

Voltammetric Assay of Silver Ions in Frog's Tissue

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Abstract : The electrochemical analysis of silver ion was performed using cyclic voltammetry (CV) and square-wave (SW) stripping voltammetry, and electrode cell systems were fabricated with graphite pencil electrode (GE) of working, reference and counter electrodes. Also electrolyte was the use of sea water as electrolyte solutions instead of ionic controlled solutions. The optimum analytical conditions for the cyclic and stripping parameters were determined using GE. The results approached the microgram working ranges of SW(ug/L) and CV(ug/L) Ag, and the optimum conditions were applied to frog's tissue and the food samples.

Keywords : Cyclic voltammetry, Square wave stripping, graphite, frog's tissue

1. Introduction

Inexpensive electrochemical voltammetric methods of detecting silver ion of Ag(I) have been developed. Usually Ag/AgCl electrodes and a platinum wire electrode are used as reference and auxiliary electrodes, respectively.

In this study, three graphite pencils were used as working, reference, and counter electrodes. The Ag/AgCl electrode and a platinum wire electrode are expensive, but graphite pencil is cheap and can be easily obtained. Renewable graphite pencil writing devices have been available for many years [1]. Graphite has been used as an electrode

system, is renewable and has the lowest cost, and can effectively detect materials. Examples are the inexpensive and renewable pencil electrode used in field-based stripping voltammetry [1], the GE electrode used for the voltammetric-behavior and square-wave voltammetric determination of trepibutone [2], and the pencil-lead bismuth-film electrodes for the determination of trace metals via anodic stripping voltammetry [3], moreover, the base and acid solution is often used as an electrolyte solution. This ionic solution is expensive and can contaminate the water controls. However sea water was used as electrolyte solutions in this paper, as it is cheap and good for the environment.

The toxicological-tolerance level of silver compounds in man is rather low (about 0.05 mg = L chronically) [4]. Further, silver has

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many technical uses, such as in photography, dentistry, and electronic compounds. It is also increasingly being used in the field of medicine and in cosmetics. Topical lidocaine in silver sulfadiazine cream has been reported to be capable of alleviating pain due to cancer or treatment-related painful skin conditions [5]. Moreover silver-based epithelial carcinoma cell lines silver [6] is highly effective in cytoselective anticancer therapies and were found in the brain tissues of the patient [7], herein various diagnostic-detection methods were investigate this purpose such as discontinuous-flow analysis[8], flame atomic-absorption spectrometry [9], flow injection analysis [10,11], ellipsometric XPS analysis [12,13], and electron spectroscopic analysis [14].

Which spectrometrics are complicate and expensive, however electrochemical voltammetric methods have also been developed in several studies. such as voltammetric detection of Ag and Hg₂ [15], behavior of silver ions in a carbon paste electrode [16], boron-doped diamond electrode [17], and microparticles Voltammetry screening method [18]. Also these methods involve complicated and cheap preparation techniques require. Thus simple GE techniques and expensive sea water electrolyte were used in this study, and the redox peak current was attained at a low detection limit. It can be applied to a frog's leg and to eundan, a supplement that helps to in vivo diagnostic assay.

2. Experimental Device and Electrode Preparation

Electrochemical instruments were used with the new Bioelectronics-1 system, which was first constructed at the authors' institute, the new version is a computerized handheld voltammetric sensor with a 2.4 V potential range, a 2 mA current range, a 10-pico A

measuring current, and a 5"4"1" instrument size, and which uses a rechargeable battery or external power and has a USB port interface with a PC. The instrument is as big as a typical cellular phone, and it can be used for the bioassay and sensor techniques for individual and laboratory applications, graphite (DongA XQ, ceramic, 60 mm, 0.9 B) was used as working and reference electrodes and as an auxiliary electrode.

