J. of Korean Oil Chemists' Soc.,
Vol. 29, No. 2. June, 2012. 302~310

Biological Assay of Mercury and Cadmium Ions Using DNA Immobilized on a Nanotube Paste Electrodes

Suw-Young Ly† · Chang-Hyun Lee* · Hong-Rak Jung** · Kwang-Ho Park**
Yong-Keun Park** · Hong-Woo Suk**

†*Department of fine Chemistry, Seoul National University of science & Technology, 172, gongreung 2-dong Nowon-gu, Seoul, 139-743, Korea .*
**Division of General Education, Pyeongtaek University, Gyeonggi-do 405-701, Korea*
***Advanced Scientific Experiment Group in Daejin High School, Seoul, Korea*
(Received June 5, 2012 ; Revised June 18, 2012 ; Accepted June 20, 2012)

Abstract : Bio assay of mercury and cadmium ions were searched using voltammetric analysis using DNA doped carbon nanotube paste electrodes (DCP). The square-wave stripping voltammetryic optimized results indicated working ranges of 1-10.0 ngL^{-1} and 20-100 ugL^{-1},Hg(II) Cd(II) within an accumulation time of 120 seconds, in 0.1-M phosphate buffer solutions of pH 6.3. The relative standard deviations of 5 ngL^{-1} Hg(II) and Cd(II) that observed were 0.14 and 0.22% (n=12), respectively, using optimum conditions. The low detection limit (S/N) was pegged at 0.1 ngL^{-1} (4.9×10^{-11}M) Hg(II) and 0.2 ngL^{-1} (1.77×10^{-10}M) Cd(II). The developed methods can be applied to assays in biological fish kidneys and water samples.

Keywords : *DNA-graphite nano tube paste electrode; Hg(II), Cd(II); voltammetry; fish kidney.*

1. Introduction

Mercury and cadmium ions are important trace metals in biologically caused lung cancer and food or environmental toxicology [1] and their assay is particularly important in trace analysis, medical treatment, and various other fields.[2] Thus, sensitive detection methods are required in analytical science such as common type of the HS-SPME-GC-MS detection method in rivers at Asturias [3], online coupling of the high-performance liquid chromatography (HPLC) separation method [4] and the inductively coupled plasma mass spectrometry (ICP-MS) method to fish tissue analysis [5]. All these methods arrived at very low detection limits, with expensive, time-consuming, and complicated fabrication systems. Recently, better simplified sensitive analytical systems are demand. Electrochemical stripping voltammetry, for example, is very sensitive and simple[6]. In trace assays, various sensitive methods have been developed for this purpose example, a rotating gold disk electrode is being used for mercury measurements in sea water, with a low detection limit of 5 ngL^{-1} Hg(II)after a deposition time of 10 minutes[7]. Here is a very long accumulation time. Square-wave anodic stripping voltammetry with low

†Corresponding *author*
(E-mail : suwyoung@snut.ac.kr)

temperature co-fired ceramics (LTCC) technology arrived at a detection limit of 0.9 ugL^{-1} Hg(II) and 0.45-ugL^{-1} Cu(II)[10], in which only Hg(II) and Cu(II) were detected with high values. Moreover, these methods involve complicated pre-treatment techniques and have a long accumulation time, and most cadmium detection methods use hanging mercury electrodes[8], which is not advisable since mercury is toxic. In addition, other more sensitive working electrodes appeared in the multi-wall carbon nano tube method {6×10^{-9} M Cd(II),4×10^{-9} M Pb(II)}[9], the bismuth film method {0.2 ugL^{-1} Cd(II) and 0.8 ugL^{-1} Pb(II), with along 300 second accumulation time} [10], the carbon paste electrode method {9.80×10^{-9}MCd(II)} and the glassy carbon film electrode method {0.23 ug/mL Hg(II)}[11]. Some of these methods had long accumulation times and arrived at poor detection limits. In this study, however, voltammetric sensitive methods were developed with a simpler DCP. The DNA and CNT properties are useful in the analytical field [12]. The CNT can serve as a metal semiconductor, and can perform electron transfer with biomolecules, which consist of a large cylindrical surface area. Thus, a number of investigations of CNTs for catalyst support and electronic equipment have been conducted. In this study, the CNT was used for electrical support. Moreover, since DNA has a specific affinity with biosensors[13], drug pollutants, and any other substance that selectively interacts with DNA probes. various papers have recently been written on the DNA immobilized electrode. In this study, the DNA and CNT mixed paste was prepared and the DCP was searched to the trace detections of Hg(II) and Cd(II). The working assay achieved lower detection ranges than those in the other papers, did not use toxic mercury electrodes, had a very short accumulation time of only 120 seconds, and easily detected the two elements. It can thus be used to detect biological and other materials requiring Hg(II) and Cd(II) analysis.

