Toxic Effect of Cadmium Assay in Contaminated Soil Earthworm Cell Using Modified Sensor

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A voltammetric toxic metal of cadmium detection was studied using a fluorine doped graphite pencil electrode (FPE) in a seawater electrolyte. In this study, square wave (SW) stripping and chronoamerometry were used for determination of Cd(II) in seawater. Affordable pencils and an auxiliary electrode were used as reference. All experiments in this study could be performed at reasonable cost by using graphite pencil. The application was performed on the tissue of contaminated soil earthworm. The results show that the method can be applicable for vegetables and in vivo fluid or medicinal diagnosis.

Key words: Cadmium, Voltammetry, Tissue, Seawater electrolyte

INTRODUCTION

Cadmium (Cd) is a toxic metal occurring naturally in the environment and as a pollutant emanating from industrial and agricultural water sources (1). Cadmium ions are easily absorbed by vegetables from water resource and, in animal-based food, are principally distributed in the liver and kidneys (2). Some studies have reported that Cd is associated with some disease such as Itai-itai (3), Alzheimer's (4), diabetes, chronic kidney diseases (5), and renal cancer (6). Many studies have been conducted to determine the toxicity of Cd. A lot of spectrometric studies have been investigated such as flame atomic absorption spectrometric determination (7,8), inductively coupled plasma optic emission spectrometry (9), thermal atomic absorption spectrometry (10,11), kinetic methods (12) sequential multi-element flame atomic absorption spectrometry (13), hydride generation atomic absorption spectrometry (14), CPE (The chemical variables affecting cloud point extraction) molecular fluorescence combined methodology (15), beam injection flame furnace atomic absorption spectrometry (16), and other absorption spectrometries (17-20). These devices are expensive and time-consuming, compared to voltammetric methods which are affordable and fast. Here, voltammetric methods have been studied actively such as carbon paste (21) modified (22) glassy carbon (23) platinum (24), mercury (25) and nanotube (26) sensors. Moreover, in this study, reasonable graphite pencil electrodes were used instead of expensive electrodes, and flourine was doped on a working electrode. All examinations were performed in seawater electrolyte solution. Analytical application was also performed on the tissue of contaminated soil earthworm. The results show that they can be used for medicinal diagnostic assays.

MATERIALS AND METHODS

Systems, reagents, electrode preparation, and experimental procedure. Experimental systems were carried out using a bioelectronics-2 structure, which was constructed by the authors' institution. Their version was fabricated to a computerized handheld voltammetric system with a 2.4 V potential windows, a 2 mA current range, a 10 pico A measuring current, and a 5"4"1" compact size. The size of the instrument was similar to that of a typical cellular phone. The FPE was prepared using coated fluorine and nafion on the graphite pencil, and graphite pencil electrode (PE) was prepared. Two pencils served as the reference and auxiliary electrodes. The supporting electrolyte was prepared using deep seawater. All the other reagents were of analytical grade. Electrolyte voltammetry was carried out in

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an open circuit. In a 5 mL conc HF solution, fluorine was doped using a 10-cycle scan with a 1.0 V initial potential, a 1.0 V switching potential, and a 0.5 Vs^{-1} scan rate. The SW stripping voltammograms used the following parameters: 0.06 V amplitude, 25 Hz, 170 sec deposition time, and −1.3 V initial potential. All experiments were performed at room temperature and without oxygen removal.

RESULTS AND DISCUSSION

First, high concentration of Cd was examined with SW using PE in seawater electrolyte. In this study, inexpensive and renewable pencils were used as working, reference, and auxiliary electrodes. Fig. 1A shows that SW examination ranges from 0 to 100 mg/L concentration using PE. At 10 mg/L, the peak current of 0.1016×10^{-3} was obtained. It increased quickly to 0.2810×10^{-3} at 20 mg/L, but decreased at 30 mg/L. After a decline, it started to increase again and oscillated. Fig. 1B illustrates the SW FPE results of micro ranges. The concentration of ranges from 0 to 10 mg/L was spiked. Two peak current were obtained at 3.13×10^{-6} A (0.1 V) and 5.073×10^{-6} A (−0.5 V) increased quickly as more concentrations of Cd spike. The curves also became sharper according to the increase of the peak currents. These linear equations was of $y = 18.527x - 32.321$, $R^2 = 0.9898$, and $y = 10.326x - 9.871$, $R^2 = 0.9809$, both can be usable for cathodic scan. Fig. 1C also shows the CV effects in microgram ranges. The anodic small peak of 1.057×10^{-4} A was obtained at 1 mg/L. After that, it increased continuously and reached 10.540×10^{-4} A. Working curve was of $y = 1.1487x + 0.3058$, $R^2 = 0.9487$. Thus the seawater electrolyte, good results were examined using FPE.

