Diagnostic *Ex-vivo* Assay of Metal Gold in Rat Droppings Using Voltammetry

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Abstract : Diagnosis with an *ex-vivo* gold sensor was done using a modified fluorine-doping sensor, and cyclic voltammetry (CV) redox potentials of 0.4 V anodic and -0.2 V cathodic were obtained. Both peak currents were optimized using square-wave (SW) stripping voltammetry, and an analytical working range of 10-80 ug/L SW was attained. The precision of the 10-mg/L Au was 0.765 (n=8) RSD under the optimum conditions, and the analytical detection limit approached 0.006 ug/L (S/N=3) with only a 60 sec accumulation time. The developed method was used to examine the mouse droppings for medicinal diagnosis.

Keywords : voltammetry, gold, dropping, ex vivo.

1. Introduction

In cancer therapy, conjugated gold nano particles are commonly used for diagnosis or cell destruction, such as for colorimetric-cancer detection [1], plasmon resonance scattering metal-colloids detection [2], gold nanorod conjugated receptor for oral cancer marker [3], and gold nanoparticle-catalyzed luminol [4]. Recently *In-vivo* therapy and the ex vivo trace-watching techniques are dependent on the photometric or resonance imaging methods, but these methods cannot be used for sensitive diagnostic analysis. Electrochemical trace assays are currently being performed for laboratory conditions [5,6,7] but are not applicable for organic or *ex-vivo* applications, for the aforementioned reason. In this study, a new type of modified fluorine-doping sensor [8] for SW stripping voltammetry that has a lower detection range than the common methods was sought. The fluorine-coated structure has accelerated ionic accumulation and catalytic specifications. Moreover, inexpensive graphite counter and reference three-electrode systems were used for gold assay, which can be applied to *ex-vivo* diagnostics for the known edible gold.

2. Experimental

2.1. Apparatus, Reagents, and Procedure
A voltammetric experiment was carried out using a bioelectronics-2 circuit from the authors' institution. The second version was
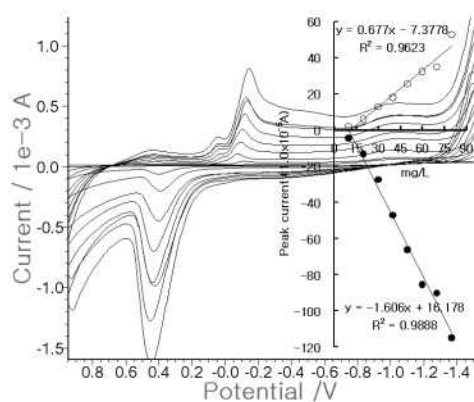
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fabricated as a computerized handheld voltammetric system whose size is similar to that of a typical cellular phone. It can be used for the bio assay and sensor techniques for individual and laboratory applications. The fluorine-doped graphite working electrode (FGE) was prepared by coating the pencil with fluorine standard. Moreover, two inexpensive pencils served as the Ag/AgCl reference and platinum counter electrodes, respectively. The supporting electrolyte that was used was deep seawater instead of an expensive electrolyte. All the reagents were prepared from analytical-grade chemicals (Aldrich), and the experiments were carried out on adult(250-270 g) rats. This study was approved by the Ethics Committee of the authors' institution and conforms with the Guide for Laboratory Animals (Korea MIFAF, revised 07.7.9).
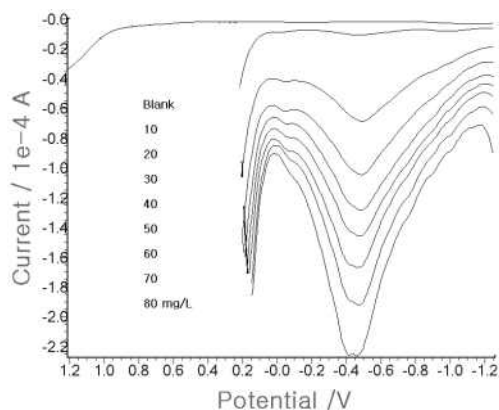
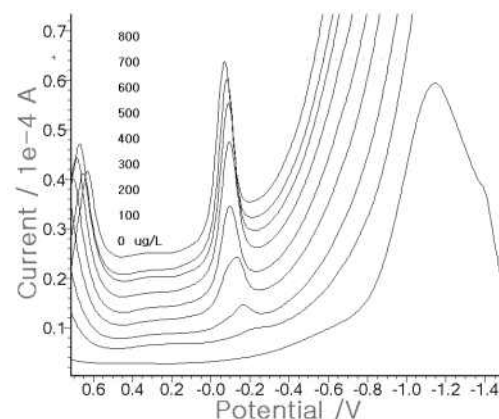## 3. Results and Discussion

