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Toxicological Investigation of Radioactive Uranium in Seawater

Suw Young Ly, Jeong Mi Bae and Jin Kim

Biosensor Research Institute, Seoul National University of Science and Technology, Seoul 139-743, Korea

Trace uranium detection measurement was performed using DNA immobilized on a graphite pencil electrode (DGE). The developed probe was connected to the portable handheld voltammetric systems used for seawater analysis. The sensitive voltammogram was obtained within only 30 s accumulation time, and the anodic stripping working range was attained at 100~800 µg/*l* U and 10~50 µg/*l*. The statistic relative standard deviation of 30.0 mg/*l* with the 15th stripping was 0.2115. Here, toxicological and analytical application was performed in the seawater survey in a contaminated power plant controlling water. The results were found to be applicable for real-time toxicological assay for trace control.

Key words: Trace, Uranium, Voltammetry, DNA immobilization

INTRODUCTION

Trace radioactive uranium (U) is commonly found (Salama *et al.*, 2003) in the groundwater of numerous nuclear energy sites (Lin *et al.*, 2005). Several reports indicate that 1.31~2.24 µg/*l* of it is present in the coasts (Djogic *et al.*, 2001). Recently, Yomiuri Online News (2011.8.7) reported the meltdown of Fukushima Nuclear Power Plant. Moreover, according to the Japanese writer Haruki Murakami, Hades (Murakami, 2011) wielded his power and caused the recent earthquake and tsunami incidents in Japan, which eventually caused the nuclear power plant meltdown, where dispersive radioactivity is connected to the horrible risks of human organic cancer (Storm *et al.*, 2006), lung cancer (Saccomanno *et al.,* 1988), kidney damage (Lestaevel *et al.*, 2005), and DNA mutagenicity. Here, for *in-vivo* or *in-vitro* diagnosis, very low analytical detection limits are demanded within nano ranges. Specific analytical methods have been sought of late for analogy purposes, such as electron impact ionization quadrupole mass spectrometry (Kahr *et al.*, 2001), inductively coupled plasma mass spectrometry (Kahr *et al.*, 2001; Pretty *et al.*, 1998), laser ablation inductively coupled plasma mass spectrometry (Sela *et al.*, 2007), and quadrupole ICP MS (Zheng and Yamada, 2006) high-resolution scanning X-ray fluorescence (Goldberg *et al.*, 2005). Some of these techniques are used for trace isotopes and radioactivity detection, but they require ion exchange, separation,

evaporation, and voltage excitation. Moreover, other detection systems are very expensive and complicated. In contrast, the voltammetric methods are simple (Ly and Kim, 2009) and fast (Ly *et al.*, 2011). Thus, more specifications of such methods have been sought for the aforementioned purpose, such as bismuth film electrode (Lin *et al.*, 2005), graphite pencil electrode (Ly *et al.*, 2010), and hanging mercury drop electrode (Cha *et al.*, 2000). The detection limits of such methods are high, however, and the mercury sensor is environmentally toxic. In this study, more sensitive DNA immobilized (Ly *et al.*, 2009) on a graphite working (Ly *et al.*, 2011) probe was sought for uranium detection. DNA on a carbon-immobilized bond has hybridization efficiency (Ly *et al.*, 2008), and its catalytic structure is capable of specific interaction (Ly *et al.*, 2009). For this reason, the developed method is sufficiently simple and more compact, with a fast stripping time of only 30 s. These advantages can enable the developed method to attain a lower detection limit compared to the existing methods applied to seawater assay for energy sites. The developed method can be used for monitoring in any nuclear site, or for *in-vivo* or *in-vitro* diagnosis.

MATERIALS AND METHODS

The instrumental system that was used, Bioelectronics-2 from these authors' version of circuitry, was computerized, and like a cellular phone, it had a ± 3.0 V potential range, a 2 mA current range, and a 10 pA detection current. It can also be used for bioassays and microorganism detection. The U standard 1,000 mg/*l* solution was obtained from Merck, (insert country). The other reagents that were used

Correspondence to: Suw Young Ly, Biosensor Research Institute, Seoul National University of Science and Technology, 172, gongreung 2-dong Nowon-gu Seoul 139-743, Korea
E-mail: suwyoung@snut.ac.kr

were of standard grade. An electrolyte was prepared through three-time distillation using 18 Mcm^{-1} graphite pencil electrode (GE) prepared from a common pencil (DongA XQ, ceramic, 60 mm, 0.9 B). The DNA-immobilized working electrode (DGE) was prepared using 20 repetitions of + -2.0 V cyclic scan in a conc-DNA solution that was used as the working electrode. Two other graphite pencils served as the reference and auxiliary electrodes (Ag/AgCl(s)/KCl(3M) reference electrode). All the experiments were performed at room temperature, and electrolyte was used for the seawater without removing the oxygen.