SW Optimizations of FPE. Fig. 2A shows the results of chronoamerometry. Every 25 seconds, each 25 mg/L Cd was spiked by 9 point at −0.6 V oxidation potential. The step current was linear from 25 to 225 mg/L variations. The peak current was obtained every time Cd was spiked. It obtained 5.092×10^{-5} A at 25 seconds and increased continuously to 34.832×10^{-5} A. The equation of these curves was sensitive for $y = 0.144x + 4.274$ and the precision $R^2 = 0.978$, can be applicable for mili ranges. Under these conditions, Fig. 2B shows the result of statistics effects using 10 mg/L constant. Based on optimum conditions, the precision was examined with 13th determination of the standard spike. The result of standard deviation was 0.13369. This shows FPE is stable for the detection of Cd. Here can be used to analytical working ranges and application.

Analytical working range and statistics. Under optimum conditions, analytical working ranges were examined using CV and SW in seawater electrolyte. Fig. 3A shows the CV results of 10~80 μg/L variations with 8 points. In the blank solution, no signal was obtained. The peak cur-

(A)

(B)

(C)
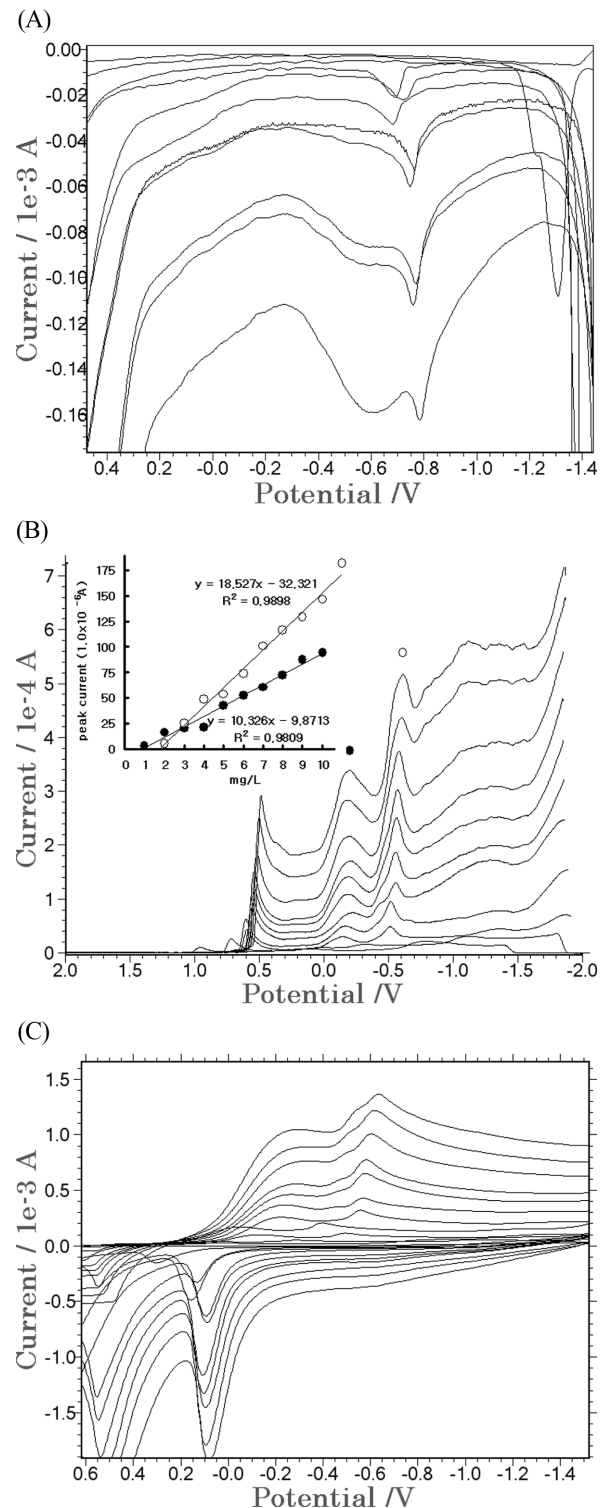
Fig. 1. (A) Stripping voltammetry using PE for Cd variations. The first curve is blank and 0~100 mg/L Cd spike with 10 points in the seawater electrolyte. (B) SW effect in lower concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/L spike fluorine-doped FPE electrode. (C) Cyclic voltammetry in the range of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/L FPE. The first curve represents the blank solution and no signal was obtained.

