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Trace Assay of Cobalt Ion Using Implant and Patch-type Probe in a Living Cell

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In this article, an assay of trace cobalt ion was searched by using carbon nanotube paste electrode (PE). Under optimized analytical conditions, linear working ranges were from 70 to 700 ngL^{-1} measured with SW stripping voltammetry, from 70 to 910 mgL^{-1} with chronoamperometry. A relative standard deviation (RSD) of 20.0 mgL^{-1} Co (II) was of 0.12% SW (n = 15). Detection limit was attained as 7.93-ngL^{-1} (1.3×10^{-10} M) Co(II). Here analytical application was performed to in vivo implanted living fish brain and a dissolved fish kidney tissue was also used for diagnostics. Moreover, the patch-type electrode system was applied to plant or leaf skin assay. The developed methods can be applied to in vivo direct assays for real time monitoring with optimum parameters.

Key words: Cobalt (II), voltammetry, brain, carbon nanotube, kidney, plant, *in vivo*

1. Introduction

In medicinal and industrial chemistry[1)], cobalt, nickel and their analogy metal compounds are widely used in such sectors as super alloys[2)], dental cast alloys[3)], hard metal (cemented carbide), diamond polishing[4)], drying agents, pigments[5)] and catalysts[6)]. Tracing cobalt ions is widely spread in food, aquatic[7)] and environmental soil systems[8)]. Being carcinogenic to humans, cobalt ions can contribute to an increase in likelihood of lung cancer[9)], cellular toxicity, metal disease[10)], and chromosomal changes[11)]. In medical diagnosis, it is necessary to trace Co even at a small amount of metal residue. To date, various analytical methods have been employed for industrial analogy techniques such as high-performance liquid chromatography[12)], multi-element trace analysis by reversed-phase, high-performance liquid chromatography[13)] liquid chromatography inductively coupled plasma mass spectrometry[14)], glow discharge quadrupole mass spectrometry[15)], electrothermal atomic-absorption spectrometry (detection limit (DL) for Co as 0.13 ugL^{-1})[16)], resonance ionization mass spectrometry[17)], and graphite furnace atomic absorption spectrometry (DL of 0.5 mg kg^{-1})[18)]. All of these techniques require pre-treated preparations and complicated detection systems such as electric or magnetic separation, fluid compression, atomization high temperature control and electric detections. Further none of these methods can be used in in-vivo direct assays.

Electrochemical techniques, however, are simple and can reduce analytical working ranges. For example, voltammetric measurement using bismuth film microelectrode can detect up to 69 ng/L (1.2×10-9 molL^{-1}) for Co(II)3); adsorptive stripping voltammetry (DL:1.7×10-11 molL^{-1})[19)]; adsorptive stripping voltammetry on a rotating-disc bismuth film electrode (DL; 70 ngL^{-1} for Co(II))[20)]; bismuth film electrode (DL: 0.08 ugL^{-1})[21)]; in-situ plated lead film electrode (DL:1.0×10-9 molL^{-1})[22)]; constant-current stripping potentiometry (DL: 10 ng/L for Co)[23)]; batch injection analysis with adsorptive stripping voltammetry (DL: 2 nM for cobalt)[24)]; and the differential pulse-polarographic determination method

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can detect Co within the range of 1-2 ppb[25)]. Here electrochemical methods can be approached to trace ranges.and applied only in vitro conditions not usable for medicinal or in-vivo direct assay for living cell. However this study was searched to in vivo diagnostics using carbon nanotube[26,27,28)] paste[29,30,31)] electrode. Which detection limit was attained to 7.93 ngL^{-1} (1.3× 10^{-10} M) Co(II), it is more sensitive than other common methods. The method was first applied to a deep brain cell and later to plant skin. It is possible to in vivo real-time monitoring and useful for analyzing to in in-vivo diagnosis.

