



## Investigation of Water Safety in Non-treated Drinking Water with Trace Toxic Metals

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The trace toxic metal copper was assayed using mercury immobilized on a carbon nanotube electrode (MCW), with a graphite counter and a reference electrode. In this study, a macro-scale convection motor was interfaced with a MCW three-electrode system, in which a handmade MCW was optimized using cyclic- and square-wave stripping voltammetry. An analytical electrolyte for tap water was used instead of an expensive acid or base ionic solution. Under these conditions, optimum parameters were 0.09 V amplitude, 40 Hz frequency, 0.01 V incremental potential, and a 60-s accumulation time. A diagnostic working curve was obtained from 50.0 to 350 µg/L. At a constant Cu(II) concentration of 10.0 µg/L, the statistical relative standard deviation was 1.78% (RSD, n = 15), the analytical accumulation time was only 60 s, and the analytical detection limit approached 4.6 µg/L (signal/noise = 3). The results were applied to non-treated drinking water. The content of the analyzed copper using 9.0 and 4.0 µg/L standards were 8.68 µg/L and 3.96 µg/L; statistical values  $R^2 = 0.9987$  and  $R^2 = 0.9534$ , respectively. This method is applicable to biological diagnostics or food surveys.

**Key words:** Trace, Toxic metal, Voltammetry, Copper, Diagnosis

### INTRODUCTION

Trace copper is an essential metal for biological components of many enzymatic reactions. Nevertheless, concentrated in vivo, a high level of trace metals is associated with neurological disorders such as Alzheimer's disease, Parkinson's disease, prion diseases (1), and ischemic heart disease (2). Here metals were found in drinking water (3), food systems of agricultural soil water (4) and in vivo fluids. For in vivo or in vitro diagnostics, very low analytical detection limits (DLs) are needed. Recently, various analytical methods have been sought such as X-ray fluorescence detection (5), X-ray microprobe assay (6), atomic absorption spectrometry (7) and the energy-dispersive X-ray fluorescence method (8). Some of these spectrics, however, require high

energy amplification, pretreated sample preparation, and other detection systems. They are complicated, time-consuming, used only in laboratory conditions, and not usable for *in vivo* or *ex vivo* direct assay. On the other hand, voltammetric stripping techniques are very simple and compact. Recently, specified trace sensors were used such as infrared photo diode electrodes (9), solid paraffin-based carbon paste electrodes (10) and others. However in this study, more sensitive mercury (11) immobilized on a carbon nanotube (9) paste (12) probe was developed, the catalytic effects of which were more sensitive and with which optimum results were attained that considerably lowered the detection limit from that of previous methods. Moreover, though expensive acid or base electrolyte solutions were not used in this study, the results of this study were applied to a directly non-treated raw fluid for *ex vivo* diagnostics.

### MATERIALS AND METHODS

**Apparatus, reagent, sensors.** The voltammetric experiments were performed with the second version of Bioelectronics-2 from the authors' institution. It was combined with three electrode systems: mercury-immobilized MCW and two graphite pencils as the platinum counter and the