RESULTS

Under cyclic conditions, the probe sensitivity was examined using -2.0 V initial potential, 2.0 V switching potential, and 0.5 mV/s scan rate. Under these conditions, the concentration effects were examined, and the results are
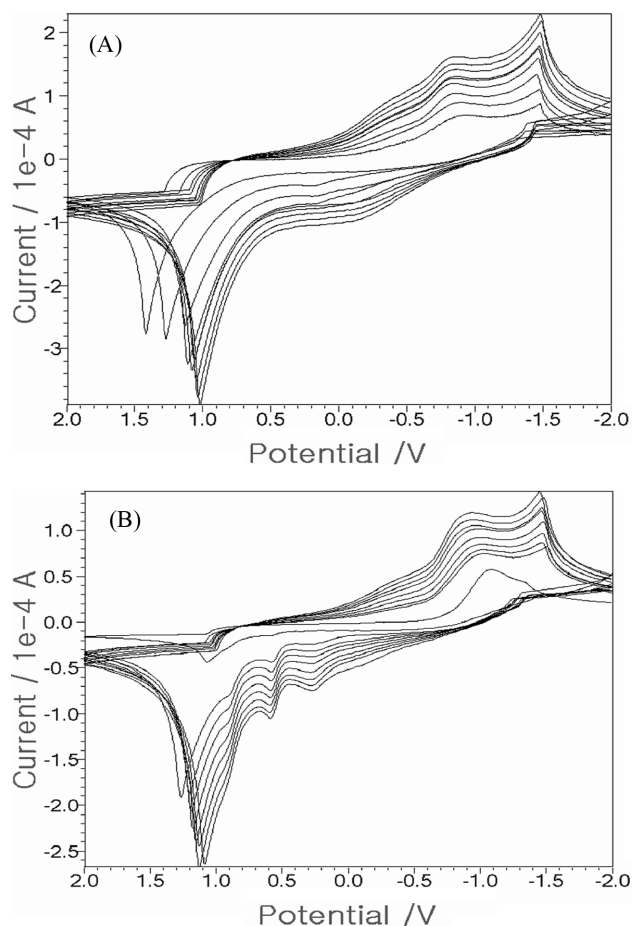

Fig. 1. Modified sensor properties of the 0.0, 10, 20, 30, 40, 50, 60, 70, and 80 mg/*l* U spikes on the common-type GE (A) and modified DGE (B) electrodes using a 0.1 M-NH$_4$H$_2$PO$_4$ electrolyte solution, at optimum conditions of -2.0 V initial potential, 2.0 V switching potential, and 0.5 mV/s scan rate, starting from the anodic direction.

shown in Fig. 1(A) and (B). In the voltammetric assay, the ionic activity depended on the electrode properties, reaction potential, and working ranges. Thus, the common-type GE and the modified DGE probe were compared using high ranges for 10~80 mg/*l* U spiking, and the 2.0 to -2.0 V potentials were used for reduction scan at a 0.5 mV/s scan rate. The voltammogram is shown in Fig. 1(A), under anodic scan for GE. The voltammogram was simple, and at 0.2 V, a small peak appeared, then a -1.0 V reduction peak was obtained, where the working slope was simple and not sensitive. Thus, the modified DGE was compared to real voltammograms, as shown in Fig. 1(B). Under anodic scan, two peaks for the 0.2 and 0.4 V oxidation activities were obtained, where the maximum peak currents were 10.54×10^{-6} and 13.50×10^{-6} A, and where one of the working curves was $y = 0.1506x + 2.1145$ and the statistic relative standard deviation was $R^2 = 0.9903$. The developed method is thus applicable for stripping voltammetry, which is more sensitive than cyclic activity. The modified DGE was thus applied to high ranges for anodic and cathodic stripping voltammetry under a 0.1 M-NH$_4$H$_2$PO$_4$ electrolyte solution, and the optimum parameters were sought.