3. Results and Discussion

3.1 Cyclic voltammetry and electrode comparison

First, sea water was used as electrolyte solutions. Usually, the Ag/AgCl and platinum wire electrodes served as the reference and auxiliary electrodes, respectively. Graphite pencil electrode was used in this solution, however, on their behalf. The working and counter electrode was also graphite. Fig. 1(A) shows the CV result using the three GE systems. No signal was obtained in the blank seawater solution. Here, 10 to 90 mg/L Ag was spiked. When 10 mg/L Ag was spiked, a small peak current (8.724×10^{-5} A) was obtained at -0.8 and -1.3 V, but when 20, 30, 40, 50, 60, 70, 80, and 90 mg/L Ag were spiked, then 13.76, 17.38, 20.65, 22.66, 23.27, 28.47, 31.11, and 44.46×10^{-5} A peak currents were obtained, respectively.

These results show that the more Ag was spiked, the higher the peak current was obtained. Fig. 1(A) and (B) show the voltammograms and the linear curves of the oxidation and reduction currents. The two curves above the x axis represent the reduction results. The linear equation of the first curve was $y = 0.4335x + 2.7586$, and the precision was $R^2 = 0.9766$. The linear equation of the second curve was $y = 0.1133x + 4.5511$, and the precision was $R^2 = 0.6949$. The others are the oxidation results. The linear equation of the third curve was $y = 0.5842x - 8.1822$, and the

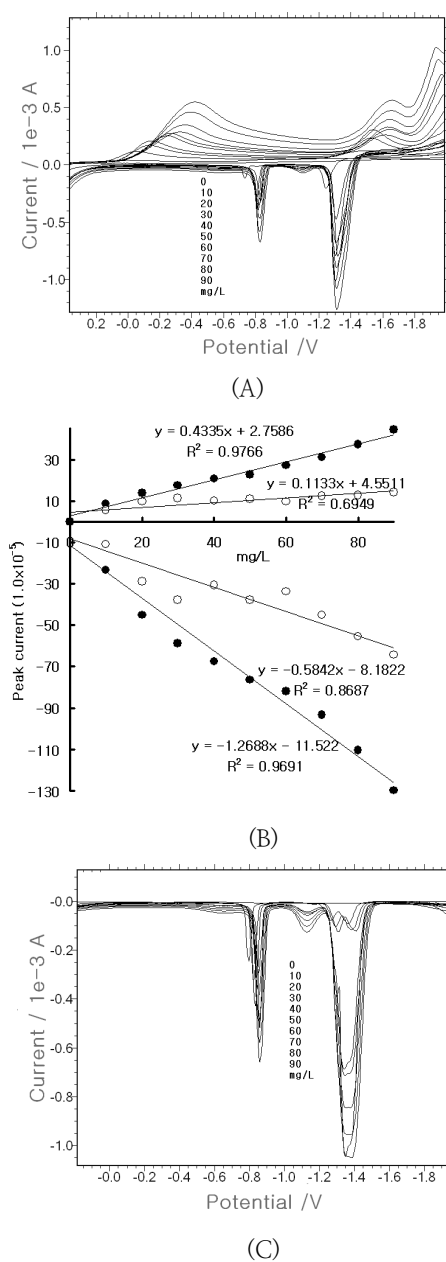


Fig. 1. (A) and (B): CV effects using GE with 0 to 90 mg/L Ag (I) variation. (C): SW Ag variation for the electrolyte solution consisting of GE (0-90 mg/L added) in seawater, using the optimum parameters.

precision was $R^2=0.8687$, the lineare quation of the fourth curve was $y=-1.2688x-11.522$, and the precision was $R^2=0.961$, the slopes of the third and fourth curves were sharper than those of the first and second. This means that oxidation is more sensitive than reduction. Fig. 1(C) shows the SW stripping voltammetry result when the graphite electrode was used, the peak current appeared at -0.8 and -1.3 V. Moreover, 10 to 90 mg/L Ag were spiked, and no signal was obtained in the blank sea water solution. First, 10 mg/L Ag was spiked, and a -24.16×10^{-5} A peak current was obtained. When 20, 30, 40, 50, 60, 70, 80, and 90 mg/L Ag were spiked, then -32.09 , -42.22 , -50.38 , -28.03 , -37.01 , -47.12 , -55.15 , and -62.37×10^{-5} A peak currents were obtained, the peak current declined until when 40 mg/L Ag was spiked. It suddenly rose when 50 mg/L Ag was spiked, but it decreased again, continuously, until when 90 mg/L Ag was spiked.