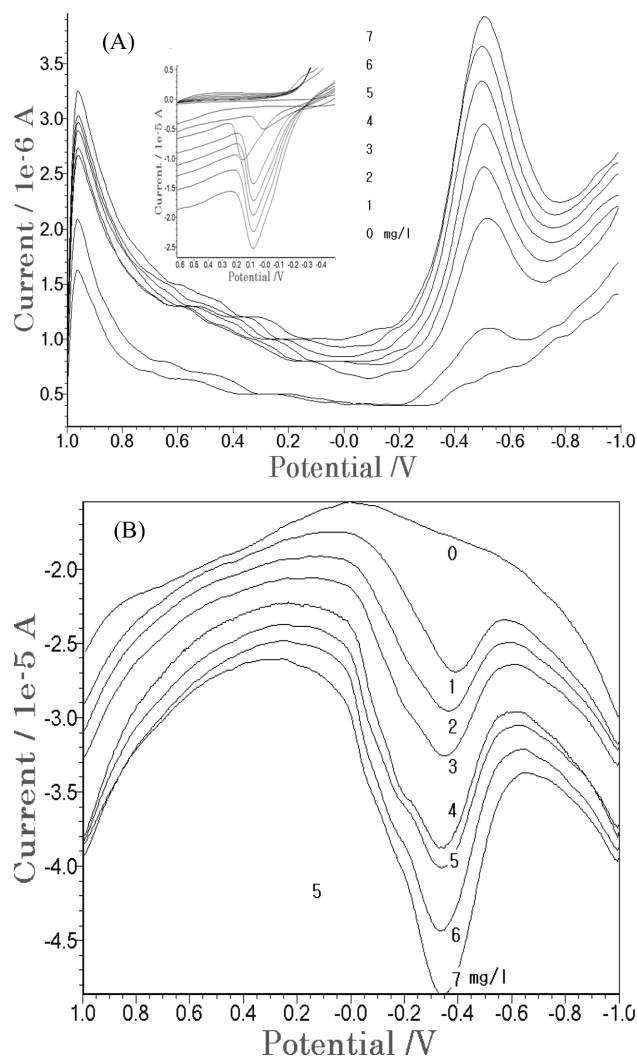
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Ag/AgCl reference, the circuit of which was a computerized, handheld compact system similar to a cellular phone and had a  $\pm 3.0$  V potential range, a 2 mA current range, and a 10 pA detection current. An atomic absorption standard 1,000 mg/L Cu(II) solution was obtained from Aldrich. All the experiment solutions were prepared with high-purity distilled water ( $18 \text{ M}\Omega\text{cm}^{-1}$ ) from a millipore water purification system. Modified MCW was prepared by mixing for 30 min weighed amounts of paste, highly purified CNT, mineral oil (Aldrich), and 1,000 ppm-standard mercury, with weight percentages of 40, 40, and 20%, respectively. Then portions of the paste were packed at the end of a handmade cylindrical tube with a 0.3 mm diameter, and a copper wire was connected to the handheld voltammetric system. All the experiments were performed at room temperature, without removing the oxygen; and under these conditions, cyclic and stripping voltammetric optimizations were performed.

