



Assay of *In Vivo* Chromium with a Hollow-fiber Dialysis Sensor

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The analytical *in vivo* chromium ion was searched for using a voltammetric hollow-fiber dialysis sensor via square wave stripping voltammetry (SW), cyclic voltammetry (CV), and chronoamperometry. Under optimum parameters, the analytical results indicated linear working ranges of 50–400 mg/l CV and 10–80 µg/l SW within a 30-sec accumulation time. The analytical detection limit (S/N) was 6.0 µg/l. The developed method can be applied to *in vivo* tissues and *in ex vivo* toxicity assay, as well as to other materials that require chromium analysis.

Key words: *In vivo*, Chromium, Voltammetry, Hollow fiber, Dialysis sensor

INTRODUCTION

Industrial chromium and nickel plating heavy metals are present in waste and surface water (Kiptoo *et al.*, 2004). Their compounding steels are widely used for food processing, stainless steel, and storage (Farinas *et al.*, 2008). *In vivo* absorbed chromium is associated with skin carcinogenesis (Uddin *et al.*, 2007), DNA damage (Hill *et al.*, 2008), lung cancer (Kondo *et al.*, 2006), and primary human cancer (Martin *et al.*, 2006). In analytical science, sensitive detection methods have been developed such as atomic absorption spectrometry (Elci *et al.*, 2008), flame atomic absorption spectrometry (Kiran *et al.*, 2008; Kiptoo *et al.*, 2004), graphite furnace atomic absorption spectrophotometry (Chen *et al.*, 2008), and laser-induced breakdown spectroscopy (Bousquet *et al.*, 2007). However photometric methods depend on expensive high-atomic-absorption techniques and require spectric separation and sensitive analytical detection systems. Electrochemical methods are inexpensive and simplified voltammetric sensor systems have been developed for chromium analysis such as differential pulse anodic stripping voltammetry (Zhu *et al.*, 2007; Bobrowski *et al.*, 2004), differential pulse cathodic voltammetry (Svanara *et al.*, 2004), liquid-liquid interface techniques (O'Mahony *et al.*, 2005), and the static mercury drop electrode method (Korolczuk, 2000). Their circuits are simple and fast-

responding. They can be used, however, only in laboratory conditions and not for *in vivo* or *vitro* direct analysis. In this study, a hollow-fiber dialysis sensor [17] was used for *in vivo* diagnosis. This sensor gives a faster response, is more cost-efficient, and uses a sensitive pre-concentration technique for *ex vivo* or *in vivo* cell tissue. The developed method achieved lower detection ranges than the other modified techniques. This method can be used for biological and pharmaceutical materials, food samples, and other materials that require chromium analysis.

MATERIALS AND METHODS

Electrode preparation and reagents. The voltammetric measurements were carried out using the *Bioelectronics-2* system, which was constructed at the authors' institute. The method of making a hollow-fiber dialysis sensor (HDS) was used with three electrode systems. HDS is the cellulose hollow-fiber dialysis sampling tube (molecular weight cutoff, 13,000; length, 32 mm; membrane thickness, 20 µm) for collecting the target Cr ions. It was connected to an intravenous rubber cylinder tube (diameter, 5 × 15 mm). In this cylinder, a graphite pencil (PE: diameter, 0.5 mm; B type) reference, auxiliary and fluorine-coated PE working (HFPE) were inserted, and the cylinder sensor was connected to electrolyte flow pumping motor systems (220 V waterproof 250 ml/min circuits). They were fabricated using the following procedure:

waste solution ← four-way HDS intravenous cylinder tube (three-electrode combination system) ← electrolyte pumping motor ← 0.1 M ammonium phosphate buffer ←

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All the systems were performed in electrolyte flowing conditions via the pumping systems. The reagent solution was prepared from double-distilled water. The chromium standard and other reagents were obtained from Aldrich and diluted as needed.