For the optimum conditions, the SW parameters were determined using a graphite electrode. Fig. 2(A) shows the SW amplitude variations for eight points in the 90 mg/L Ag spike. In this experiment, 10 ml seawater was used as an electrolyte solution, when 0.05 V amp was given, a -13.87×10^{-5} A peak current was obtained. After that, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, and 0.4 V amp were given, and -202.1 , -262 , -293.9 , -319.7 , -310.1 , -295.2 , and -274.5×10^{-5} A peak currents were obtained. As the maximum current was obtained at 0.25 V, it was chosen as an optimum condition.

Fig. 2(B) shows the SW increment potential variations for eight points in the 90 mg/L spike, when a 0.005 V increment was given, a -102.3×10^{-5} A peak current was obtained. After that, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, and 0.04 V increments were given, and -105.1 , -105.1 , -103.6 , -101.7 , -111.4 , -112.2 , and -109.2×10^{-5} A peak currents were obtained. As the maximum current was obtained at the 0.035 V increment, 0.035 V was chosen as an optimum condition, besides

the optimum condition of the amplitude and the increment, the other parameters of SW frequency, initial potential, and time accumulation were examined, final results were 10 Hz, -1.5 V, and 250 s, respectively (not shown here). Under these conditions, the analytical working ranges were examined.

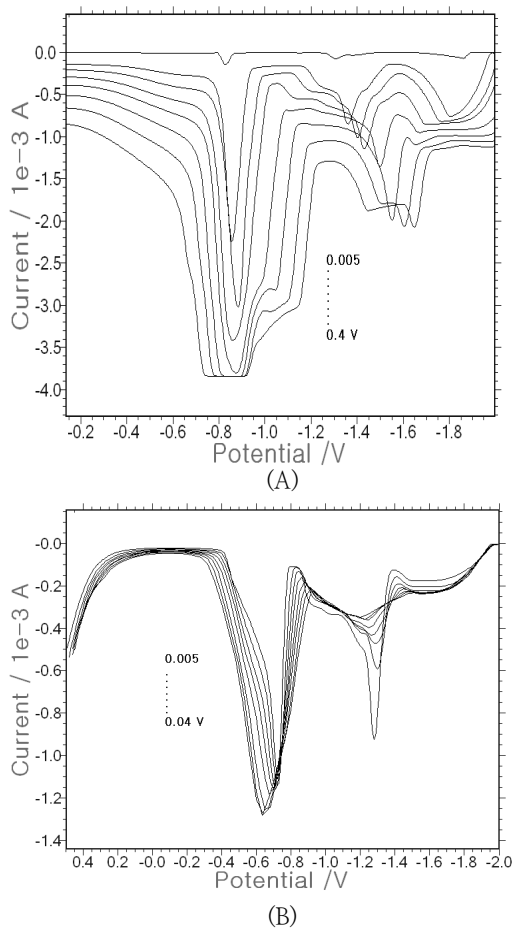


Fig. 2. (A): SW amplitude variations from 0.05 to 0.35 V. (B): SW increment potentials from the 0.01 to 0.04 V variations.