## RESULTS AND DISCUSSION

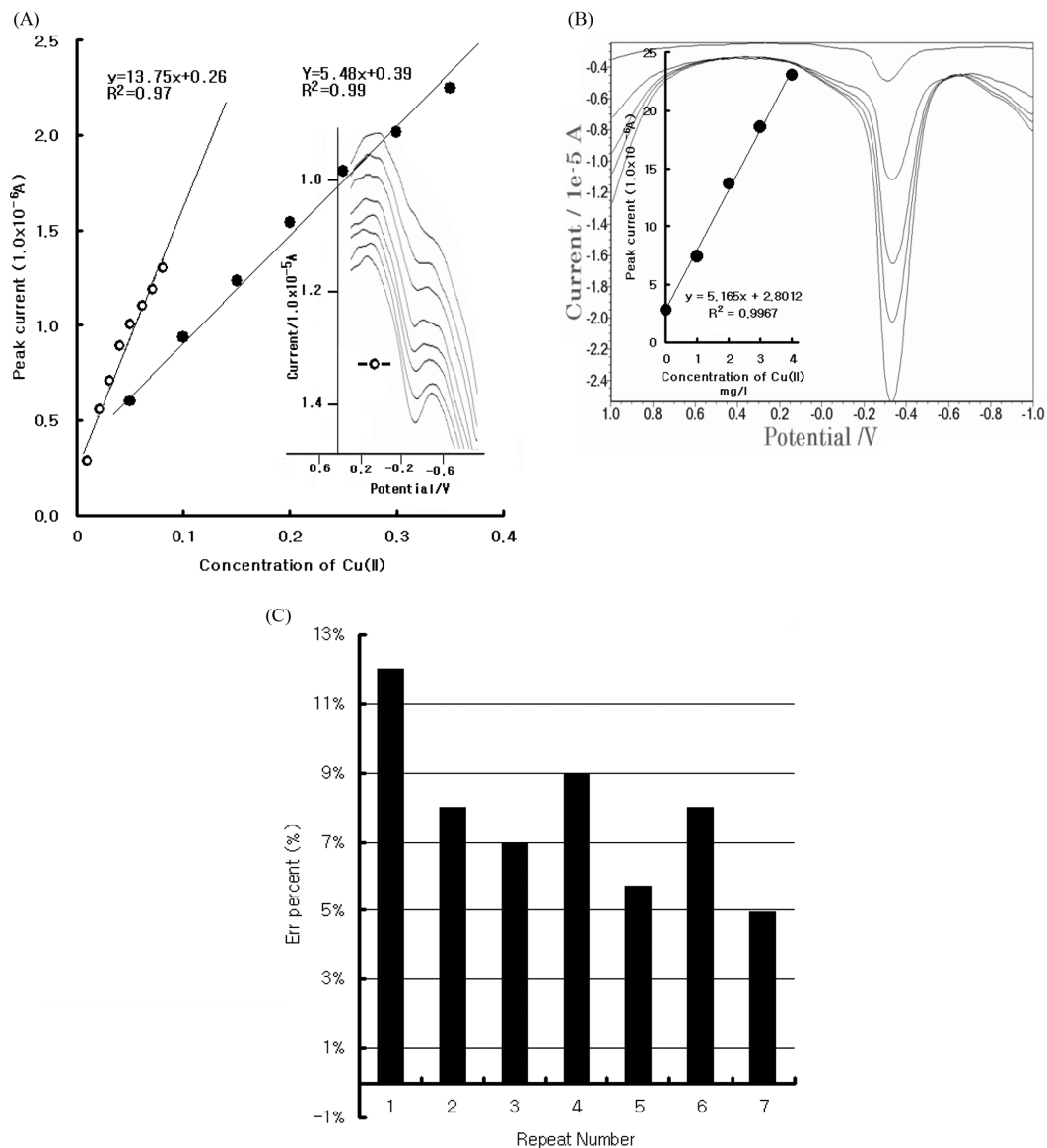
**Cyclic and stripping voltammetry.** *In vivo* or *ex vivo* surveys are dependent on the analytical detection limit. For this reason, optimum voltammetric parameters were examined via cyclic and stripping voltammetry. Moreover, better and inexpensive tap water was used instead of the expensive electrolyte using 10 ml non-treated water with  $-2$  V initial  $+2$  V switching potentials and a  $0.5$  mV/s scan potential. The cyclic peak potentials are shown in the Fig. 1A inset curves, and the main curves show the results of the cathodic stripping voltammetry, wherein the water blank is simple and no peak appeared. Thus, the  $1$  mg/L Cu(II) standard  $1,000$  ml concentration was spiked with a  $0$ -sec accumulation time, while there was no peak. Thus, a  $30$ -sec accumulation time was used. The second curve is that of  $0.319 \times 10^{-6}$  A, where a  $-0.5$  V cathodic potential appeared, after which spiking was continued at a  $2\sim 7$  mg/L standard. The statistical linear equation was  $y = 0.302x + 0.247$ , and the relative standard was  $R^2 = 0.955$ . Under these conditions, anodic stripping voltammetry was performed, and the voltammetric results are shown in Fig. 1B. The spiking was varied at  $1, 2, 3, 4, 5, 6,$  and  $7$  mg/L, and the peak current increased continually from  $0.549$  to  $1.751 \times 10^{-6}$  A. The working curve was  $y = 0.2x + 0.2999$ , and the statistic was  $R^2 = 0.9844$ , which is usable for high ranges, though reduction stripping is more sensitive and a big current appeared. Cyclic oxidation is also better than reduction. Under these conditions, the stripping voltammetric optimization was examined, and the final results were a  $0.09$  V amplitude, a  $40$  Hz frequency, a  $0.01$  V incremental potential, and a  $60$ -sec accumulation time. These conditions were used to determine the analytically possible interference effects, and were calibrated using standard addition methods. Under these conditions, the analytical working ranges and diagnostic applications were used.



**Fig. 1.** Sensor properties. (A)  $1\sim 8$  mg/L Cu(II) variation with a cathodic SW; the inset curve is the CV for a  $1\sim 7$  mg/L continuous spike. (B)  $1\sim 7$  mg/L variations via SW anodic stripping, under a 10 ml tap water electrolyte. For the CV, the following conditions were used: a  $-2.0$  V initial potential, a  $2.0$  V switching potential, and a  $0.5$  V/s scan rate. For the SW, the optimum parameters were used.