2. Experimental Design

2.1 Systems, reagents, and experimental conditions

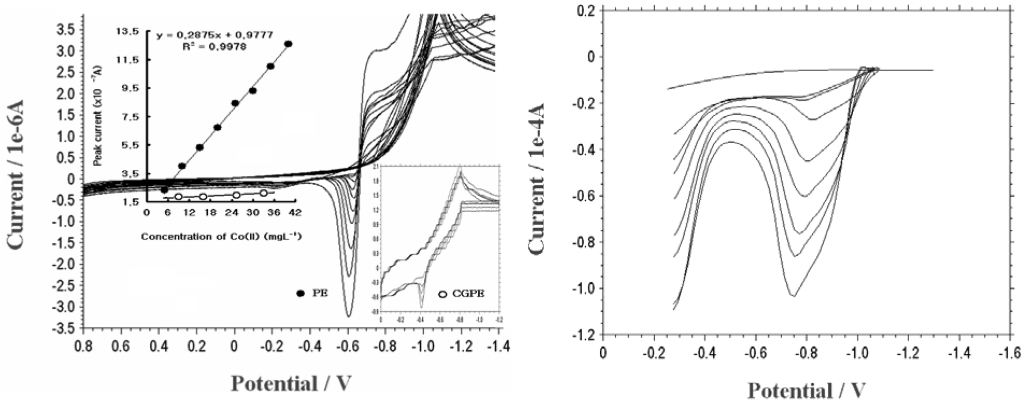
The measurement systems used with CHI 660A electrochemical workstation (CH Instruments, Inc., Cordova). Working paste electrode was prepared using with a multi-walled carbon nanotube, catalytic CVD. Its external diameter is 8 nm; its internal diameter is 2-5 nm; its length is 0.5-200 nm (Nanostructured & Amorphous Materials, Inc.) and which was washed through magnetic stirring in a 2 M nitric acid (from Merck) solution and triple-distilled pure water (Milli-Q). Paste was prepared by mixing the carbon nanotube and the mineral oil (Sigma) (40:20(W/W)) was placed in a needle-type plastic syringe with a diameter of 0.3 mm, with a copper wire connected to the electric system. A counter Pt electrode measuring 0.3 mm in diameter and 3 mm in length was prepared. Chloride-coated (0.2 mm-diameter) silver wire (Merck) was used as the reference electrode. The three-electrode system was optimized in a solution of 10-mL ammonium phosphate. Application was done on living cell systems.

The stock solution of Co(II) was prepared by dissolving 1000 mg/L (Merck standard). All other reagents were of analytical grade and supplied by Aldrich (England), Merck (Germany) and Sigma (USA). The experiments were performed at room temperature without removing oxygen. A fish (a carp 13 cm length, 120 gram) was anesthesized using diluted ether [(C2H5)2O] with tap water by 50%:50% dilution. Later, the kidney (0.25 grams) was extracted from this fish and diluted in 0.1M HCl solution. Analytical applications were carried out using pond fish and their living plant, near the industrial area of the Chinchen (-C-) metropolis in Korea and subsequently on another live fishing site in Lin Po (-L-) town in Korea. The two groups of fish were examined by using 250 gram weight live and die A handmade implantable macro type three-electrode systems were prepared with a macro size of 0.3 mm diameter, 3 mm length, and a reference for chloride-coated 0.2 mm silver-wire electrode. In an anesthetic condition induced by using ether working electrode was inserted into the fish's brain core with 7 mm deep hole using a micro dental drill. The counter and the reference electrode were implanted into fish back tail. The three-electrode cable was connected to an electrochemical workstation. In actual swimming conditions, a live water hyacinth plant leaf in the –C– site were also examined. A tape-type (5 mm diameter circle) working-electrode was attached on the leaves' skin surface, which was connected to the copper wire. Other wire-type counter and reference electrodes were attached on the leaves skin with the same method, and were connected using a copper wire, then stripping voltammetry was performed in live conditions.