Stripping activity and statistic probe stability. Under oxidation and reduction stripping, the reaction potentials were examined using the same electrolyte. The real voltammograms are shown in Fig. 2(A) and (B). Fig. 2(A) shows oxidation for the DGE properties, and the anodic working ranges were varied from 0.0 to 8.0 mg/*l* add. Here, the oxidation peaks of -0.4, 0.4, and 0.8 V were obtained at three points, among which 0.4 V was found to be sensitive and the working conditions sharp. Such potential was thus used for stripping voltammetric optimization, where the peak current was varied from 0.12×10^{-5} to 1.5×10^{-5} A. Under these conditions, cathodic stripping was performed to the same ionic strength, with 0.0~8.0 mg/*l* U spikes. The real voltammograms are shown in Fig. 2(B). Moreover, two peaks for the 0.5 V weak and -1.0 V big activities appeared. Here, the -1.0 V peak current increased to 1.0~5.9×10^{-6} A, whose working curve was found to be $y = 0.062x + 0.713$, and whose statistic was $R^2 = 0.752$. The probe stability, however, depended on the working repetition and signal oscillations. Thus, the DGE probe was examined for the statistic voltammograms with 15th stripping, with 3.0 mg/*l* U constant and 30 s accumulation time as optimum parameters. The results are shown in Fig. 2(C), where the first point is electrolyte blank, after which the 3.0 mg/*l* U spike was increased to 7.81×10^{-4} A then oscillated to 8.09~8.67×10^{-4} A. Here, the statistic standard deviation was 0.2115, indicating that the probe is sensitive and stable enough for analytical application. The stripping sensitivity, however, depends on the voltammetric redox direction, accumulation time, and other parameters, which were set using 3.0 mg/*l* U add (results not shown here). The points were varied by eight
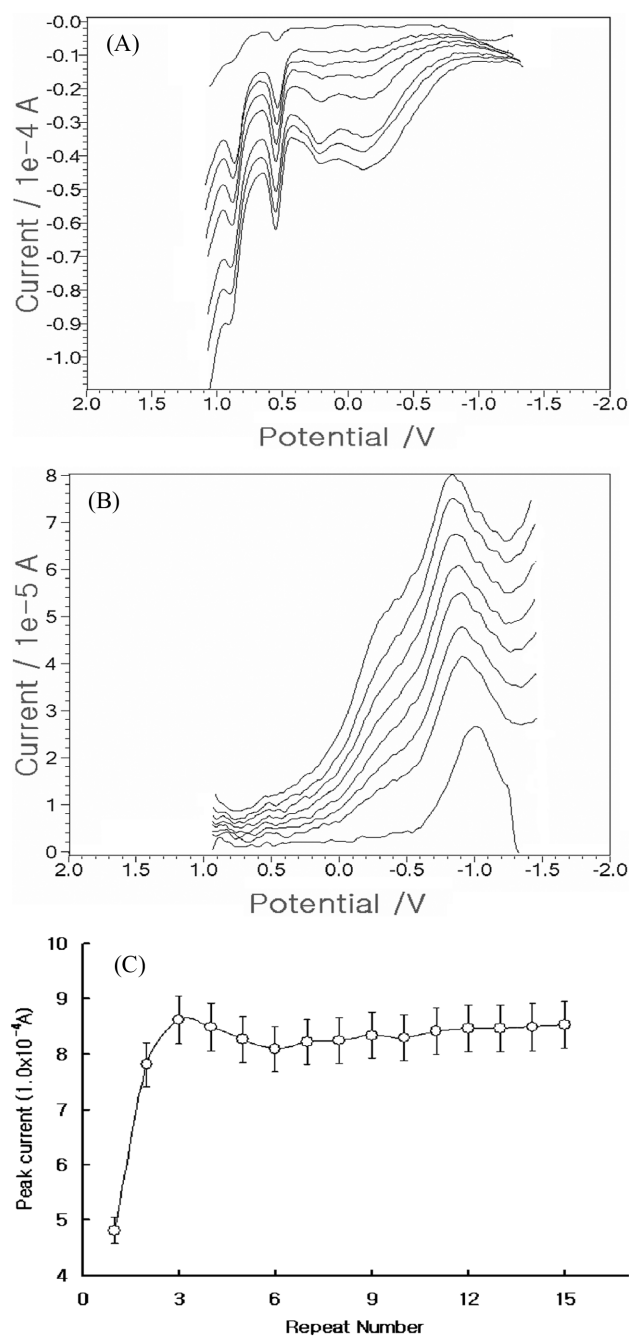
Fig. 2. (A) SW stripping anodic scan for the -2.0 V accumulation potential and 2.0 V switching potential. (B) Cathodic scan for the 2.0 V accumulation potential, -2.0 V switching potential, and ionic-strength variation for 10, 20, 30, 40, 50, 60, 70, and 80 mg/*l* add using DGE. (C) Statistics for the 3.0 mg/*l* U constant through 15th repeated stripping, with 30 s accumulation time, using the same conditions as in (A).