RESULTS

Voltammetric peak potentials of PE and HFPE. For the reaction potentials, a 0.1 M ammonium phosphate buffer was used for the electrolyte flowing migration. Under a 250 ml/min velocity, HDS was put in the electrolyte blank solution and obtained for the base voltammogram, after which the Cr standard was spiked in the blank solution at 50–400 mg/l and 2.0 V to –2.0 V via oxidation and reduction scans. A fast reaction was continuously achieved, and the raw voltammograms are shown in Fig. 1(A), in which an anodic peak can be detected only at –0.4 V. It was recognized that the Cr ion could be detected via oxidation. After such recognition, better sensitive stripping voltammetry was performed using anodic and cathodic stripping, and no cathodic peak was obtained. Fig. 1(B) shows the anodic stripping voltammograms, with a simple electrolyte and no signals, whereas a 100-mg/l Cr spike was obtained for the –0.8 V and –0.0 V anodic peak potentials, and the peak current increased from 2.83×10^{-6} A to 29.66×10^{-6} A (0.0 V) and 3.23×10^{-6} A to 39.88×10^{-6} A (–0.8 V). These results are applicable to high-range detection. Under these parameters, the results of the chronoamperometry was compared with those of common-type PE and specialized HFPE sensors. Fig. 1(C) shows the –0.0 V constant potentials, with 25–225 mg Cr standard additions in the 0.1 M ammonium phosphate buffer solution at flowing conditions, and the exchanged working electrode. The Cr concentration also changed with the same methods. The sensitive HFPE current was better than that of PE, and the first curve is lower than that of PE, as with the initial peak width and the peak sharp. Moreover, the signals' growth definitely appeared. These results show that HFPE is suitable as the working electrode for *in vivo* ranges.

Stripping optimization for the HFPE statistics. At the 0.1 M ammonium phosphoric buffer with the 100 mgL^{-1} Cr standard, SW amplitude variations were searched for. Fig. 2(A) shows their voltammograms. Under anodic stripping, amplitude variations were examined from 0.01 V to 0.08 V, and their peak current increased from 0.39×10^{-5} A to 13.71×10^{-5} A and then decreased. The peak potential at 0.07 V was big and had a sharp width. Under this parameter, the SW frequency was determined at 5–40 Hz (Fig. 2(B)) variations. At 10 Hz, the peak current was 13.7×10^{-5} A and the other point decreased. Thus, at the 0.07 V amplitude, the 10 Hz frequency was fixed, after which the SW increment potential, the SW initial potential, and the strip-