3.2. Working Ranges, Statistics, and Application

Under the optimum conditions, the analytical working ranges were examined using a graphite electrode. Fig. 3(A) shows the CV

results of the microgram variation from 100 to 800 $\mu\text{g/L}$ Ag. When 100 $\mu\text{g/L}$ Ag was spiked, no signal was obtained, but when 200 $\mu\text{g/L}$ Ag was spiked, the oxidation of the 40.64×10^{-5} A peak current was attained. When 300, 400, 500, 600, 700, and 800 $\mu\text{g/L}$ Ag

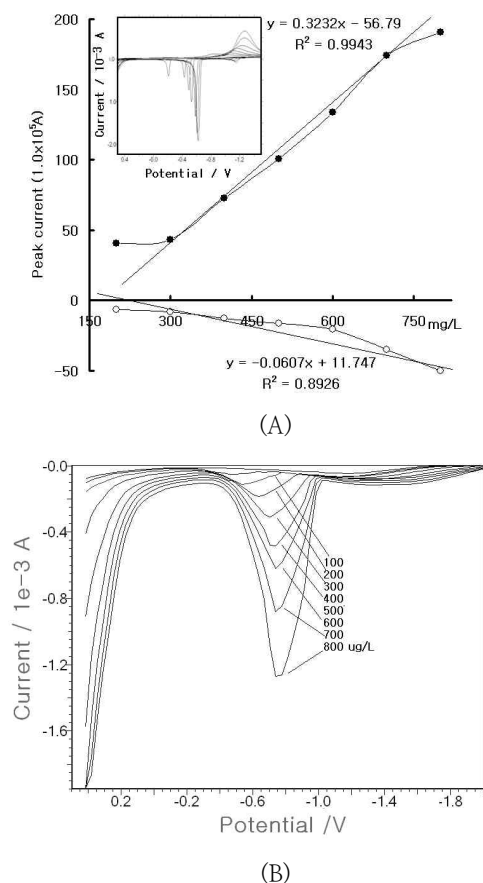


Fig. 3. (A): CV variations for the 100, 200, 300, 400, 500, 600, 700, and 800 $\mu\text{g/L}$ Ag spikes in the seawater solution, under the optimum conditions. (B): SW anodic variations for the 100, 200, 300, 400, 500, 600, 700, and 800 $\mu\text{g/L}$ Ag spikes in the seawater solution, under the optimum conditions. (C): SW anodic variations for the 10, 20, 30, 40, 50, 60, 70, and 80 $\mu\text{g/L}$ Ag spikes in the seawater solution.

were spiked, the 42.91, 72.84, 100.7, 133.5, 174.2, and 190.9×10^{-5} A peak currents were obtained. The width of the Ag peak narrowed at 0.6 V, with a slope sensitivity of $y=0.3232x-56.79$ and a precision of $R^2=0.9943$. Moreover, -0.357 , -8.317 , -12.98 , -16.43 , -20.41 , -34.97 , and -49.84×10^{-5} A peak-current reductions were obtained, individually, when 200, 300, 400, 500, 600, 700, and 800 $\mu\text{g/L}$ Ag were spiked, the linear equation was $-0.0607x+11.747$, and the precision was $R^2=0.8926$. Fig. 3(B) shows the SW stripping voltammetry result of the microgram variation from 100 $\mu\text{g/L}$ to 800 $\mu\text{g/L}$. When 100 $\mu\text{g/L}$ Ag was spiked, a 2.47×10^{-5} A peak current was obtained. When 200, 300, 400, 500, 600, 700, and 800 $\mu\text{g/L}$ Ag were spiked, 7.994, 14.57, 26.31, 42.47, 55.08, 80.32, and 117.2×10^{-5} A peak currents were obtained. Fig. 3(C) shows the SW stripping voltammetry results in various lower concentrations. All in all, 10- to 80- $\mu\text{g/L}$ Ag were spiked. When 10 $\mu\text{g/L}$ Ag was spiked, no signal was obtained, but when 20- $\mu\text{g/L}$ Ag was spiked, a 0.1191×10^{-5} A signal was obtained. After that, 30-, 40-, 50-, 60-, 70-, and 80 $\mu\text{g/L}$ Ag were spiked, and 0.3576, 0.8867, 1.286, 1.831, 2.147, and 2.438×10^{-5} A peak currents were obtained.