### Analytical working ranges, application, and statistics.

Under the optimized SW conditions, the analytical working ranges were examined using a  $120$  s accumulation time. Fig. 2A shows the two ranges of the working curves. The first curve was obtained at  $10, 20, 30, 40, 50, 60, 70,$  and  $80$   $\mu\text{g/L}$ . The inset voltammograms show the anodic stripping, wherein the statistic slope was  $\Delta x/\Delta y = 13.75$ , and the relative standard deviation was  $R^2 = 0.97$ , which was applied to drinking water. In this figure, a higher range was obtained at  $50, 100, 150, 200, 250, 300,$  and  $350$   $\mu\text{g/L}$ , and the slope sensitive was  $\Delta x/\Delta y = 5.48$  and the relative standard deviation,  $R^2 = 0.99$ . That can be used for food composition and concentrated soil analysis. Fig. 2B shows the applications

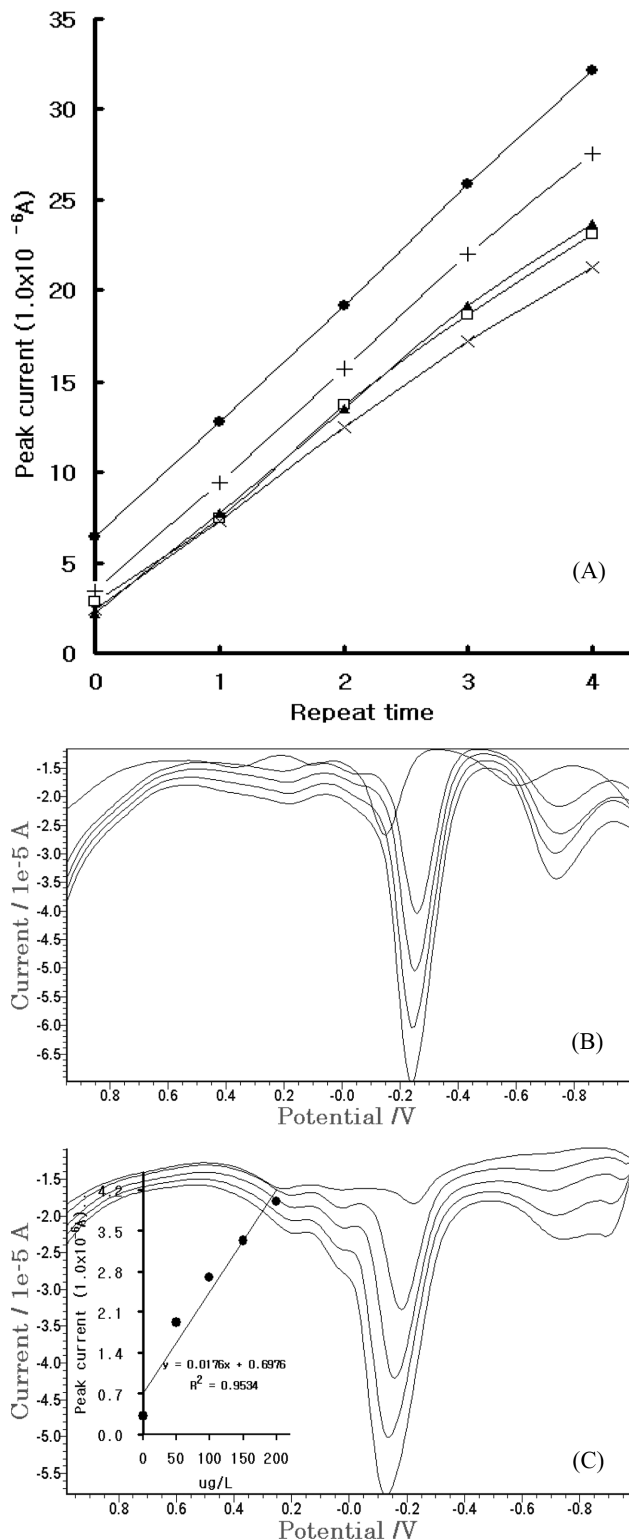


**Fig. 2.** (A): Square wave micro-working range of 50~350 ppb  $\mu\text{g/L}$  and 10~80  $\mu\text{g/L}$  Cu(II) variations. (B): Standard addition method for 1.0 mg/L Cu(II) in tap water with a pH of 7.0, using an SW amplitude of 0.08 V, a frequency of 25 Hz, an incremental potential of 10 mV, an accumulation potential of  $-1.1$  V, and an accumulation time of 120 s. (C): statistic 7th repeated standard addition methods of a 1 mg/L tap water constant.