2. Experimental Method

2.1. Apparatus, Reagents, and Working Electrode Preparation

Experimental measurements were carried out using a CHI660A (from Voltammetry Systems, Inc.). A three-electrode cell was used to monitor the voltammetric signal. DCP was used as the working electrode, with saturated Ag/AgCl/KCl as the control. Platinum wire was used as the auxiliary electrode. All electrolyte solutions were prepared using double-distilled water (18 M ohm cm^{-1}). Double-stranded calf thymus DNA(dsDNA) and other reagents were obtained from Aldrich and were diluted as needed. Multi-walled carbon nano tubes (15-40 nm in diameter) for the CVD method were obtained from Nanotech in Korea. The conventional paste electrode was prepared by mixing 70 % graphite powder with 20 % mineral oil. This mixture was homogenized in a mortar for 30 minutes. The mixed paste was inserted into a plastic syringe needle with a diameter of 3.0 mm, and a copper wire was connected to the electric system. The DNA carbon paste and the DNA nano tube paste electrode were constructed using the same methods, and raw DNA and nano tube powder were used directly after 30 minutes of homogenization. Then, glassy carbon electrodes (BAS stationary voltammetry electrodes MF-2012 with a 3.0 mm diameter) were used. The pencil electrode was prepared using a Pentel pencil, i.e., model P205, with a diameter of 0.9 mm. The metallic part that holds the lead in place inside the pencil was soldered to the lead for electrical contact. The gold electrode was used for the 0.5 mm diameter pure metal. The three electrode system was immersed into a 15 mL cell containing electrolyte solutions of 0.1 M phosphate and ammonium

phosphate solution, while the other parameters were maintained at optimal conditions. All the experiments were performed at a room temperature of 24± 0.5°C, without removing oxygen.

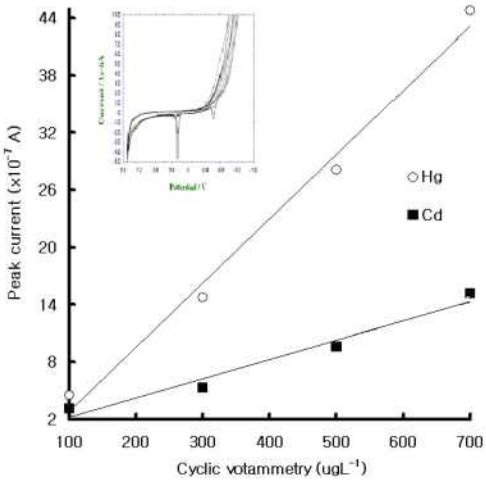
2.2. Experimental Procedure

Various electrolyte solutions of acid and base buffers (all 0.1 M solutions) were initially examined in search of a possible supporting electrolyte. Phosphoric acid buffer solution was found to be the most suitable, yielding a sensitive peak response from the background currents. The electrolyte concentration effect of the phosphate solution was studied within a low and a high range. A 0.1 M solution with a pH of 6.3 was found to be most suitable. The following square-wave anodic optimized parameters were considered: the accumulation potential of -1.3 V, the final potential of 1.1 V, the increment potential of 0.04 V, the amplitude of 0.4 V, the frequency of 200 Hz, and the accumulation time of 120 seconds. Electrode cleaning for every measurement was also found to be unnecessary. Consequently, all the experiments were found to be capable of being performed in an open circuit.