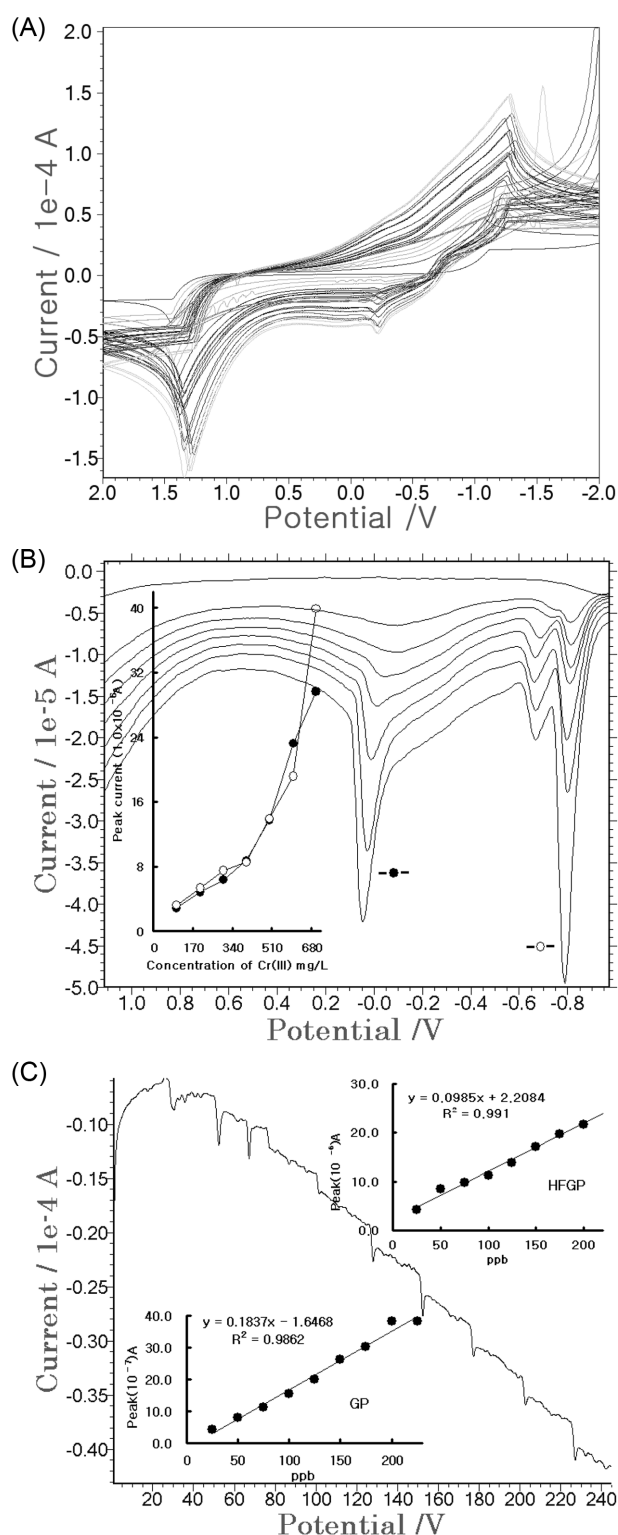


Fig. 1. (A) CV concentration effects of 50, 100, 150, 200, 250, 300, 350, and 400 mg/l of Cr(III) using PE. (B) SW concentration effects of 100, 200, 300, 400, 500, 600, and 700 mg/l of Cr(III) using PE. (C) Chronoamperometric concentration effects of 25, 50, 75, 100, 125, 150, 175, 200, and 225 mg/l of Cr(III) using PE and HFPE.

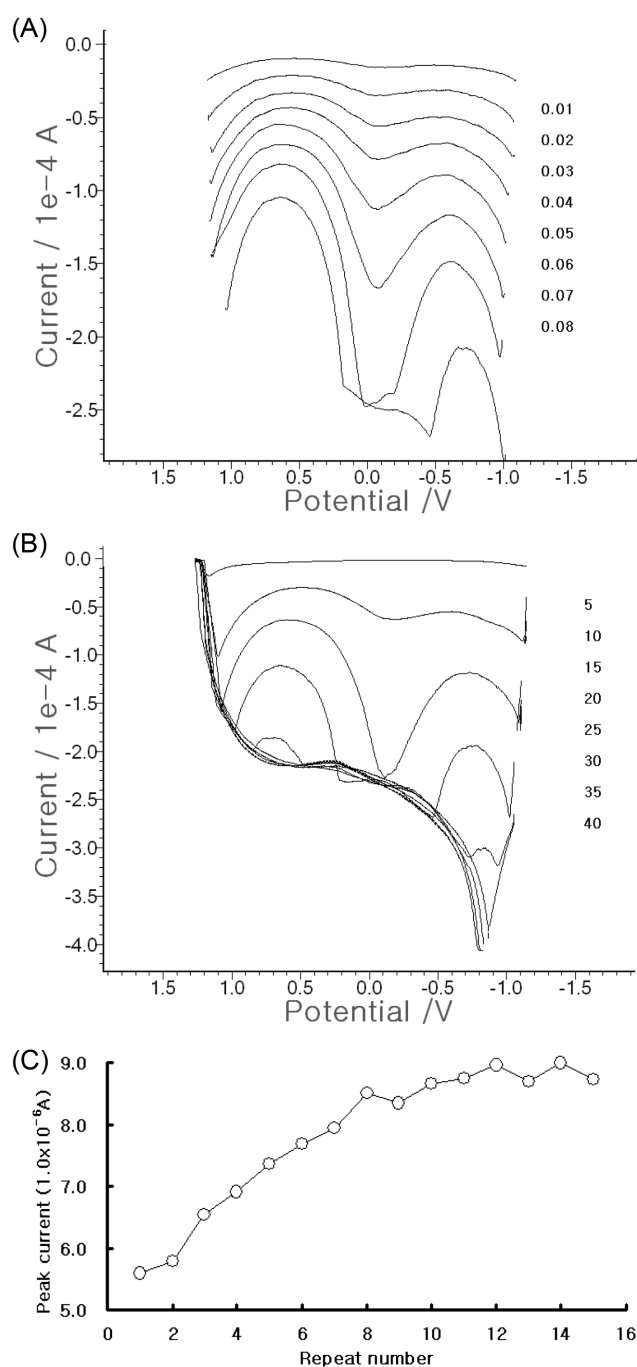


Fig. 2. (A) SW stripping amplitude variations for 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 V. (B) SW frequency variations for 5, 10, 15, 20, 25, 30, 35, and 40 Hz. (C) Statistics for the 15th repetition in the 100ppm Cr constant using optimum SW parameters.

ping accumulation times were determined. The results were obtained for the -0.8 V initial potential and the 0.004 V incremental potential, and within a 30-sec accumulation time (not shown here). Under these conditions, the sensor stability was examined using 100 mg/l of Cr for the 15th repeti-