3.1. Cyclic and Stripping Potentials

For the reaction potential, reversed scan was performed using seawater electrolyte solution, and cyclic voltammetry was performed from -2.0 to 2.0 V reverse potential. The electrolyte blank was also simple, and no current appeared. Fig. 1(A)

(b)

(c)

Fig. 1. (A) CV redox voltammograms, with a scan rate of 100 mV/s for seawater and with 2.0 V initial potential, 2.0 V switching potential, and spiking concentration variations of 10, 20, 30, 40, 50, 60, 70, and 80 g/L Au. (B) SW anodic-concentration variations of 10, 20, 30, 40, 50, 60, 70, and 80 g/L Au spike. (C) Cathodic variations of 100, 200, 300, 400, 500, 600, 700, and 800 g/L Au. The other parameters were under optimum conditions.

(A)

shows a high range of variations for reverse scan, from 0 to 80 mg/L add. The first peak was linear and simple, then a 10 mg/L spike was obtained for 0.4 V oxidation and -0.2 V reduction peak potentials. Linear spiking was

then obtained at the -4.54×10^{-5}A$\sim$ -114.80×10^{-5}A oxidation peak currents, and the reverse scan increased to 1.87×10^{-5}A$\sim 53.02 \times 10^{-5}$A cathodic. The positive scan was twofold more sensitive than the negative current, and the positive scan of the redox voltammograms are usable for stripping scan. Stripping voltammetry was thus performed using an FGE sensor. Fig. 1(B) shows the results of the anodic stripping voltammetry for the 10 to 80 mg/L Au variations, only 0 sec accumulation time was used, but the peak potential was shifted to negative and increased to 8.124×10^{-5}A$\sim 14.260 \times 10^{-5}$A. The peak width was sensitive and usable for optimization. More sensitive cathodic stripping was then performed, as shown in Fig. 1(c), for the 100 – 800 ug/L spike. The potential window that was used was 1.8 V initial potential, the final potential was –1.9 V cathodic, the deposition time was 60.0 sec, the peak current was obtained at 0.51×10^{-6}A$\sim 32.24 \times 10^{-6}$A, the linear equation was y=0.06x–5.19, and the statistic was R^2=0.95, whose working range can be used only for *ex-vivo* diagnostics. A more sensitive range was thus sought through stripping optimization.

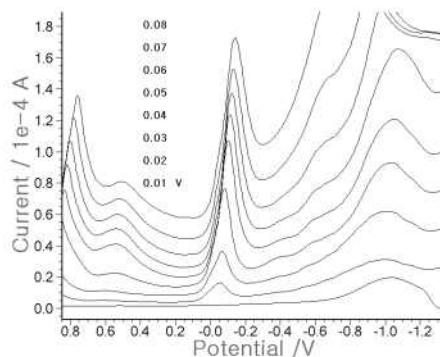### 3.2. Stripping Voltammetric Optimization

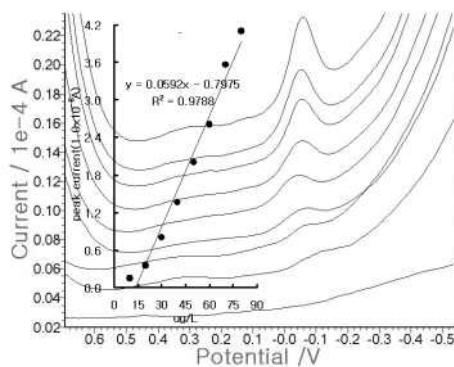Using cathodic stripping, the following optimum conditions were examined. Fig. 2(A)

