



Real-time Detection of Trace Copper in Brain and Kidney of Fish for Medical Diagnosis

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For the detection of trace copper to be used in medical diagnosis, a sensitive handmade carbon nanotube paste electrode (PE) was developed using voltammetry. Analytical optimized conditions were found at 0.05 V anodic peak current. In the same conditions, various common electrodes were compared using stripping voltammetry, and the PE was found to be more sharply sensitive than other common electrodes. At optimum conditions, the working ranges of 3~19 μgL^{-1} were obtained. The relative standard deviation of 70.0 μgL^{-1} was determined to be 0.117% ($n = 15$), and the detection limit (S/N) was found to be 0.6 μgL^{-1} (9.4×10^{-9} M). The results were applied in detecting copper traces in the kidney and the brain cells of fish.

Key words: Paste electrode, Kidney, Brain cells, Fish, Copper

INTRODUCTION

In biological science, metal ions are essential in human body systems, while copper and their trace analogy redox interactions with cholesterol in brain tissue are related to medical conditions such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (1,2), therefore, effective assay on these metal ions is important in diagnostic detection fields (3-6). Sensitive analytical methods have been developed that achieved very low detection limits (DL), such as the ETV-ICP-MASS technique (DL: 0.8 μgg^{-1}) (7), EDXRF (8), HPLC-MASS (9), and ETAAS-USN-ICP-OES (DL: 0.04 μgL^{-1}) (10). However, all these methods are fabricated with flame atomization, ionic separation, mag-

netic or electric attraction, and require other photometric or electrical detection systems. Also, these methods are impossible to be used for *in vivo* direct assay. In contrast, the voltammetric method such as the cyclic and the stripping method are easy to use and inexpensively compact (11-13). These systems depend on various working electrode techniques and were developed for the much more modified electrode, as for example, the glassy carbon electrode (DL: 39 μgL^{-1}) (14), (DL: 1.73 ppb) (15,16), paste electrode (DL: 0.5 μgL^{-1}) (17), (DL: 3×10^{-9} M) (18-20), mercury electrode (DL: 10^{-9} M) (21), (DL: 1.4×10^{-8} M) (22), and gold electrode (23). All these are very useful and have sufficiently addressed the need to determine the amount of copper ions in the environment, foods, or pharmaceuticals, especially when testing is undertaken under laboratory conditions and used only pretreated electrolyte conditions. However, in this study, which represents the first direct *in vivo* assay for such a purpose, real-time nondestructive diagnosis was performed using handmade carbon nanotube paste electrode directly inserted deep into the brain core of live fish. The method approached lower detection limits than other common voltammetric methods, which uses carbon nanotubes that have a specific set of physical and chemical properties reported in other papers (24-26), and that is simple enough for handmade preparation and can be used in medical diagnosis through direct assay and other analysis.

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MATERIALS AND METHODS

Apparatus and experimental procedure. Experimental systems were taken using a CHI 660A instruments electrochemical workstation (CH Instruments Inc., Cordova, TN, USA) equipped with a three-electrode system and was used in the cyclic and square-wave stripping voltammetry. All working electrodes were prepared with handmade techniques. The reference electrode was an Ag/AgCl electrode (saturated KCl), and the auxiliary electrode was a platinum wire. The PE working electrode was made by mixing 80% carbon nanotube graphite powder (choongnam Korea Zip 330-816, nanotechco., LTD, Korea, CVD, 15-4-diameter, 30~50 μm length, 95% purity) with 20% mineral oil. This mixture was homogenized in a mortar for 30 min. The paste was inserted in a plastic syringe needle with a diameter of 3 mm, and a copper wire was connected to the electric system (27,28). Then the carbon fiber microelectrode was prepared from a 7- μm -diameter, 15-mm-long carbon fiber attached to a copper wire via silver paint (29,30) and was inserted into a polyethylene tube with a 0.3 mm diameter. The electrode was sealed by heating. Another unmodified carbon paste electrode was prepared with common graphite powder (31). All electrolyte solutions were prepared from double-distilled water (18 MOhm/Cm⁻¹). Other reagents and standard stock solutions were obtained from Aldrich and diluted within the required time. Electrical measurements were performed in the 0.1 M-(NH₄)H₂PO₄ and the Cu(II) was scanned at -1.90~1.60 V. A cyclic voltammogram was recorded at 300 mVs⁻¹ scan rates. We found the Cu(II) peak current of anodic 0.05 V in cyclic voltammograms (32), then we also found that the electrode cleaning time is not necessary for every measurement. In this study, we first tested several electrolyte solutions such as phosphoric acid, nitric acid, acetic acid, and sulfuric acid (all in 0.1 M) as possible supporting electrolytes. The phosphoric acid solution was found to be the most suitable medium, yielding the best peak separation from the background currents. Then the effect of the ionic activity was studied, and the high peak signals were obtained in the concentration range of 0.1~0.08 M.