Fig. 3. (A) Stripping working ranges for 0, 100, 200, 300, 400, 500, 600, 700, and 800 μg/*l* U anodic spiking. (B) Micro ranges of 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 μg/*l* U ion add. In a 0.1 M-NH$_4$H$_2$PO$_4$ electrolyte strength with a pH of 4.0 and 0.35 V SW square wave amplitude, 40 Hz square wave frequency, 1 mV increment potential, -2.0 V accumulation potential, and 240 s accumulation time.

points per parameter, and the maximum peak currents were recorded. Then the analytically possible interference ions were calibrated using the standard addition method. Under these conditions, the analytical working ranges and statistic applications were examined.
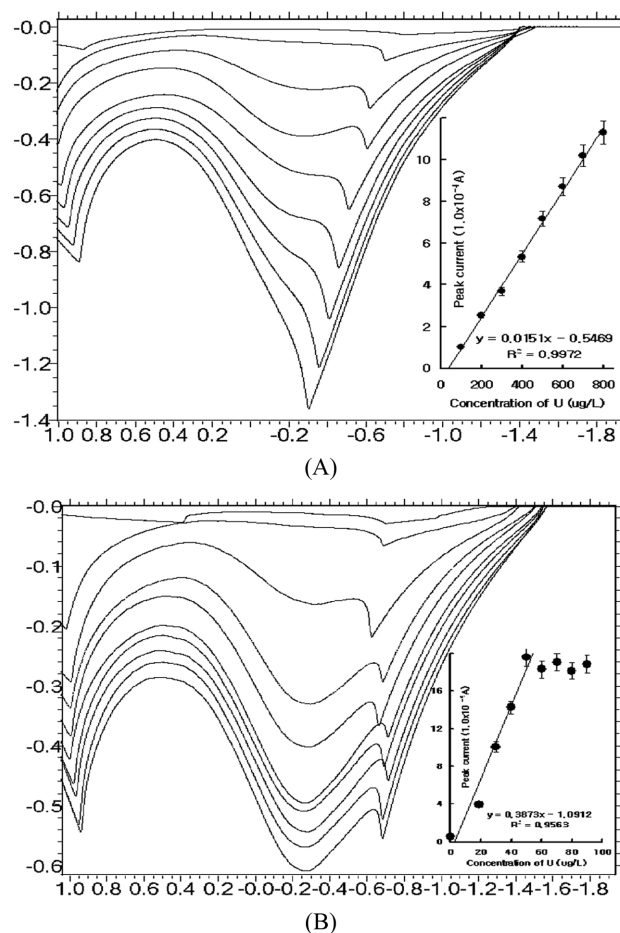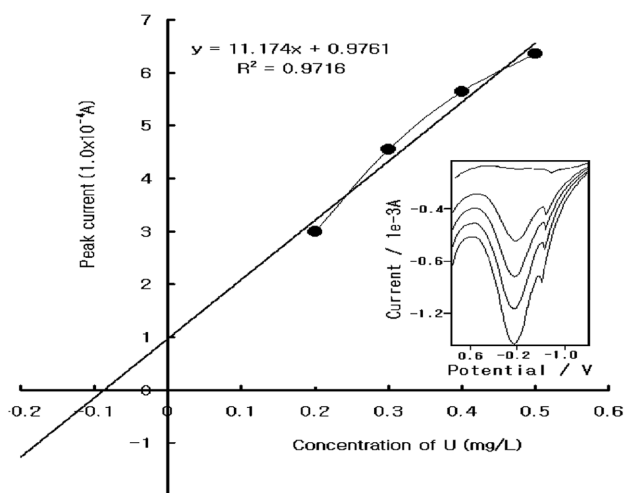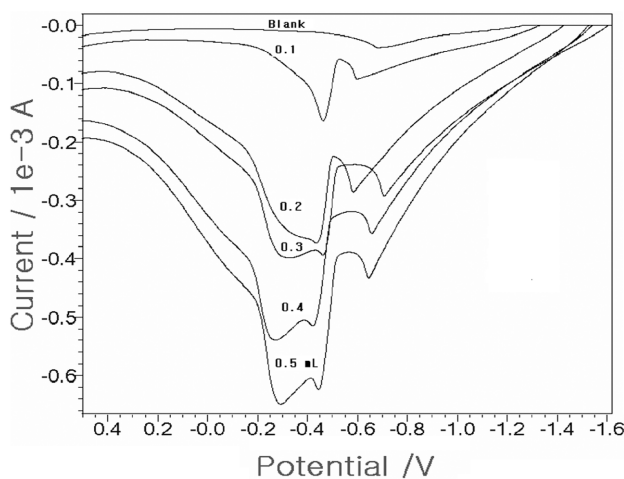
Analytical working ranges and statistics. Under the optimum conditions, the mili and micro working ranges were examined. Fig. 3 shows the real anodic-stripping voltammograms at 0.35 V amplitude, 40 Hz frequency, 0.001 V increment potential, and -2.0 V stripping potential. Under optimum conditions, the analytical linear working ranges were examined. Fig. 3(A) shows the micro range of 100.0~800.0 μg/*l* U spike by anodic stripping under 0.1 M electrolyte strength, using the optimum parameters. Here, the working slope was Δx/Δy = 0.015, the intercept was -0.55, and the relative standard deviation was R^2 = 0.9972. The anodic peak potential appeared at -0.3 V, and the first peak appeared for 2.782×10^{-4} A then increased from 3.05 to 5.68×10^{-4} A, where the error percentage was shown to be