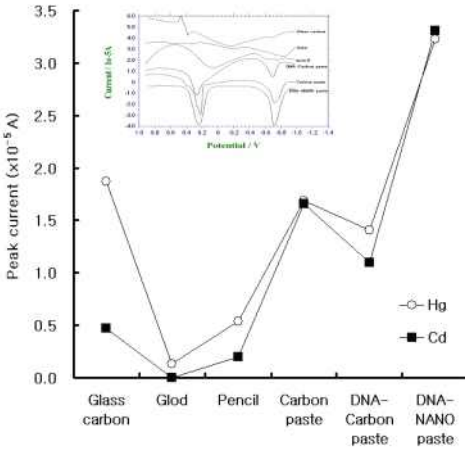
3. Results and Discussion

3.1. Cyclic Voltammetric Effects of Hg(II) and Cd(II), and Comparison with Conventional Electrodes

In Fig. 1, the cyclic voltammetry concentration effects of 0.1, 0.3, 0.5, and 0.7 mgL^{-1} Hg(II) and Cd(II) were examined using DCP in a blank solution. No peak signals were detected; thus, small amounts of 0.1, 0.3, 0.5, and 0.7 mgL^{-1} mercury and cadmium standards were spiked, after which the positive scans obtained Cd(II) and Hg(II) peak currents of -0.7 V and 0.3 V, respectively, higher-current mercury peak signals appeared, their working curves were



(A)



(B)

Fig. 1. (A) : Cyclic voltammetric concentration effects of 0.1, 0.3, 0.5, and 0.7 mgL^{-1} Hg(II) and Cd(II) in pH 6.2 phosphate buffer solutions using a scan rate of 100 mVs^{-1} in DNACNTPE. (B) : Stripping voltammetric electrode comparisons of common glassy carbon, gold, pencil, carbon paste, DNA carbon paste, and DNA nano tube paste electrodes In 1 mgL^{-1} Hg(II) and Cd(II).

plotted, and calculated sensitivities of $\Delta x/\Delta y$=0.9886 Hg(II) and $\Delta x/\Delta y$=0.9659 Cd(II) were obtained. In these equations, mercury

responded more sensitively. Afterwards, conventional electrodes and the developed DCP electrode were compared using square-wave stripping voltammetry. The stripping voltammetric measurement of the Hg(II) and Cd(II) peaks was commonly carried out at 0.2 V and -0.8 V. Other parameters, such as a frequency of 200 HZ, an accumulation potential of -1.3 V, an amplitude of 0.4 V, an increment potential of 0.04 V, and an accumulation time of 120 seconds, were also tested. At these conditions, conventional electrodes and the developed DCP electrode were compared using the response currents in Fig. 1(B), which shows the results of the current ratio and the real voltammograms. The electrode signals at identical conditions for 1 mgL^{-1} Hg(II) and Cd(II) were examined, and all optimized analytical conditions were used. The glassy carbon electrode showed a small peak signal that appeared at 0.4 and -0.8 V, whereas the pencil and gold electrodes did not show any peak current, but the conventional carbon paste and DNA carbon paste electrodes responded with significantly increased peak signals of the same potential, and a half-width of very narrow signals was obtained. Upon examination, the DNACNTPE showed a very sensitive increased peak height of 3.225×10^{-5} A Hg(II) and 3.306×10^{-5} A Cd(II), and at this time, DNA carbon paste electrodes were obtained only at 1.40×10^{-5} A Hg(II) and 1.10×10^{-5} A Cd(II), which showed poor response signals. Following this, the DNA and graphite nano tube mixing weight ratios of 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, and 1:5g were examined. At 5:1 to 1:2 Hg(II) and Cd(II), the peak currents continually increased; at 1:1 to 1:4, the peak currents linearly decreased; at 1:2, the maximum increased peak current appeared, and mercury and cadmium responses of 9.56×10^{-5} A Hg(II) and 6.63×10^{-5} A Cd(II) were obtained; and at 5:1, the peak current was very poor and 3.97×10^{-5} A Hg(II) and 4.47×10^{-5} A Cd(II) responses were obtained. Thus, the 1:2 ratio was used for all experimental mixing weights.