RESULTS AND DISCUSSION

Cyclic voltammograms and electrode comparison.

At a high concentration, the cyclic peak potential was determined using various ionic strengths for 1~8 mgL⁻¹ Cu(II) with eight points and was found at 0.05 V peak potential with anodic scan and increased continuously and sensitively as shown in Fig. 1A. However, in blank solution, no noise signals appeared in the cathodic scan, and there was no reduction obtained, while a more sensitive peak current was determined using stripping voltammetry. Then various commonly usable electrode types were tested for suitabil-

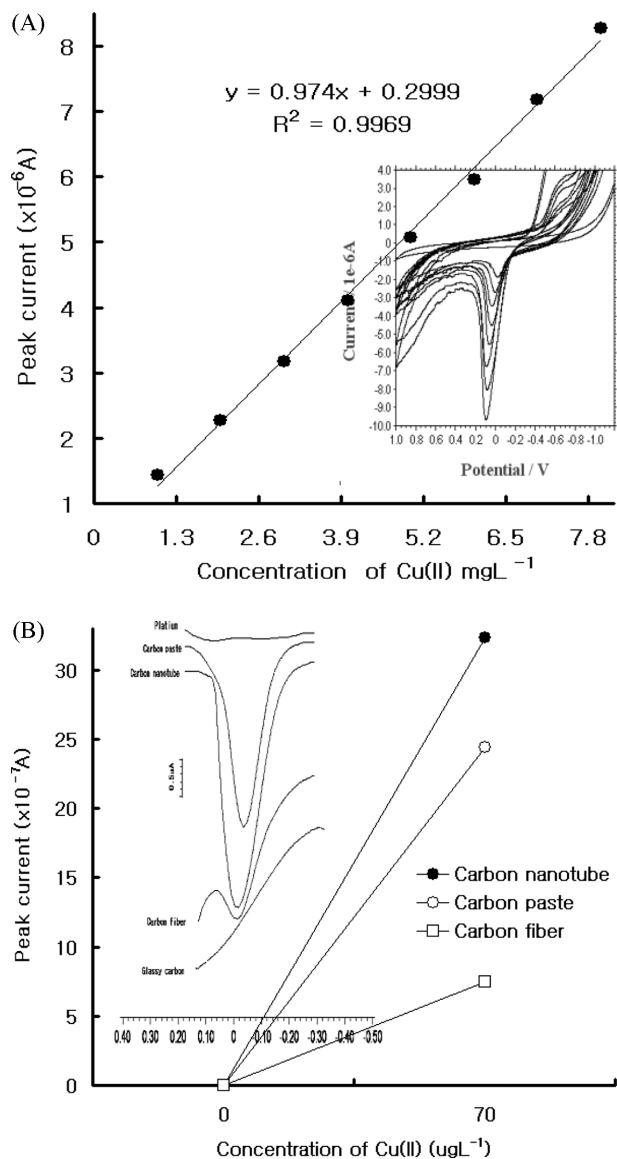


Fig. 1. (A) Cyclic voltammograms for 1, 2, 3, 4, 5, 6, 7, and 8 mgL⁻¹ of Cu(II), with 0.5 Vs⁻¹ scan rate. (B) Comparison for various common electrodes in 70 μgL^{-1} Cu(II) spike at the optimum SW condition.

ity. Fig. 1B shows three types of electrodes, and all electrodes were performed in one cell, at this condition, carbon nanotube (32.31×10^{-7} A) is sensible, carbon paste (24.44×10^{-7} A), and carbon fiber (7.45×10^{-7} A) was found and all their peak potentials appeared in the same potential, thus all other SW were examined using carbon nanotube PE.

Optimizations for the voltammetric effect for accumulation time and initial potential and other parameters.

