP-MS shown in Table 1.

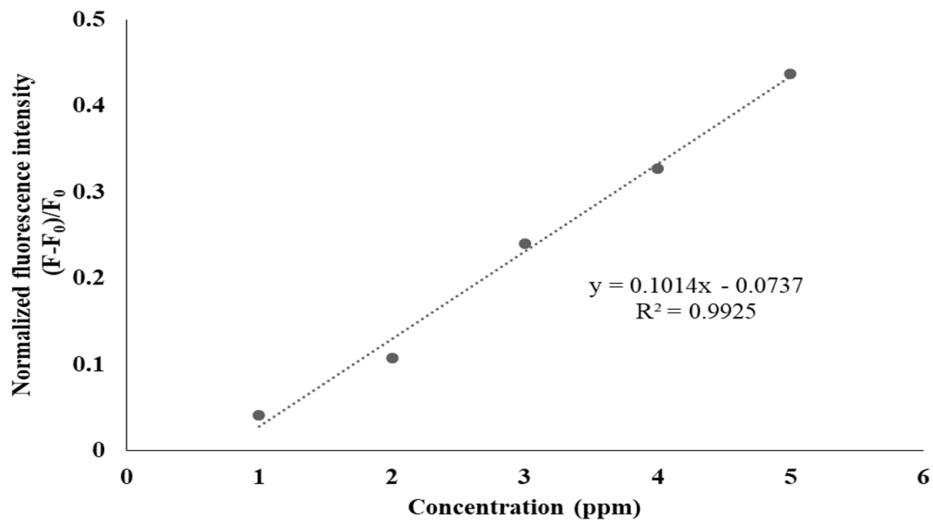


Figure 5. The standard curve of CdS-Cys-DAB nanoprobe in range 1.0 ppm to 5.0 ppm.

Table 1. The analytical performance of CdS-Cys-DAB nanoprobe

Method	Added (mg kg ⁻¹)	Found (mg kg ⁻¹) (±SD, n = 5)	Percentage recovery (±SD, n = 5)	% RSD
This study	50.00	45.65	91.3 (±2.4)	3.2%
ICP-MS	50.00	48.80	97.6 (±2.6)	1.3%

5. Determination selenium(IV) in onions sample

The proposed procedure was applied to measure of Se(IV) in onions sample. The sample was treated and determination by the proposed method. The Se(IV) level was found to be average 1.42 ppm for onion sample.

Conclusions and Discussion

Water soluble functionalized CdS-Cys-DAB nanoprobe was synthesized for use as a luminescent probe for selenium(IV) detection. This nanoprobe is based on the fluorescence enhancement of selenium ions, which interacts with functionalized CdS-Cys-DAB nanoprobe. The calibration plot was linear in the range between 1.0 ppm and 5.0 ppm with a correlation coefficient of 0.9925. The detection limit of this sensor was found to be 0.12 ppm. There is a little or no interference from many metal ions that normally coexist with Se (VI) ion. In addition to its good sensitivity, other advantages of this method include its simplicity and rapidity.

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