for the contaminated water sample, which was fixed by a solution that contained the standard 1 mg/L Cu(II), in which 5 ml of the known sample and 5 ml of the tap water solution were stripped under the optimum parameters. Anodic stripping is also shown in this figure. The first curve shows a  $-0.4$  V peak potential with  $2,412 \times 10^{-6}$  A obtained, after which the 1,000 mg/L standard 0.01 ml spiked sequential anodic voltammograms increased at the same  $-0.4$  V potential from 7.31, 12.49, 17.18, and  $21.29 \times 10^{-6}$  A. The statistical working curve was  $y = 5.16x + 2.80$ ,  $R^2 = 0.99$  and the error rate was 9%. 1.09 mg/L Cu(II) obtained, the results were sensitive, and the peak voltammograms were linearly

sharp. They can be used for food or any other system. The probe was statistically examined, and the 7th repeated standard addition methods were examined using a 1 mg/L tap water constant. Fig. 2C shows the results on a histogram. Each of the results was calculated at 12, 8, 7, 9, 6, 8, and 5% error rates, and the relative standard deviation was 0.023. Under these conditions, a real sample was examined to develop a commercial-market food drinking water.

**Standard Verification and Applications for non-treated drinking water.** The developed results were used to detect the trace range in the copper-rich soil, at which site



**Fig. 3.** (A) SW application for standard addition by a copper-rich soil radish using five different purchased samples. (B) Market drinking water sample of cola. (C) Soda pop using an SW amplitude of 0.09 V, a frequency of 40 Hz, an incremental potential of 10 mV, an accumulation potential of  $-1.1 \text{ V}$ , and an accumulation time of 60 s.

the same samples were examined five times. Fig. 3A shows a picture of the 5th statistical analysis results for the radish sample for the different purchased soil samples. The calculated values were 0.93, 0.98, 1.08, 1.09, and 1.12 mg/L, which indicate that the same slopes and the same sensitivity can be applied to other types of soil. Then more advanced applications were examined. Fig. 3B shows the following standard addition methods for market cola. The first curve represents the 5 ml electrolyte with 9.0  $\mu\text{g/L}$  Cu(II) contain and 5 ml drinking cola that had a voltammogram, for a  $1.61 \times 10^{-6} \text{ A}$  peak current at a  $-0.18 \text{ V}$  peak potential. Thus, the sequential 0.5, 1.0, 1.5, and 2.0 mg/L standards were spiked, and a continuous peak current was obtained from 2.67, 3.61, 4.66, and  $5.52 \times 10^{-6} \text{ A}$ . The linear equation was  $y = 0.019x + 1.651$ , the statistic was  $R^2 = 0.9987$ , and the containing was 8.68  $\mu\text{g/L}$ . A more advanced experiment was performed directly on a real non-treated water sample. Fig. 3C shows raw voltammograms of commercial drink solutions for soda pop. The voltammetry was directly performed using 4.0  $\mu\text{g/L}$  Cu(II) contained non-treated water. First, an undiluted 10 ml original fluid was oxidized via stripping voltammetry. The voltammogram is shown in this figure. The first curve shows the same potential of 0.2 V and the current of  $0.311 \times 10^{-5} \text{ A}$ . Thus, standard 50, 100, 150, and 200  $\mu\text{g/L}$  sequence spiking procedures were performed, and the oxidation peaks appeared at 1.93, 2.69, 3.35, and  $4.01 \times 10^{-6} \text{ A}$ , the statistic linear equation was  $y = 0.0176x + 0.6976$ , the relative standard deviation was  $R^2 = 0.9534$ , and the calculated value was 3.963  $\mu\text{g/L}$ . Thus, the developed methods can be applied directly using carbonated soda drinks.

The novel experiments described in this paper showed that trace metal ranges in MCW can be investigated for drinking water systems. Under optimum conditions, the developed parameters were a 40 Hz SW frequency, a 0.01 V incremental SW potential, an 0.08 V amplitude, and a 1.0 V anodic accumulation potential. These conditions, the simplified probe sensors were shown to be more sensitive than common spectro photometric and voltammetric methods. The handmade combination sensors were successfully used to directly detect trace metals in non-treated raw water without using any electrolyte.

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