In Fig. 2, the effect of various accumulation times was determined for the ranges of 10~190 s, with 10 points. The range of 90~110 s showed much greater signals than what

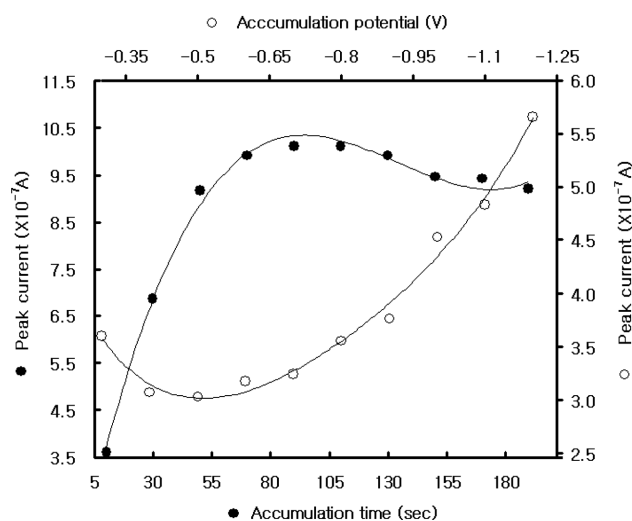


Fig. 2. SW anodic voltammograms of various accumulation times for 10, 30, 50, 70, 90, 110, 130, 150, 170, and 190 s with 1.4 ppm Cu(II) spike. Various SW accumulation potentials of -1.2 , -1.1 , -1.0 , -0.9 , -0.8 , -0.7 , -0.6 , -0.5 , -0.4 , and -0.3 V with 1.4 ppm Cu(II) spike at other conditions were used for the optimum SW condition.

was obtained for the peak current of 10.11×10^{-7} A. The greater ranges of 130–190 s did not produce much increase and peak width only showed a slow increase. Thus, all other experiments were performed with 90 s accumulation time. In these conditions, the accumulation potential was examined for the ranges of -1.2 to -0.3 V in the anodic scan, and the results are shown in this figure. From -1.2 V to -0.6 V, the heights of peak currents decreased gradually with $5.65\sim$

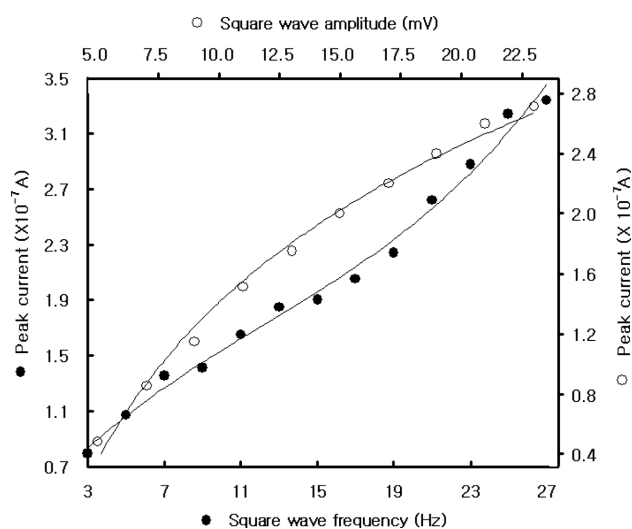


Fig. 3. SW signal from diverse frequencies of 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27 Hz with 1.4 mgL^{-1} Cu(II). The change of amplitude in 5, 7, 9, 11, 13, 15, 17, 19, 21, and 13 mV with Cu(II) of 1.4 mgL^{-1} .

3.17×10^{-7} A. Then, there were increases between -0.5 and -0.3 V with $3.03\sim 0.60 \times 10^{-7}$ A. Based on the result, -1.2 V was chosen as the optimum accumulation potential for Cu(II), other experimental parameters are the same as indicated in Fig. 3.