(A)

(B)
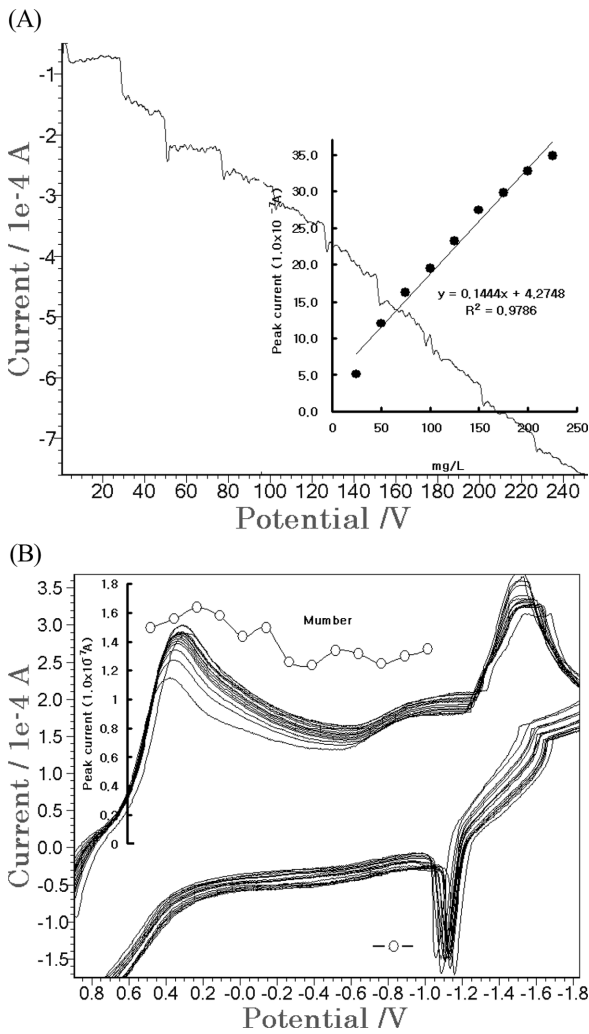
Fig. 2. (A) The results of chronoamerometric concentration effects for 25-225 mg/L Cd add with 25 seconds, −0.6 V oxidation potential. (B) The statistics using 10 mg/L Cd FPE electrode with CV with optimum conditions.

rent increased from 6.143×10^{-6} A to 51.220×10^{-6} A oxidation. The curve became sharper and linear. After that, the SW anodic and cathodic scans were examined in micro ranges. Fig. 3B shows the anodic scan from 10 to 80 μg/L with 8 point spike. The curve was narrowed forward to the bottom. Linear equation was $y = 0.8031x − 15.206$ and $R^2 = 0.884$, which can be applicable for any field. Moreover, Fig. 3C shows the SW examination on cathodic scan from 10 to 80 μg/L. The peak current increased continuously from 1.357×10^{-6} A to 6.961×10^{-6} A. Linear equation of $y = 0.0781x + 1.0428$ and precision of $R^2 = 0.9599$ were obtained. After the working ranges, low detection limits were examined and the application was performed.

In vivo applications. The examination of Cd in the earthworm tissue was examined using the standard addition

(A)

(B)

