Diagnostic Assay of Toxic Zinc in an Ex Vivo Cell Using Voltammetry

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Voltammetric detection of the toxic Zn ion was investigated using a fluorine-doped graphite pencil electrode (FPE). It is notable from the study that pencils were used as reference and working electrodes. In all the experiments, a clean seawater electrolyte solution was used to yield good results. The analytical working range was attained to 10µgL⁻¹. The optimized voltammetric condition was examined to maximize the effect of the detection of trace Zn. The developed sensor was applied to an earthworm’s tissue cell. It was found that the methods can be applicable to in vivo fluid or agriculture soil and plant science.

Key words: Diagnosis, Toxic zinc, Ex vivo, Cell, Voltammetry

INTRODUCTION

Among in vivo cell systems, the Zn(II) ion is essential in the human body for the implementation of multiple functions in the organisms. However can be toxic harmful at in vivo high concentration (Zheng and Hu, 2007). Zn is also related to the Wilson’s disease, Alzheimer’s, Blackfoot and cutaneous diseases (Santon et al., 2003; Miller et al., 2006; Horng and Lin, 1997; Fabris et al., 2006). Here in vivo detections are demand for there control and diagnostics. Recently many detection methods have been adopted such as Chelating Fluorescent Protein Chimeras, high-performance liquid chromatography, online ICP MS detection, thermospray flame furnace atomic, low-level g-ray spectrometry, two-column ion exchange, slurry sampling electrothermal vaporization, atomic absorption spectrometric, isotope dilution thermal ionization, FAAS high performance nebulizer, flow injection diode array, ICP-AES, and X-ray fluorescence spectrometry (Evers et al., 2007; Capitan-Vallvey et al., 2002; Zheng and Hu, 2007; Nascentes et al., 2004; Kohler et al., 2000; Pohi and Prusisz, 2007; Lu and Jiang, 2001; Zaporozhets et al., 1999; Ayoub et al., 2002; Zareba et al., 2005; Azubel et al., 1999; Bianchi et al., 2007; Marcó et al., 2001). Some these methods are complicated, expensive, and unusable for in vivo diagnosis, but simple and inexpensive voltammetric methods have been developed for these purpose such as film electrodes, bismuth poly film electrodes, hanging mercury drop electrodes and stationary mercury electrodes (Kefala et al., 2003; Wu et al., 2008; Nedeltcheva et al., 2005; Locatelli and Torsi, 2000). There are still toxic and complicated modification techniques. However in this study, a non-toxic and sensitive fluorine modification method was used to make a working electrode. It was also low-cost as it used pencils for the auxiliary, reference and working electrodes. Moreover, clean seawater was used for the electrolyte solution to yield good results in all the experiments. This developed prove was applied to an earthworm’s cell and good results were obtained.

MATERIALS AND METHODS

Systems, reagents, electrode preparation, and procedure. The voltammetric systems were carried out using the Bioelectronics-2 circuit, which was constructed by the authors’ institution. Its second version was fabricated to a computerized handheld voltammetric system with a 2.4 V potential window, a pico A measuring current, a rechargeable battery or an external power supply, and a USB interface with a PC. The instrument’s size was that of a typical cellular phone.. The FPE fluorine coating was performed using conc-HF solution, a 10-cycle scan with a 1.0 V initial potential, a 1.0 V switching potential, and a 0.5Vs⁻¹ scan rate. Other two pencils each served as the reference and auxiliary electrodes. The supporting electrolyte was prepared with clean seawater. All the other reagents were of analytical grade. The voltammetry was carried out at an open circuit.. The initial analytical SW conditions were used with a 0.06 V amplitude, 25 Hz frequency, 170-sec deposition time,
and −1.3 V initial potential. All the experiments were performed at room temperature and without oxygen removal. Under these conditions, cyclic peak potentials were examined.

RESULTS AND DISCUSSION

First, a high concentration of Zn was investigated with cyclic voltammetry using FPE in clean seawater electrolyte. Pencils served as auxiliary and reference electrodes. Fig. 1(A) shows the CV results that ranged from 1 to 10 mg/l variations. The peak current reached 1.2427×10⁻⁵ A in a 1 mg/l spike via an oxidation scan for the 0.3 V peak potential. It increased quickly to 34.15×10⁻⁵ A, then dropped slightly at 5 mg/l before it increased again and approached

Fig. 1. (A) Cyclic voltammetry for the concentration effects of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/l of Zn(II) in seawater electrolyte, -2.0V initial potential, 1.2V switching potential, and 0.5 mV/sec scan rate. (B) Examination using anodic SW range for 1, 2, 3, 4, 5, 6, 7, and 8 mg/l of Zn(II) with optimum conditions.