With the results from the earlier experiments, the response of the square-wave stripping voltammetric peak current for Cu(II) was studied in various pH levels between pH 3.65–7.51. The peak currents grew relatively gradually between pH 3.65 and 6.03 with $6.5\sim 9.46 \times 10^{-6}$ A. After that, the height of the peak currents suddenly decreased with $9.42\sim 0 \times 10^{-6}$ A. Not shown here. pH 6.03 had the highest peak current at 9.46×10^{-6} A and was determined to be the optimum pH. At this condition, the SW step potentials in the range of 1–17 mV were examined. This curve was also almost a straight line because the peak currents increased steadily with $1.37\sim 4.54 \times 10^{-7}$ A. The highest current, 4.54×10^{-7} A, at 17 mV was selected as the optimum step potential. The optimum parameters, accumulation time of 90 s, accumulation potential of -1.2 , and pH 6.03, were concerned in this examination, which were detected in previous tests. The measurements for frequency were performed with the optimum parameters detected in the previous five examinations. On the SW graph, the current curves appeared with almost the same space at around 0.05 V, which is not shown in this paper. Like other parameters, the curve from this examination is similar to a direct proportion graph in the range of $0.79\sim 3.34 \times 10^{-7}$ A as shown in Fig. 3. It is recognized that 27 Hz is the optimum frequency that had the strongest current, 3.34×10^{-7} A. As shown in figure, the peak currents of amplitude were raised and yielded an almost linear curve. The range of 5–23 mV reached at $0.48\sim 2.71 \times 10^{-7}$ A. The curves from this examination also had regular intervals and existed at around 0.05 V on the SW graph (data not shown). Among the ranges, 23 mV had the highest current at 2.71×10^{-7} A. Based on the result, 23 mV is deemed the optimum parameter for Cu(II). This examination was carried out with the above optimum parameters similar to other previous detections.

Interference test and application. At the optimized conditions, various interference-organic matters were studied by adding several other organic matters such as Cu(II), adenine, guanine, cytosine, thymine, glucose, dopamin and catechol. This examination was performed in this order with 100 mgL^{-1} Cu(II). These substances had an influence on the Cu(II) with 100%, -41.58% , 93.54% , 0.82% , 12.72% , -7.87% , 7.50% , and -7.10% . As shown in Fig. 4A, it was determined that adenine is the most effective organic matter against Cu(II) with -41.58% , 2.18×10^{-7} A. With the final results, the concentration of Cu(II) at the level of μgL^{-1} was tested for usable working ranges using the optimized conditions. Fig. 4B shows the obtained well-defined Cu(II)