3.2. SWSV Effects of Various Parameters, such as pH, Accumulation Time, and Square-Wave Step Potential

In the DCP, various parameters of the electrolyte solution's pH, accumulation time, and accumulation potentials were examined (not shown here). First, the hydrogen ionic activity of the stripping voltammetric peak current in a 1.0 mgL^{-1} concentration of Hg(II) and Cd(II) was examined for the pH levels of 3.3, 4.3, 5.3, 6.3, 7.3, 8.3, 9.4, 10.4, and 11.1. The hydrogen ionic activity was changed using 0.1-M HCl and 0.1-M NaOH solutions. The mercury and cadmium ionic force increased quickly from 3.3 to 6.0 at a pH of 6.3 when the anodic peak signals reached the maximum level. The peak's shape and width symmetrically narrowed, and the maximum peak height of Hg(II) : Cd(II) = 3.68×10^{-5} A : 6.14×10^{-5} A was increased. Beyond this pH, the rate of the activity of the peak current quickly decreased. There was no linear response when the pH range was at 7.3 - 11.0. Therefore, the 6.3 pH level could be used for optimum conditions. Other effect parameters of accumulation times were then examined, while the zero times did not respond and a very small peak appeared, where as at the continually increasing times of 10, 20, 30, 40, 50, 60, 70, 80, 100, and 120 seconds, both signals very quickly increased with the same ratios. As no response was acquired at more than 120 seconds, 120 seconds was used for the optimal accumulation conditions. The height concentration range of 5 to 10 mgL^{-1} Hg(II) and Cd(II) was tested, and the same results appeared at 120 seconds. An examination of the influence of varying pre-concentration potentials was then carried out within the negative range of -1.35, -1.3, -1.25, -1.2, -1.15, -1.1, -1.05, and -1.0 V. From -1.35 to

-1.3V, the peak current increased very sharply with a narrow half-width. The peak signal decreased non-linearly at the potential range of -1.25 ~ -1.0 V. The peak's width and sharps did not change, and only the peak height decreased. Moreover, other interfering effects did not appear. Therefore, the optimal potential of -1.3 V was chosen for the adsorptive stripping voltammetric detection of both ions.

3.3. SW SV Effects of Various Parameters, such as Amplitude, Frequency, and Increment Potential

Fig. 2(A) illustrates the voltammetric peak current in a 1-mgL^{-1} concentration of Hg(II) and Cd(II) as a function of varying square-wave amplitudes (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, and 0.4 V) after a pre-concentration time of 120 seconds. The range of amplitude increased very quickly from 0.05 to 0.25 V, and then to 0.35-0.4 V after the peak current did not increase. At this amplitude, an Hg(II) and Cd(II) peak height ratio of 17.92×10^{-5} A : 4.42×10^{-5} A appeared. Thus, the mercury ions responded more sensitively than did the cadmium ions. Moreover, the peak width and peak sharps responded sharply, and other examined mercury and cadmium concentrations showed the same results. The square-wave frequencies for Fig.2 (B) were then tested. The results were in the range of 25, 50, 75, 100, 125, 150, 175, and 200 Hz, while from 25 to 100 Hz, the mercury ions did not respond, and from 125 to 200 Hz, they increased very quickly, but the cadmium ions linearly and slowly increased. Thus, optimum conditions resulte data frequency of 200Hz. The low stripping voltammograms are shown in the same figure, whose peak width and sharps also sensitively responded. Both maximum peak current heights of 14.4×10^{-5} A Hg(II) and 3.97×10^{-5} A Cd(II) were obtained. The effect of the square-wave increment potential on peak currents was then studied within the