(b)

(c)

(d)

(a)
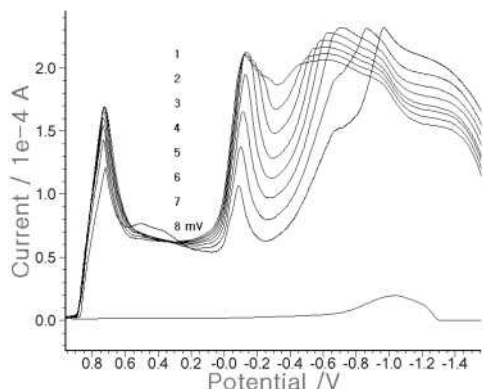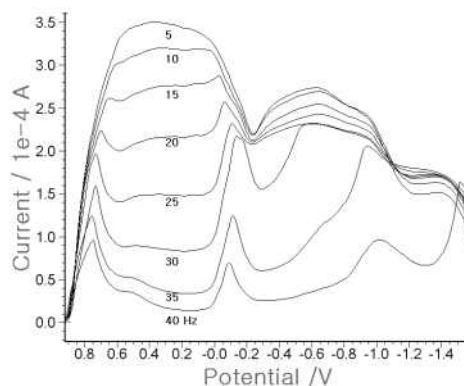
Fig. 2. (A) Peak current of varying SW increment potentials for 0.001, 0.002, 0.003, 0.004, **0.005,** 0.006, 0.007, and 0.008 V. (B) SW frequencies of the 5, 10, 15, 20, 25, 30, 35, and 40 Hz variations. (C) SW amplitude variations for 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 V by the 10.0 ug/L Au constant.

shows the increment potential for the 0.001 – 0.008 V variations with 8 points. The peak current was varied to 1.94 ~ 2.88x10^{-5}A, where 0.006 V was big and maximum, whose peak width narrows. Thus, the 0.006 V increment potential was fixed at this point, and the SW frequency variations were examined up to 5~40 Hz using the same concentration. Fig. 2(B) shows raw voltammograms, and the peak currents were varied to 1.99 ~ 3.16x10^{-5}A. As 10 Hz is more sensitive, however, than the other points, and as the peak width was sharp, the 0.006 V increment potential and the 10 Hz frequency were fixed under both conditions. The SW amplitude was examined, and its real voltammograms are shown in Fig. 2(C), where the peak current varied from 0.29 to 1.78x10^{-5}A and 0.08 V was big. The optimum parameters were thus fixed at 0.006 V increment potential, 10 Hz frequency, and 0.08 V amplitude. Under these conditions, the micro working ranges were examined. Fig. 2(D) shows the results for the 0.01 to 0.08 ug/L Au variations, and their slope ratio of $\triangle x/\triangle y=0.0592$, whose response was very sensitive at the precision of R^2=0.9788. The estimated detection limit was 0.006 ug/L, based on the signal-to-noise ratio(S/N=3). For the stability of FGE, the statistics were examined using 1 mg/L Au spike with 15-time repetition. The peak current varied from 3.54 to 4.03x10^{-5} A, and the standard deviation was 0.38, which is stable for application purposes. These results can be used for *in-vivo* or *ex-vivo* applications.