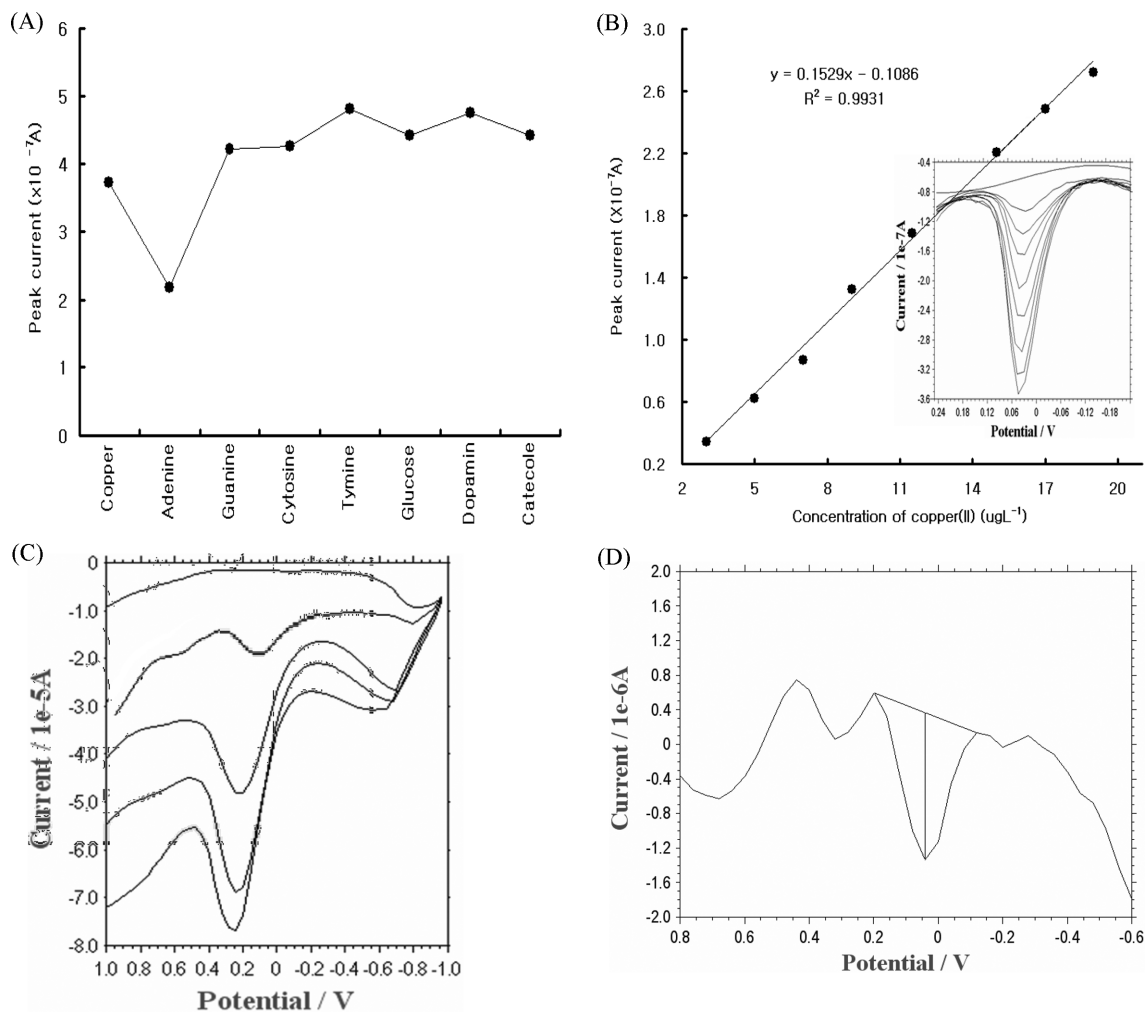


Fig. 4. (A) Interference test of Cu(II), adenine, guanine, cytosine, thymine, glucose, dopamine, and catechol in the ratio of 1 Cu(II) to 1 other materials. (B) Stripping voltammetric working ranges of 3, 5, 7, 9, 11, 15, 17, and 19 μgL^{-1} Cu(II). (C) Applications of fish kidney, first curve is blank electrolyte solution, and diluted kidney solution 1 mL spike, then standard copper ions of 1, 2, 3 ppm spike curves. (D) In real-time fish brain core, detected copper signal, 90 s accumulation time, at -1.1 V accumulation potential, frequency of 27 HZ, potential step of 17 mV, and amplitude of 23 mV. Other experimental conditions are used as in Figs. 2 and 3.

peaks following the 90 s accumulation time with microgram ranges of $3\text{--}19\ \mu\text{gL}^{-1}$, eight points, linear equation was obtained for $y = 0.1529x - 0.1086$ and precision of $R^2 = 0.9931$ ($y = \text{peak current} \times 10^{-7}$ A, $x = \text{Cu(II) concentration, } \mu\text{gL}^{-1}$) were obtained. The results were applied to the analytical utility of the sample of real fish in a contaminated pond. In this pond, live fish was obtained, from which was extracted 1.63 g of kidney sample and diluted in 0.1 M hydrochloride 5 mL solution, then examined using standard addition methods. Results are shown in Fig. 4C, at this condition, linear equation of $y = 8.79x + 0.3$, precision of $R^2 = 0.978$, results of $0.3\ \mu\text{gL}^{-1}$ in 1 ml kidney solution was obtained. A more expanded application was performed using S-company products vitamin complex with known contents of $2\ \text{mgg}^{-1}$, which was examined three times, the Cu(II) recovered from the samples had satisfactory values

ranging from 95% to 105.5%. Finally, real-time application was performed as an *in vivo* assay using anesthetized pond fish with the insertion of the electrode using a micro-hand-drill, leaving it inserted for few seconds into the fish brain, then the working electrode (3 mm diameter, 5 mm length) was inserted and dental bonding agent was coated, other reference and counter electrode was used with Ag/AgCl wire (0.2 mm diameter) and Pt wire (0.2 mm diameter), the wire-type reference and counter electrode was inserted in the fish's backbone, all electrodes were connected to electrochemical systems with 0.2 mm diameter enamel-coated copper wire. Then all experiments were performed in living state. The results are shown in Fig. 4D. Under the optimized conditions, various SW reactions were scanned giving the same peak potentials. The study showed that qualitative real-time assay *in vivo* can be carried out, and

the results usable in any direct-approach medical diagnosis.

The developed method used concentration ranges of 3–19 μgL^{-1} Cu(II) that were obtained with the use of handmade carbon nanotube PE, as were the optimized conditions for the hydrogen ionic strength of 6.0 pH, 0.1 M ammonium phosphate solution, with a 90 s accumulation time, the detection limit was achieved at 0.6 μgL^{-1} (9.4×10^{-9} M). The three-electrode system was directly inserted into a fish brain to detect copper signals without any pretreatment. The method can be used in medical diagnosis and in direct *in vivo* analysis.

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