3. Results and Discussion

3.1 Comparison of common graphite pencil electrode (CGPE) and PE in cyclic and square-wave stripping voltammetry

CGPE's voltammetric peak potential and sensitivity were compared to those of PE. Fig. 1 shows the an analytical working ranges of the CGPE (insert curve) and PE, within the range of 5, 10, 15, 20, 25, 30, 35, and 40 mgL^{-1} PE. CGPE varied at 10, 15, 25, and 35 mgL^{-1}. The CGPE peak was poor and not sensitive enough oxidation peak. When PE was used, however, a sharp peak potential appeared at -0.6 V positive scan. In the PE conditions, the linear equation was $y = 0.2875x + 0.9777$; the slope ratio produced reliable results, precision was R2 = 0.9978, and a peak current of



(A) *(B)*

Fig. 1. (A) Electrode comparison: the CGPE (insert curve) for 10- to 35-mgL^{-1} Co(II) CV. PE for 5- to 40-mgL^{-1} Co(II) CV, in 0.8V initial and -1.4 V switching potential, using a 0.5 Vs^{-1} scan rate. (B) PE for SW 100- to 800-mgL^{-1} Co(II) spike, -1.6 V initial potential, 0.04 V increment potential, 0.25 amplitude, 450Hz frequency, 100 sec accumulation time, using an 0.1 M ammonium phosphate electrolyte solution.

2.29×10-7~12.59×10^{-7} A. This result shows that PE is more sensitive than common CGPE. More sensitive working ranges are required in in-vivo systems. Thus, SW stripping voltammetry was examined for the optimum parameters by using PE.

The SW stripping voltammetric concentration effects and peak potential were examined. In anodic scan, identical peak potentials were obtained. Cathodic reaction, however, did not appear at all, as shown in Fig. 1(B). Within a concentration range of 0, 50, 100, 200, 300, 400, 500, 600, 700, and 800 mgL^{-1} spike, the peak height of 1.099×10^{-5}~9.575×10^{-5} was obtained. A big current appeared. In this potential, the optimum SW parameter was determined.

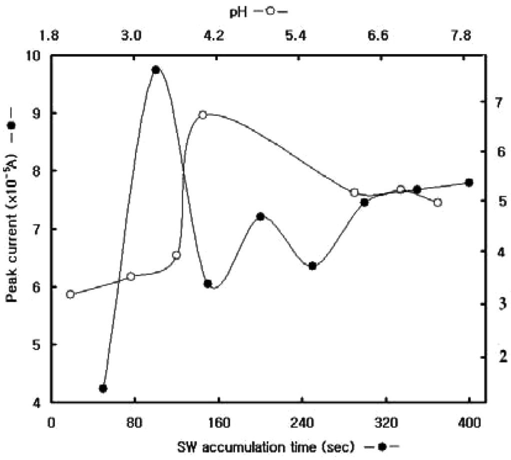
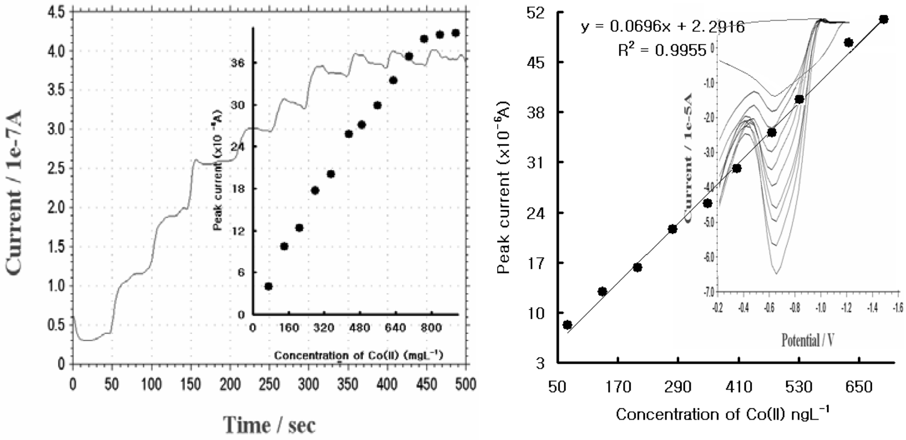


Fig. 2. Various accumulation times of 50-400 sec, and 2.08-7.43 pH in a 0.1 M $NH_4H_3PO_4$ electrolyte solution, using 10-mgL^{-1} Co(II) with 0.25V amplitude, 200 Hz frequency, 100 sec accumulation time, 4.01 pH. Other parameters were used for the optimum conditions.