3.3. Analytical Statistics and *ex-vivo* Applications

One week before the use of rat forage, 1000 mg/L standard of a 10-ml solution was fed to the experimental rat for one day. Overnight, a rate of 5 g dropping was obtained, which was diluted to a 10-ml concentrated nitric-acid solution and 10-ml water. Under optimum conditions, the

dropping solution was examined via SW stripping anodic and cathodic voltammetry. Fig. 3 shows the results of the use of the standard addition methods for anodic. The first sample obtained 0.42x10^{-5} A, then the 0.1, 0.2, and 0.3 mL standard spikings were 1.89, 3.49, and 5.49x10^{-5} A with –0.6 V. It yielded a good result of 0.89 ug/mL, with 85.0 % (n=5) recovery. Also cathodic was obtained same results.
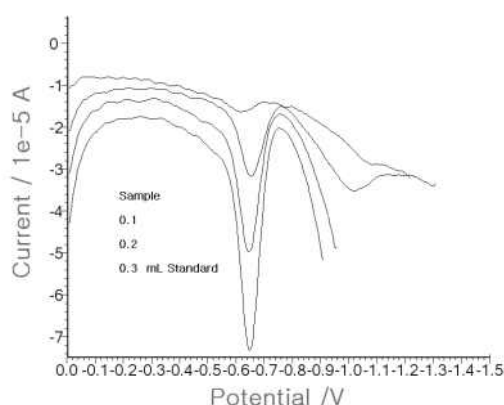

Fig. 3. The *ex-vivo* applications for the rat droppings in the 10 ml electrolyte. The first curve is the 0.1 1 sample spike, followed by the 0.1, 0.2, and 0.3 ml standard add, under optimum SW conditions.

4. Conclusions

In this study, fluorine-doped graphite working, graphite counter, and reference electrodes were used. These inexpensive three-electrode systems were found to be stable in gold assay and more sensitive than the common methods. The optimized SW analytical conditions were 0.006 V increment potential, 10 Hz frequency, and 0.08 V amplitude. It had lower detection limits compared to the micro range. Moreover, the analytical detection time that was used was only 60 sec accumulation time. This indicates

that FPE is useful for *ex-vivo* or other diagnostic applications for therapeutic conditions.

References

1. W. Lu, S. R. Arumugam, D. Senapati, A. K. Singh, T. Arbneshi, S. A. Khan, H. Yu and P. C. Ray, Multifunctional Oval-Shaped Gold- Nanoparticle-Based Selective Detection of Breast Cancer Cells Using Simple Colorimetric and Highly Sensitive Two- Photon Scattering Assay, *ACS Nano,* **4**, 1739 (2010).
2. R. Hu, K. T. Yong, I. Roy, H. Ding, S. He and P. N. Prasad, Metallic Nanostructures as Localized Plasmon Resonance Enhanced Scattering Probes for Multiplex Dark-Field Targeted Imaging of Cancer Cells, *J. Phys. Chem. C*, 113, 2676 (009).
3. X. Huang, I. H. Sayed, W. Qian and M. A. Sayed, Cancer Cells Assemble and Align Gold Nanorods Conjugated to Antibodies to Produce Highly Enhanced, Sharp, and Polarized Surface Raman Spectra: A Potential Cancer Diagnostic Marker, *Nano lett,* **6**, 1591 (2007).
4. Z. F. Zhang, H. Cui, C. Z. Lai and L. J. Liu, Gold Nanoparticle-Catalyzed Luminol Chemiluminescence and Its Analytical Applications *Anal. Chem.* **77**, 3324 (2005).
5. M. Pumera, M. Aldavert, C. Mills, A. Merkoc¸I and S. Alegret, Direct voltammetric determination of gold nanoparticles using graphite-epoxy composite electrode, *Electrochim Acta* **50**, 3702 (2005).
6. A.C. Almon, Trace multi element determinations in reactor moderator water: simultaneous determination of copper, gold, silver and mercury using differential-pulse stripping voltammetry, *Analy Chim Acta,* **249**, 447 (1991).
7. N. Gan, Z. Wang and X. U Jianguo, Amperometric detection of gold by differential pulse voltammetry using a DNA biosensor, *Rare Metals,* 26, 169 (2007).
8. S. Y Ly and J. H Lee, Human-Urine Diabetes Assay and In Vivo Rat Bladder Assay Using a Fluorine-Doped Carbon Nanotube Catheter Sensor, *Ann Biomed Eng,* 37, (2009).