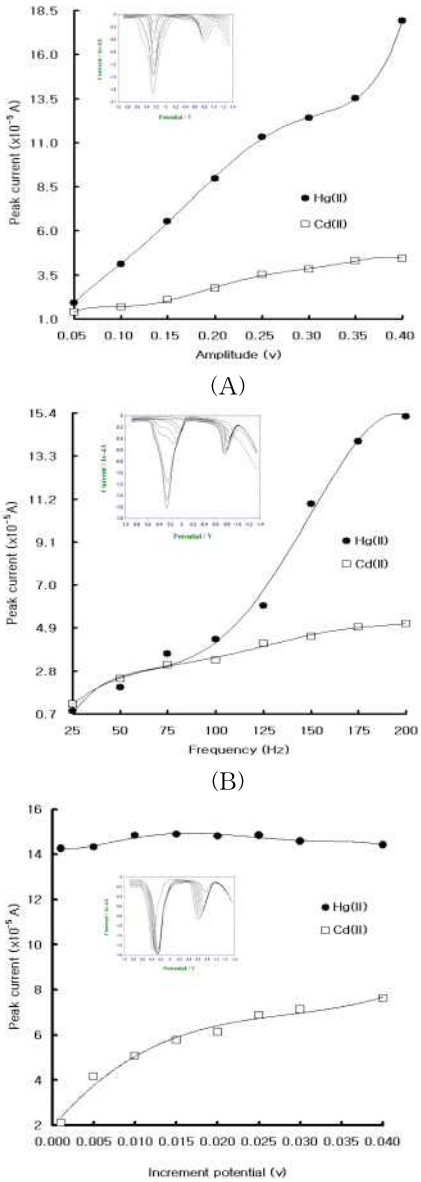


Fig. 2. (A): Peak current of varying square-wave amplitudes (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 35, and 0.4 V); (B): square-wave frequencies (25, 50, 75, 100, 125, 150, 175, and 200 Hz); and (C): incremental voltages (0.01, 0.05, 0.01, 0.015, 0.02, 0.025, 0.03, and 0.04 V) in 1.0 mgL^{-1} Hg(II) and Cd(II). Other parameters in Fig.3 were held constant.

range of 0.01, 0.05, 0.01, 0.015, 0.02, 0.025, 0.03, and 0.04 V, given a concentration of 1.0 mgL^{-1} Hg(II) and Cd(II). Other experimental parameters in Fig.2 (A) were held constant. The Hg(II) current's response linearly increased, and that of Cd(II) quickly responded from 0.01 to 0.02. Optimal conditions were arrived at 0.04 V, and at the optimized conditions, analytical working ranges were studied.

3.4. Statistical Results, Interference, and Applications of Pond Water, Cigarettes, andRiver Fish Kidney Tissue

Fig. 3 shows the effects of varying Hg(II) and Cd(II) concentrations using a 120-second deposition time. All these results are raw and calibrated equations. Fig 3(A) illustrates the low-concentration 1- to 10 ngL^{-1} resulting curve of increments in the calibration. At this time, the regression equations of y = 1.9956x-1.636 Hg(II) and y = 0.0959+0.1789 Cd(II) [correlation coefficient of 0.9908 Hg(II) and 0.962 Cd(II) = 10 points; y = current A; and x = Hg(II) and Cd(II) concentrations, ngL^{-1}] were obtained, in which ranges the Hg(II) ions responded more sensitively. More increased micro-concentration ranges were then obtained (not shown here), and the calculated working ranges of 20, 30, 40, 50, 60, 70, 80, 90, and 100 ugL-1, and the equations y = 0.0753x+5.7496 Hg(II) and y = 0.0282x-0.2301 Cd(II) [correlation coefficient of 0.9732 Hg(II), 0.9933 Cd(II)] were obtained, at which time the mercury ions responded more sensitively than did the cadmium ions. Other higher concentration effects were examined, from which high concentration ranges of ppm levels finally appeared. Fig. 3(B) shows these effects, the working range of 0.1 mgL^{-1}, and the linear equations for the slop ratio of y = 0.0073x-0.1207 Hg(II) and y = 0.0039x-0.1178 Cd(II) [correlation coefficient of 0.9985 Hg(II) and 0.999 Cd(II)] that were sensitively obtained. At this condition, both

