Diagnosis of Trace Toxic Uranium Ions in Organic Liver Cell

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(Received March 28, 2014; Revised June 17, 2014; Accepted June 30, 2014)

Uranium is toxic and radioactive traces of it can be found in natural water and soils. High concentrations of it in biological systems cause genetic disorders and diseases. For the in vivo diagnosis, micro and nano range detection limits are required. Here, an electrochemical assay for trace toxic uranium was searched using stripping voltammetry. Renewable and simplified graphite pencils electrode (PE) was used in a three-electrode cell system. Seawater was used instead of an electrolyte solution. This setup can yield good results and the detection limit was attained to be at 10 µg L⁻¹. The developed skill can be applied to organic liver cell.

Key words: Uranium, Voltammetry, Fish’s liver, Fluorine

INTRODUCTION

Uranium presence in nature is widespread, as it is found in minute quantities in all rocks, sand, soil (1) and in animal internal organs (2). As it is radioactive and toxic (3,4), its presence in the environment may affect human health and cause medical disorders such as chemical kidney toxicity, functional and histological damages (5), cancer (6) and sterility (7) and can even affect human body systems. There is therefore a need for a sensitive, accurate and reproducible method that can be used on real samples in the biological, environmental and geological fields (8). For detection purposes, several methods have been carried out, such as kinetic phosphorescence analysis (9), multi-group analysis (10), inductively coupled plasma mass spectrometry (11), extraction chromatography (12), alpha particle spectrometry (13), absorption spectrum analysis (14-16), discharge mass spectrometry (17-19) and electrochemical polarographic (20) mercury (21,22) dropping (23,24) electrode. Herein photometric or mass spectrometric methods are demand of spectric light separation, electric acceleration and high energy pumping techniques are required. Moreover complicated electric mercury electrode is toxic in most voltammetric studies and not usable for in vivo or in vitro low concentration ranges. In this study, a simple and reasonable fluorine-doped pencil sensor was used as a working electrode. The said sensor does not need much preparation time and is nontoxic. Moreover, a reference and an auxiliary electrode were used with graphite pencils unlike other voltammetric methods. Also, deep seawater was used as the electrolyte solution. With the use of pencil electrodes and clean sea water solution, the experiment’s expense was kept at a minimum. Reasonable techniques were applied to biological organic cell systems for medical science.

MATERIALS AND METHODS

The experiment was carried out using Bioelectronics-2 circuit, which was constructed by the authors’ institution. The second version of this device was fabricated as a computerized handheld voltammetric system whose size is similar to that of a typical cellular phone. It can be used for bio assay and sensor techniques for individual and laboratory applications. The fluorine-doped graphite electrode (FPE) was prepared by coating the pencil with fluorine. Also, two pencils served as the Ag/AgCl reference and platinum counter-electrode, respectively. The supporting electrolyte was prepared with clean deep seawater. All the other reagents were of analytical grade. Electrolyte voltammetry was carried out on an open circuit. A uranium standard was obtained from Merck. Highly purified water was prepared through three-time distillation using 18 MΩm⁻¹ of Milli-Q Ultra-Pure Water System (Millipore, Bedford, USA). Fluorine immobilization was performed using a 10-cycle scan with a 1.0 V initial
potential, a 1.0 V switching potential, and a 0.5 V/s-1 scan rate. The SW stripping voltammogram used optimized parameters. All experiments were performed at room temperature and without oxygen removal.

RESULTS AND DISCUSSION

Cyclic voltammetry (CV) and stripping voltammetry using FPE. In this study, clean seawater was used as the electrolyte solution, and the working, reference and auxiliary electrodes were made from inexpensive graphite PE. First, cyclic voltammetry and stripping voltammetry were examined using FPE in high concentration. Fig. 1A shows the cyclic scan in the range of 0, 10, 20, 30, 40, 50, 60, 70 and 80 mgL\(^{-1}\) U variations, using \(-2.0\) V initial potential, \(-2.0\) V switching potential, 0.1 V/s scan rate. In the blank solution, the peak current was clear. By spiking U variations, it began to increase for a \(-0.2\) V peak potential. It continually increased from \(6.545 \times 10^{-5}\) to \(24.7 \times 10^{-5}\) A. The inset figure shows that the linear equation was \(y = 0.247x + \ldots\)
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Square wave (SW) stripping voltammetry. The low concentration of U was also examined using CV and SW. Fig. 2A shows the cyclic ionic activity in the range of 100, 200, 300, 400, 500, 600, 700 and 800 µg/L U concentration. When 100 µg/L U was spiked in the clean seawater, it obtained peak currents for 3.402 × 10⁻⁶ A, after which the peak current started to increase from 3.402 × 10⁻⁶ A to 7.341 × 10⁻⁵ A without a decline, with y = 0.0056x + 2.6587, statistics of R² = 0.9927. Then Fig. 2B shows the SW scan ranging from 1 to 8 mg/L U concentration. The greater the concentration of U was spiked, the more sensitive peak current was obtained. It increased from 2.557 × 10⁻⁵ A to 10.37 × 10⁻⁵ A. The equation of the SW result was y = 1.184x + 1.000 and the precision was R² = 0.986 can be useful for trace detection. Fig. 2C shows interference by other ions. Various analytical interference ions were examined by adding other metals and analog ions into the medium containing 10 µg/L of Uranium, for the tenfold spiking of 100 µg/L of Fe, Zn, Cr, Al, Ge, Cd, Pb and Cu ion. This yielded results of 100%, 455.03%, -68.28%, 23.33%, -13.06%, -100.00%, 0.00% 0.56% and 0.57%, respectively. The analytical interferences were effectively corrected using standard addition methods.

Statistics and application. Fig. 3A, showing the statistics for sea water blank, and 10 µg/L U constant was examined for 15 times. Here, standard deviation was 1.9158 (blank) and 1.3889 (10 mg/L U spike). Both are stable and sensitive. After that, more sensitive working range was examined using 200 sec accumulation time. Fig. 3B shows the SW effects in the microgram variations. The concentration of 0, 10, 20, 30, 40, 50, 60, 70 and 80 µg/L U was spiked and it increased from 4.954 × 10⁻⁶ A to 15.95 × 10⁻⁶ A. The linear equation was y = 0.166x + 3.321 and the precision was R² = 0.980. Despite micro ranges, SW using FPE was sensitive and effective in trace analysis. The analytical application was performed under such conditions for in vivo or ex vivo analysis. Fig. 3C shows the SW results of the application to fish liver. Standard addition method was used. The fish liver was dissolved in 1ml nitrate and this solution was diluted with 10ml distilled water. 1 ml solution was spiked and examined using SW with FPE. It obtained 1.717 × 10⁻⁶ A. After that, 2, 3 and 4 ml U standard was spiked, obtaining 2.945, 3.456, 3.75 × 10⁻⁶ A, individually. These results were obtained for 1.887 µg/ml U. These results show that the sensor can be used for diagnosis in the human body or in animals.
After the comparison of CV and SW, it was found that the SW was effective in detecting trace U ions in the milli or micro ranges. Under optimized conditions, the analytical detection limit was 8.0 µg/L. The developed sensor was applied to diagnostic liver assay in fish cells, and the results of the application shows that it can be used in fields that require the detection of certain toxins, in medical science and for ex vivo detection purposes at an affordable cost.

ACKNOWLEDGEMENT

This study was supported by the Research Program funded by the Seoul National University of Science and Technology.

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