(C)
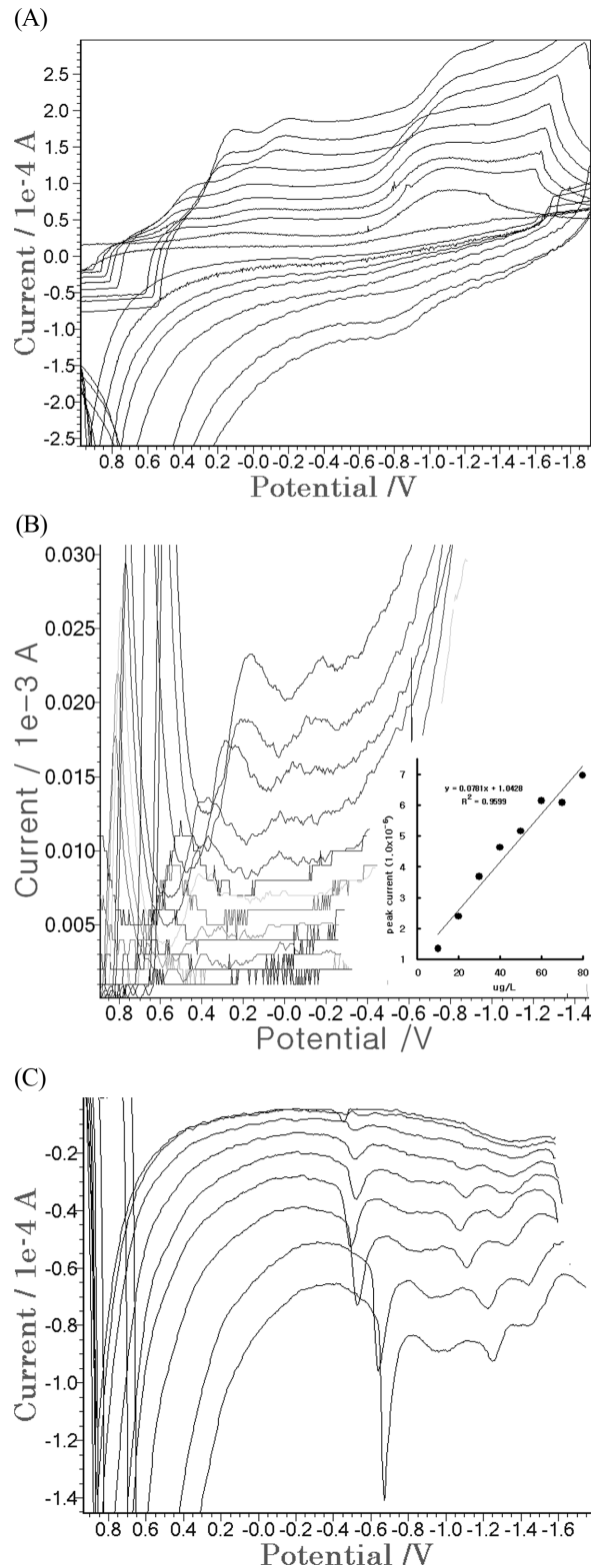
Fig. 3. (A) The CV for analytical working ranges of 10, 20, 30, 40, 50, 60, 70, and 80 μg/L Cd ion spike using FPE electrode. (B) The SW anodic working ranges from 0 to 80 μg/L. (C) The cathodic scan of SW ranges of 0, 20, 30, 40, 50, 60, 70, and 80 μg/L Cd ion spike in seawater electrolyte under optimum conditions.
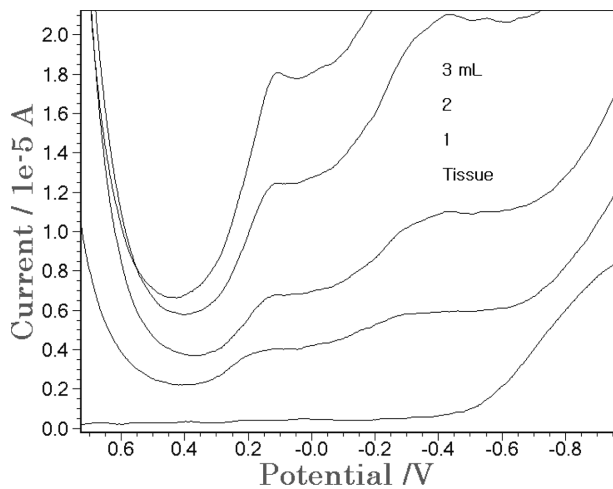
Fig. 4. Determination of Cd in the contaminated site of earthworm cell using the standard addition method. The bottom curve is the result of the blank solution. The unknown solution that dissolved the earthworm tissue in the nitrate was spiked. Then 1, 2, and 3 standard Cd solution was spiked in order based on optimum condition.

method in seawater electrolyte. Fig. 4 shows the results of the analytical detection in the range of microgram additions. Before experimenting on the application, an unknown cell tissue was prepared. This solution was made by dissolution of earthworm tissue for 0.5 g/10 mL nitrate with heating in the 100 mL distilled water. 2 mL unknown solution was spiked at first, then the 1, 2, 3 mL Cd standard was spiked in order. The first curve represents the blank solution. The peak current was obtained at 7.224, 14.620, 32.72, and 42.84×10^{-7} A, respectively. Here, the working equation was y = 14.386x − 11.947, R^2 = 0.9414, and standard addition methods were obtained for 0.6801 mg/L containing tissue solutions. The results, which were significant and sharp, are usable for medicinal diagnosis. The developed technique can be applied to in vivo fluid diagnosis of live organs.

Stripping voltammetry was examined for detecting Cd using FPE in seawater solution. The optimum condition was as follows: −0.035 V amplitude, 5 Hz frequency, and 60 seconds accumulation time for cathodic scan; and 0.04 V amplitude, 15 Hz frequency and 90 seconds accumulation time for anodic scan (not shown here). Under these conditions, the detection limit was obtained at 6.0 μg/L by using this developed sensor. The standard addition method was used for application in earthworm cell. The result of the application shows that it can be applied for based food systems or in vivo diagnosis. It can also be applied for medical fields that require Cd detection.

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