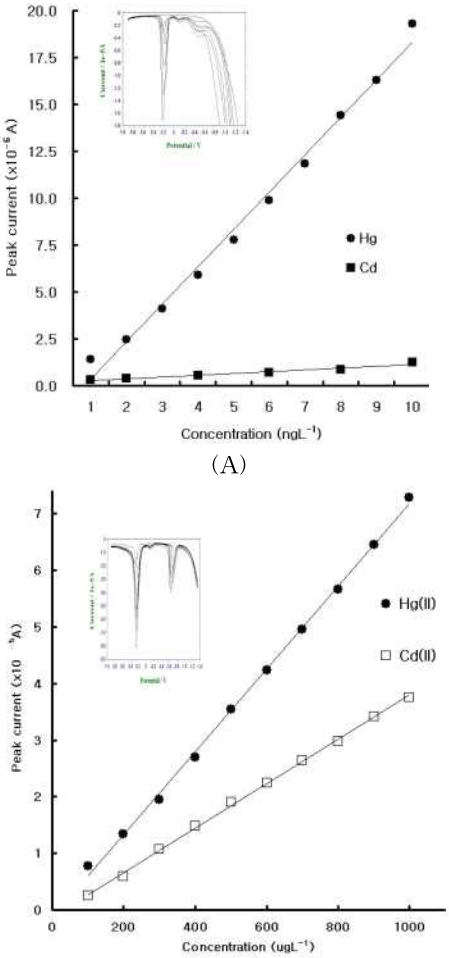


Fig. 3. Varying Hg(II) and Cd(II) concentrations. (A): 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ngL^{-1}; and (B): 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mgL^{-1}on DNACNTPE, with an electrolyte solution of 0.1 M phosphate buffer at 6.3 pH, and with deposition at 120 seconds. Other experimental parameters in Fig. 2 were held constant.

peaks similarly increased, and the slop ratio sensitively responded. The statistically analytical detection limits (S/N) were examined, and the following results were obtained: 0.1 ngL-1 for Hg(II) (4.9×10^{-11} M)

and 0.2 ngL^{-1} for Cd(II) (1.77×10^{-10} M). The calculated values are more sensitive than those derived from other common methods, and the DNAGNPE is considered more advantageous. At optimal conditions, various interference metal ions were studied by adding several other ions into the medium containing 100 ugL^{-1} of the Hg(II) and Cd(II) ion, the existence of 100 ugL^{-1}, such as Bi(II), Ni(II) Ba(II), Pb(II), Ge(IV), Ca(II), Ag(I), Cr(III), and Cu(II), resulted in -54.1 %, -0.52 %, -10.8 %, -18.5 %, -13.9 %, 6.4 %, -20.4 %, -19.3 %, and 43.4 %, and the presence of a threefold excess was changed into -19.3 %, -12.4 %, 19.8 %, -89.8 %, 10.7 %, -11.4 %, -193.7 %, -66.1 %, and 120 %, respectively. The presence of other ions was also effectively removed using standard addition methods.

Various analytical applications were examined, although they are not shown here. Fig. 4(A) first shows the pond water results.