3.2 SW stripping optimizations using PE

In the 0.1 M ammonium phosphate electrolyte solution, various SW parameters were examined. First, SW amplitude range of 0.05-0.4 V and SW frequency range of 200-900 Hz using eight points were examined, with the peak current elevated to the ranges of 1.07×10^{-5} A~2.45×10^{-5} A and 2.3×10^{-5} A~2.0×0^{-5} A, under 0.25 V amplitude and 200 Hz. Thus, the 0.25 V SW amplitude and 200 Hz remained constant (results not shown here). Under these conditions, the SW accumulation time was examined. Fig. 2 shows the oscillated peak currents within the range of 50, 100, 150, 200, 250, 300, 350, and 400 sec. At 100 sec, the maximum peak current of 9.74×10^{-5} A was obtained. The peak current was more sensitive than the frequency, amplitude, and pH effects. In this condition, electrolyte pH parameters were examined.

In Fig. 2, the electrolyte's pH effect was shown within the range of 2.08, 2.96, 3.62, 4.01, 6.21, 6.89, and 7.43 pH. It was changed by using 0.1 M HCl and 0.1 M NaOH spikes. At 4.01 pH, maximum peak current of



(A) *(B)*

Fig. 3. (A) Shows the results of chronoamperometric working ranges from 70 to 910 mgL^{-1} Co(II) in a -0.8 V potential with 500 sec. (B) SW anodic working ranges from 70 to 700 ngL^{-1} Co(II), using 0.25V amplitude, 200 Hz frequency, 100 sec accumulation time, 4.01 pH, 0.05 V increment potential using a 0.1 M $NH_4H_2PO_4$ electrolyte. Optimum conditions for other parameters were also used.

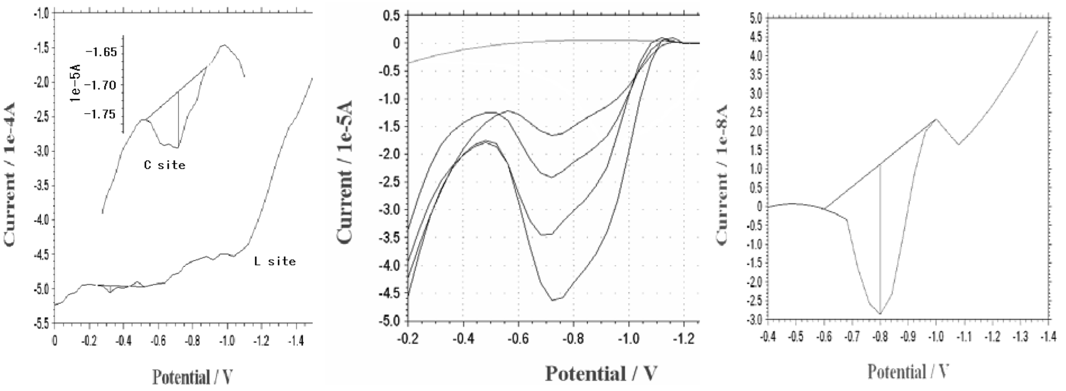
6.82×10^{-5} A was obtained, which was bigger than the increment potential, the accumulation potential, and the SW frequency. At 4.01 pH strength, SW increment potentials from 0.005 V to 0.04 V by using eight points were examined, 2.788×10^{-5} A~2.168×10^{-5} A peak current was obtained. At 0.05 V, the maximum peak appeared, which is as sensitive as the other parameters. The final optimum conditions for results were as follows of 0.25 V amplitude, 200 Hz frequency, 100 sec accumulation time, 4.01 pH, and 0.05V SW increment potential were obtained.