in a linear curve. The developed method can thus be used for contaminated power plants or for cooling seawater, for reviving agricultural lands, and for preventing food contamination. More sensitive low ranges were sought, however, for micro working. Fig. 3(B) shows that for the 10~90 μg/*l* U add of 10 points, the first curve was electrolyte blank, where the voltammogram did not have any noise. Then 10 μg/*l* U spiking appeared with a small peak of -0.7 V, and the current was only 3.952×10^{-5} A. Then two peaks appeared for 10.0×10^{-5} A and increased to 19.57×10^{-5} A, where the working curve was y = 0.3873x - 1.0912, the relative standard deviation was $R^2 = 0.9563$, and the error per-

centage was shown to be within the curve. Moreover, another 5 points were linearly oscillated. These results can be used for low-range applications such as *in-vivo* or *in-vitro* detection. Within these ranges, the statistic detection limits were examined using KSb/m (k = 3, n = 15, m = (x/y)), and reached 7.0 μg/*l* U, showing that the developed method is much more sensitive than the other common analytical methods. The results of this study are highly reproducible and sensitive, making them suitable for use in any assay.

The analytical applications in the standard solution and the seawater assay were examined. Under optimized conditions, the standard addition method was used to examine the known solution. Fig. 4(A) shows the real voltammograms, with the first curve being electrolyte blank and with 20 μg/*l* U constant spike. Here, a 1.099×10^{-4} A peak current was obtained, then three points were spiked to 30~50 μg/*l* U standard. The peak current increased linearly from 4.55 to 6.35×10^{-4} A, and the working slope was 11.17, the intercept was 0.976, and the statistic was precise. As these results can be applied to any sample, the unknown seawater was examined. Fig. 4B shows the same method, where the first peak that was obtained was 1.099×10^{-4} A followed by $1.60~3.14 \times 10^{-4}$ A, the linear equation was y = 5.168x - 0.554, $R^2 = 0.9932$, and the content was 1.72 μg/*l* U. The developed method can thus be applied to determine the possibility of their use in a radioactive or any nuclear site in a real-time interface.

(A)

(B)

Fig. 4. (A) Analytical application by the standard addition method using the known solution, where the first point was 20 μg/*l* U 10 m*l* standard, then 30, 40, and 50 μg/*l* U standard spikes, using the same parameters as in Fig. 3(A). (B) Unknown-seawater analysis and standard spike, where the first curve was 10-m*l* electrolyte blank and the seawater sample was 1 m*l*, with 20, 30, 40, and 50 μg/*l* U spikes, under optimum conditions.

DISCUSSION

Radioactive uranium detection was performed using the novel DNA immobilized on a graphite DGE probe with a handheld system. The optimum parameters were 0.35 V amplitude, 40 Hz frequency, 0.001 V increment potential, and -2.0 V stripping potential, and only 30 s accumulation stripping time was used. Under these conditions, 100~800 μg/*l* and 10.0~50.0 μg/*l* U working ranges were attained. Moreover, simple and compact systems can attain lower detection limits of 7.0 μg/*l* (signal/noise = 3). The study results were shown to be more sensitive than the spectric and electrochemical assays. Here, the parameters were successfully applied to the toxicological analysis of the micro-range seawater samples. The developed method can also be applied to raw power plant sites and in other toxicology fields that require interfacial diagnosis.

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