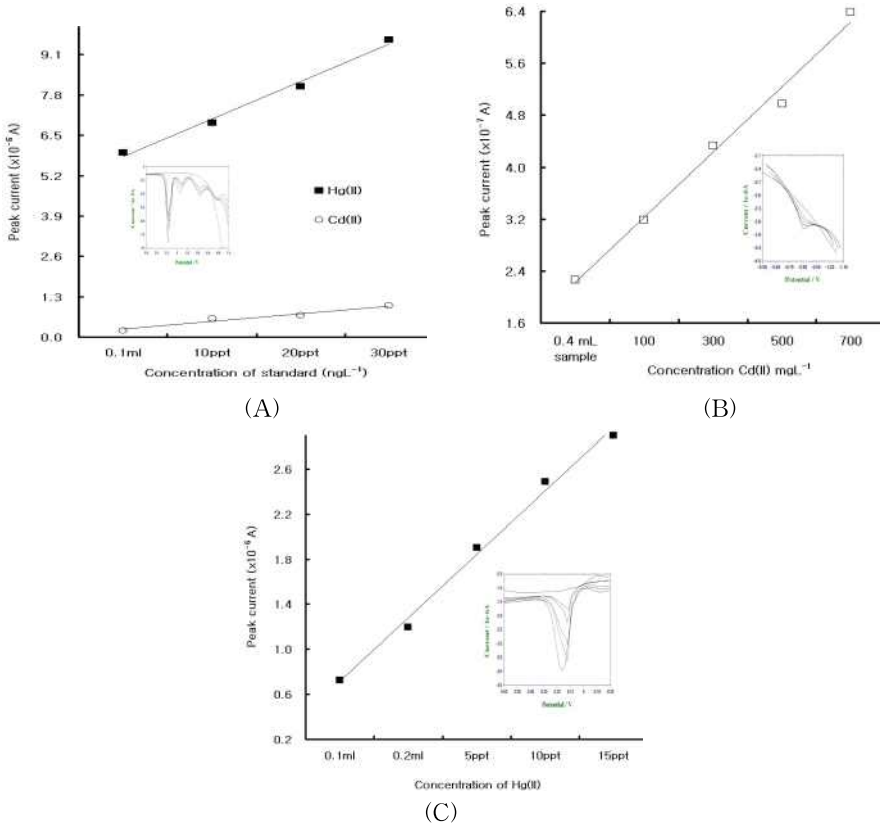


Fig. 4. Applications of pond water (A): 10 mL blank electrolyte solution, 0.1 mL sample, and 5.0, 10.0, and 15.0 ngL^{-1} Hg(II) and Cd(II); of cigarettes (B): 10 mL blank electrolyte solution, 0.4 mL diluted sample, and 100, 300, 500, and 700 ugL^{-1} Cd(II); and of fish kidney (C): 10 mL blank electrolyte solution, 0.2 mL diluted fish kidney, and 5, 10, and 15 ngL^{-1} Hg(II) on DNACNTPE, with an electrolyte solution of 0.1 M phosphate buffer solution. All the raw voltammograms and calibration curves shown had a deposition time of 120 seconds. Other experimental parameters in Fig.3 were held constant.

Blank solutions are shown, which manifest that no noise signal was obtained, after which the 0.1-mL sample solutions were spiked, and the mercury and cadmium peak currents were obtained. Thus, another standard of 5.0, 10.0, and 15.0 ngL^{-1} Hg(II) and Cd(II) was spiked, continued peaks were calibrated, and 0.59 and 0.13 ngL^{-1} Hg(II) and Cd(II) were obtained [R^2 = 0.9902 Hg(II) and R^2 = 0.9672 Cd(II)]. The raw voltammograms are shown in the figure. Commonly used cigarette samples were then examined, from which only 0.14 ngL^{-1} Cd(II)/1.89 g cigarettes [R^2 = 0.9902 Cd(II)] appeared. A river water fish was examined next, with a body weight of 85 g and an extracted kidney weight of 0.57 g, which was diluted in a 10 mL concentrated HCl solution. Fig. 4(C) shows the raw voltammograms and the calibrated equation results. Cd(II) cannot be obtained merely with detected mercury ions. In the blank solution, no signal appeared, and the 0.2 mL diluted kidney sample solutions were spiked, at which time only the mercury peak current was obtained. Other standard Hg(II) solutions of 5, 10, and 15 ngL^{-1} were spiked, and their calculated values (9.25 ppt / 0.57 g kidney / 85 g body weight) [R^2 = 0.9928 Hg(II)] were obtained. Other river fishes (at the same site) were examined, and the same results were obtained.

4. Conclusions

The developed methods were fabricated with DNA carbon nanotube paste electrodes using square-wave stripping voltammetry. The electrode response arrived at was linearly related to the Hg(II) and Cd(II) concentration, which ranged from 1-10 ngL^{-1}, $20^{-1}00$ ugL^{-1}, and 0.1 mgL^{-1} at an accumulation time of 120 seconds. The peak currents reached the maximum at a phosphate buffer electrolyte solution pH of 6.3, and various interference ions were removed using standard addition methods. Since the method used in this study had a lower detection limit, the analytical applications of the cigarette, fish kidney, and pond water samples were examined. The proposed method can also be applied in other fields requiring Hg and Cd ion analysis.