3.3. In-Vivo Application on Frog's Tissue

When the analytical working ranges were obtained, under optimum conditions, applications were performed in frog's tissue. A frog was exposed to chloroform and 0.5 ml Ag standard (1000 mg/L Ag) was injected into the frog's hind leg. After 30 min, 1 gram of the frog's hind leg tissue was cut and put in 2 ml nitrate to dissolve the tissue. It was then diluted with a 100 mL water solution. Fig. 4(A) shows the examined Ag ion in nitrate tissue solution, where the frog's tissue was dissolved via SW stripping voltammetry. In the blank solution, no peak current appeared, but in the 1 mL sample spike, a 4.86×10^{-5} A peak

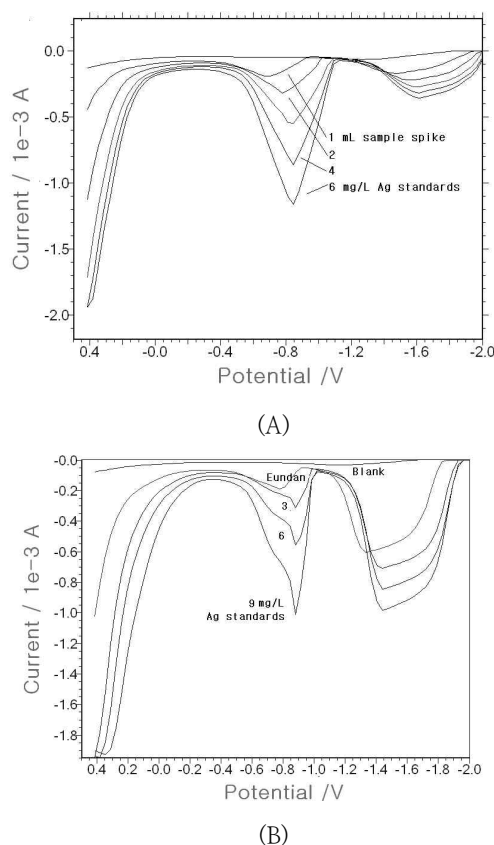


Fig. 4. (A): The SW stripping voltammetry result of the application to frog's tissue using GE under the optimum conditions. (B): The SW stripping voltammetry result of the application to Eundan using GE under the optimum conditions.

current was obtained. Then 2, 4, and 6 mg/L Ag standards were spiked, and 31.37, 54.62, and 89.47×10^{-5} A peak currents were obtained. Here, the same peak potential can be shown, whose techniques can be used for in vivo and in vitro diagnostic application. Further, more advanced applications were performed in Eundan, a supplement often used in South Korea and coated with silver metal powder that helps smokers stop smoking. Fig. 4(B) shows the Ag ion in Eundan that was examined via SW stripping voltammetry. First,

a Eundan was put in the mortar and ground, after which it was weighed. It was found to weigh 0.00125 g. It was then put in the 2 ml nitrate. In the blank solution, there was no signal, and the 0.1 mL sample spike obtained a 1.326×10^{-5} A peak current. When 3, 6, and 9 mg/L Ag standards were spiked, 2.456, 4.711, and 9.185×10^{-5} A peak currents were obtained. It was thus confirmed that the proposed technique can be used for food and in-vivo analysis.

4. Conclusion

Different concentrations of the Ag ion in microgram were examined via SW stripping voltammetry. The optimum conditions of 0.25 V amplitude, 10 Hz frequency, 0.035 V increment potential, -1.5 V initial potential, and 250 s accumulation time were obtained. These optimum conditions were applied to frog's tissue and to food systems. After the experiment, it was found that sea water could yield good electrolytes.

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