3.3 Working range, statistics, and application in vivo

Under optimum conditions, the analytical linear working range was determined. The results of the chronoamperograms are shown in Fig. 3(A), which was conducted at a -0.8 V negative potential, a concentration range was varied to 70, 140, 210, 280, 350, 431, 490, 560, 630, 700, 770, 840, and 910 mgL^{-1} Co(II) add, herein a regression equation was obtained of $y = 0.00444x + 4.0606$, statistics of $R2 = 0.9719$, and a current range of 3.948×10^{-8}~40.21×10^{-8} A was varied. A wide working range and a sensitive slope ratio appeared. In Fig. 3(B) the SW stripping voltammetry results for 70, 140, 210, 280, 350, 420, 490, 560, 630, and 700 ngL^{-1} Co(II), and the slope for △x/y△=0.0696 soon appeared. High precision (R2 = 0.9955) was attained, and a narrow peak width was obtained. It was used in application, statistics, and analytical detection

Table 1. Comparisons of analytical detection limits

Methods	DL	Reference
Here developed method	7.93 ngL^{-1} (1.3×10^{-10}M)	
Electrothermal atomic absorption spectrometry	0.13 ugL^{-1}	16
Graphite furnace atomic absorption spectrometry	0.5 mg kg^{-1}	18
Voltammetric measurement using bismuth film microelectrode	1.2×10-9 molL^{-1}	3
Adsorptive stripping voltammetry	70 ngL^{-1}	20
Bismuth film electrode	0.08 ugL^{-1}	21
In-situ plated lead film electrode	1.0×10^{-9} molL^{-1}	22
Constant-current stripping potentiometry	10 ngL^{-1}	23
Adsorptive stripping voltammetry	2 nM	24



(A) (B) (C)

Fig. 4. (A): Test result are shown in C site and L site. L site indicates a non-contaminated-pond fish's brain cell and. C site shows contaminated-pond fish's brain cell. (B): the standard addition method using the fish kidney in C site; the curve on the top is produced in electrolyte blank, the next curve is produced in 0.5-mL kidney solution spike,other curves are yielded in 10, 20, and 30 mgL^{-1} Co(II) respectively. (C): The curve represents Co in plant leaves from C site pond. Other conditions were used as optimum conditions.

limits. The values show a very low detection limit of 7.93 ngL^{-1} SW than common methods, results are shown at Table 1.

In these results, analytical precision was tested with 15th replication, 20 mgL^{-1} Co(II) detection, 100 sec accumulation time, from which the relative standard deviation of 0.1189 (n = 15), and high precision were attained.

Fig. 4(A) shows a sharp peak of the C site curve which is a -0.75 V peak potential as well as a -5.329× 10^{-7} A peak. We had contaminated with Co(II) ion. The L site curve has no peak,meaning that is the fish not contaminated. Fig. 4(B) shows the results of the kidney sample in contaminated-pond fish from C site. The first curve depicts a 10 mL electrolyte blank-solution. The second curve, a 0.5-mgL^{-1} dissolved kidney sample was spiked, and 10 mgL^{-1}, 20 mL^{-1} and 30 mL^{-1} standard spike was add. Calibrated results of a 9.14±0.6 mgL^{-1}. Fig. 4(C) shows that the exact peak width was obtained the same -0.8 V potential was obtained as well as a peak height of -3.990×10^{-8} A. thus, the method can be used for other plant skin and brain cells. These results can apply to real-time assays on living tissues.

4. Conclusion

Using a handmade carbon nanotube paste electrode, the optimized SW analytical conditions were of 0.25 V amplitude, 200 Hz frequency, 100 sec accumulation time, 4.01 pH electrolyte solution, and 0.05 V increment potential were obtained. In the conditions, analytical detection limit was attained at 7.93- ngL^{-1} Co(II). It is more sensitive than when common[16,18] voltammetric methods[3,20,21,22,23] are employed. Handmade electrode systems can be implanted into the living cell, monitored using cyclic voltammetry, chronoamperometry, and SW stripping voltammetry, which could be used in real-time assays for one month and beyond. Moreover, the patch-type electrode prepared in this study was used on a live skin, which responded well to in-vivo assay. The results show that the proposed methods here can be used with any other living cell system requiring real-time monitoring.

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