Fig. 2. (A) The SW amplitude variations for 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, and 0.04 V. (B) The SW frequency variations of 5, 10, 15, 20, 25, 30, 35, and 40 Hz. (C) The SW accumulation time variations of 0, 30, 60, 90, 120, 150, 180, 210, and 240 sec for the 80 mg/l Zn(II) ion spike in the clean seawater electrolyte.
8.28×10^{−6} \text{ A}. The reduction peak was not reached, though. Under these conditions, the anodic and cathodic SW conditions were examined. Fig. 1(B) shows the cathodic results in the high concentration. The anodic SW results were not obtained. The cathodic values of the 1, 2, 3, 4, 5, 6, 7, and 8 mg/l of Zn(II) were spiked. The bottom curve shows the result of the blank electrolyte and the simple. At 1 mg/l, it started to have a small peak, and the peak increased linearly. It became sharp, and the peak currents increased from 1.079×10^{−6} \text{ A} to 13.93×10^{−6} \text{ A}, the linear equation was y = 4.2946x − 7.6953, and the statistic relative standard deviation was R^2 = 0.9936. These results can be used for SW optimization.

**SW optimizations of FPE.** The optimum SW conditions were examined to obtain sensitive Zn detection results. First, the SW amplitude variations were studied by maintaining the 80 mg/l Zn spike in the 10ml seawater electrolyte. Fig. 2(A) shows the results that ranged from 0.005 V to 0.04 V amplitude. They increased from 2.109 to 71.680×10^{−6} \text{ A} without a decline, due to which 0.04 V amplitude was chosen as the optimum condition. Under this condition, Fig. 2(B) shows the variations of the SW frequency of 5, 10, 15, 20, 25, 30, 35, and 40 Hz. The width of the curve became narrow, and the peak current increased from 9.594 to 25.080×10^{−6} \text{ A}. The maximum current was attained at 40 Hz. Under there conditions, Fig. 2(C) shows the SW accumulation time variation. At 30 sec, the peak current was 1.013×10^{−7} \text{ A}, and it increased to 1.236×10^{−5} \text{ A} at a 60-sec accumulation time. After that, no current bigger than 1.236×10^{−5} \text{ A} was obtained. Therefore, 60 sec was chosen as the optimum condition. Under this condition, the analytical working range and application were performed using the seawater electrolyte.

**Analytical working range and statistics.** Under the optimum SW conditions, the analytical working range was examined using cathodic. Fig. 3(A) shows the chronoamperometric results according to the time variations spiking. Every 30 seconds, the 100-mg/l concentration was added. The linear curve increased from 100 to 700 mg/l, and the linear equation was y = 0.0959x − 5.1363, with R^2 = 0.9859. The peak currents can be used for milli ranges. Under these conditions, Fig. 3(B) shows the SW milli ranges of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg/l. The peak current was attained at 5.846×10^{−6} \text{ A}, and the linear equation was y = 24.512x + 2.6231, with R^2 = 0.976. can be used for low ranges, but Fig. 3(C) shows the results of more sensitive micro ranges. The concentrations from 10 \mu g/l to 80 \mu g/l were spiked in order. The small peak of 3.162×10^{−7} \text{ A} was attained at 10 \mu g/l. The bigger currents started to be obtained, and reached 7.513×10^{−7} \text{ A}. These results can be used for trace detection. Moreover, seawater electrolyte can help yield good results. Under these condi-
tions, the analytical detection limit was calculated as 10 µg/l. These results can be used for the biological *in vivo* and *vitro* diagnostics.

**In vivo applications.** The diagnostic application was performed in the earthworm’s cell using contaminated soil. Fig. 4(A) shows the results of the standard addition method in the range of microgram additions. The first curve represents the blank solution, in which no signal was obtained. Then the 0.1 ml cell solution (Here the 1.0 g earthworm cell was dissolved in a 1.0 ml nitrate concentrate and diluted with 100 ml distilled water) was spiked and obtained a 9.114×10⁻⁶ A peak current within a 90-sec accumulation time, which is zinc’s peak potential. Thus, the 0.05 ml Zn standard was added with three points, and the peak currents linearly increased from 12.2Xe⁻⁶ to 16.44Xe⁻⁶ A, and y = 2.4468x + 4.5472 and R² = 0.9853. Thus, 1.86-µg/ml Zn(II) was obtained. The results can be used for medicinal diagnosis, specifically for *in vivo* fluid diagnosis of live organs. Fig. 4(B) shows the results of the CV voltammograms. The same peak potential and the same results were obtained.

The novel fluorine-doped FPE sensor described in this study is for the diagnosis of trace Zn ions in *in vivo* fluids. The optimized conditions that were examined in this study consisted of a 0.04 V amplitude, a 25 Hz frequency, and a 60-sec accumulation time. Under these conditions, the developed sensor approached the detection limit of 10 µg/l⁻¹. The application in the earthworm’s cell was performed using a standard addition method. The results of the application showed that the developed method can be used for any *in vivo* or *in vitro* diagnostic application. It can also be applied in other fields that require medicinal diagnosis of humans.

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**Fig. 4.** (A) Detection of Zn(II) in an earthworm’s cell using standard addition methods by SW and (B) CV with optimum conditions. The first curve represents the electrolyte blank. The second curve represents the result of the solution in which the earthworm’s cell was dissolved. After that, the 0.05 µg/l Zn standard spiked three times based on optimum conditions.
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