References

1. E. Tatrai, Z. Kovacikova, A. Hudak, Z. Adamis and G. Ungvary, "Comparative *in Vitro* Toxicity of Cadmium and Lead on Redox Cycling in Type II Pneumocytes", *J. Appl. Toxicol*, **21**, 479 (2001).
2. P. Suciu, M. Vega, and L. Roman, "Determination of Cadmium by Differential Pulse Adsorptive Stripping Voltammetry", *J. Pharmaceut Biomed*, **3,** 99 (2000).
3. G. Centineo, E. B. Gonzalez and A. S. Medel, "Multielemental Speciation Analysis of Organometallic Compounds of Mercury, Lead and Tin in Natural Water Samples by Headspace-solid Phase Microextraction Followed by Gas Chrmatography-mass Spectrometry", *j . Chromatogr A*, **1034,** 191 (2004).
4. L. Liang, G. B. Jiang, J. F. Liu, and J. T. Hu, "Speciation Analysis of Mercury in Seafood by using High-performance Liquid Chromatography On-line Coupled with Cold-vapor Atomic Fluorescence Spectrometry Via a Post Column Microwave Digestion", *Anal Chim Acta,* **477,** 131 (2003).
5. C. F. Harrington, S. A. Merson, and T. M. D' Silva, "Method to Reduce the Memory Effect of Mercury in the Analysis of Fish Tissue using Inductively Coupled Plasma Mass Spectrometry", *Anal Chim Acta*, **505,** 247 (2004).
6. S. Y. Ly, J. I. Chae, Y. S. Jung, W. W. Jung, H. J. Lee, and S. H. Lee, "Electrochemical Detection of Ascorbic

Acid (vitamin C) using a Glassy Carbon Electrode", *Nahrung/Food,* **48,** 201 (2004).
7. R. D. Riso, M. Waeles, P. Monbet, and C. J. Chaumery, "Measurements of Trace Concentrations of Mercury in Sea Water by Stripping Chronopotentiometry with Gold Disk Electrode: Influence of Copper", *Anal Chim Acta*, **410,** 97 (2000).
8. M. R. G. Rubio, M. B.A. Fontes, Z. M. d. Rocha, E. M. Richter, and L. Angnes," LTCC Manifold for Heavy Metal Detection System in Biomedical and Environmental Fluids", *Sensor Actuat* B-Chem **103,** 468 (2004).
9. K. Wu, S. Hu, J. Fei, and W. Bai," Mercury-free Simultaneous Determination of Cadmium and Lead at a Glassy Carbon Electrode Modified with Multi-wall Carbon Nanotubes", *Analy Chim Acta.* **489,** 215 (2003).
10. S. B. Hocevar, J. Wang, R. P. Deo, and B. Ogorevc, ''Potentiometric Stripping Analysis at Bismuth-Film Electrode", *Electroanal*, **14,** 112 (2002).
11. K. Fanta and B. S. Chandravanshi, "Differential Pulse Anodic Stripping Voltammetric Determination of Cadmium(II) with N-p-chlorophenyl-cinnamohydroxamic Acid Modified Carbon Paste Electrode", *Electroanal* **13,** 484 (2001).
12. T. H. Degefa, B. S. Chandravanshi, and H. Alemu, "Differential Pulse Anodic Stripping Voltammetric Determination of Lead(II) with N-p-Chlorophenylcinnamohydroxamic Acid Modifid Carbon Paste Electrode", *Electroanal*, **17,** 1305 (1999).
13. H. Karadeniz, B. Gulmez, F. Sahinci, A. Erdem, G. I. Kaya, N. Unver, B. Kivcak, and M. Ozsoz, "Disposable Electro-chemical Biosensor for the Detection of The Interaction Between DNA and Lycorine Based on Guanine and Adenine Signals", *J. Pharmaceut Biomed,* 2003, **33,** 295 (2003).