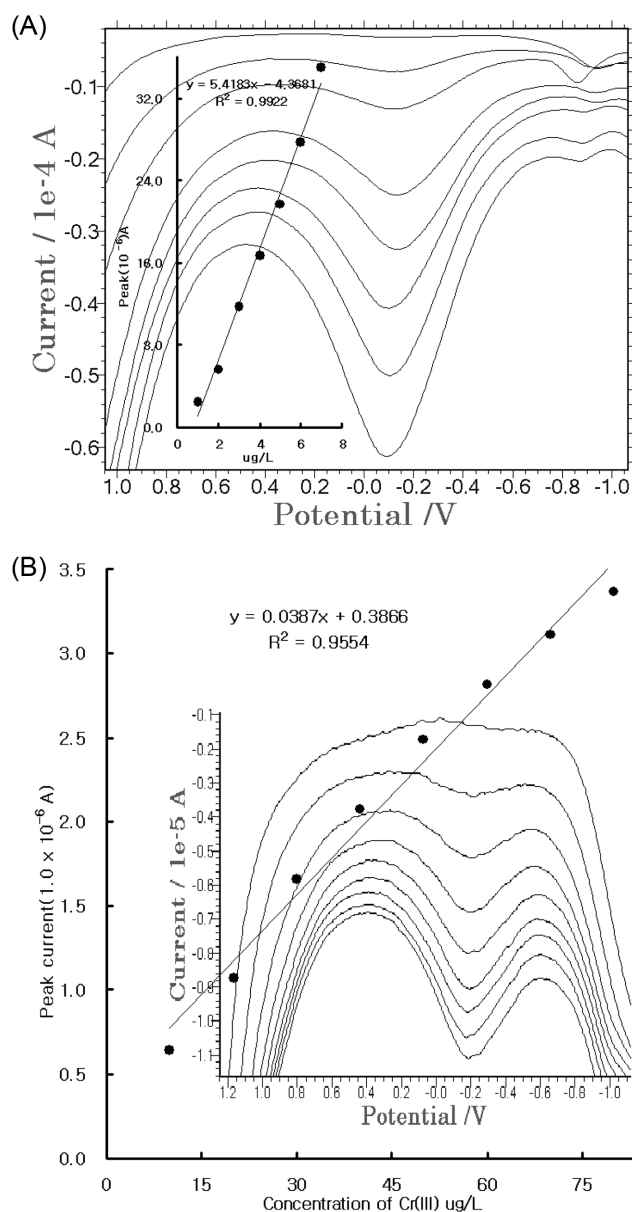


Fig. 3. (A) Concentration effects of 10, 15, 20, 25, 30, 35, and 80 $\mu\text{g/l}$ Cr spikes within a 30-sec accumulation time. (B) Diagnostic application of the diluted flog cell, with each curve an electrolyte blank, to the cell tissue with 1, 2, 3, 4, 5, 6, and 7 ml spikes using optimum parameters within a 120-sec accumulation time.

tion. Fig. 2(C) shows the results. The first six points varied from 5.60×10^{-6} A to 7.67×10^{-6} A, then stabilized from 8.51×10^{-6} A to 8.95×10^{-6} A. These results can be used for *ex vivo* or *in vivo* toxicity analysis. Thus, the SW low ranges were examined at optimum parameters.

Working range, statistics, and applications. Fig. 3(A) illustrates the Cr working curves for the micro-ranges. Herein, the electrolyte blank is simple and a 10 $\mu\text{g/l}$ spike

was obtained for the -0.2 V peak potentials with a peak current of 0.642×10^{-6} A, and increased to 3.367×10^{-6} A. The slope was sensitive, and the relative standard was stable. These micro-ranges can be used for *in vivo* or *ex vivo* applications. Then the contaminated bull frog cells were examined. A 250 g-heavy, 35 cm-big reptilia was obtained from the raising company. For the contamination in a 1,000 ml/ fishing port with a living frog, the 100 ml/ Cr standard (1,000 mg/l) was spiked for one day overnight, and ethyl ether was injected into it as an anesthesia, after which 4.34 g of a leg muscle was extracted, and the tissue was diluted in a 100 ml/ mass flask using 10 ml/ conc-HCl. This contaminated muscle solution was examined using the standard addition method. Fig. 3(B) shows the calibration curves. The first curve is that of the electrolyte blank solutions, and a diluted muscle solution for the 1~7 ml/ spike, with the linear equation $y = 5.41x - 4.36$ at $R^2 = 0.9922$, was obtained. These results can be qualified for *in vivo* adsorbed chromium ions.

DISCUSSION

Trace chromium ion was detected using a hollow-fiber dialysis sensor and cylindrical combination electrode systems. The voltammetric response was found at the linear working range of 10~80 $\mu\text{g/l}$ within a 30-sec accumulation time. The chromium activity peaked at a pH of 4.92, an amplitude of 0.07 V, a frequency of 10 Hz, an initial potential of -0.9 V, and an incremental potential of 0.004 V. Since the results of the developed method had a lower detection limit of 6.0 $\mu\text{g/l}$, it could be applied to the *ex vivo* toxicity assay of cell tissues and in other fields that require diagnostic *in vivo* or *ex vivo* analysis.

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