



Research Paper

Assay of caffeine in popular drinks of food systems and in vivo organs using carbon fiber Microelectrodes

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ABSTRACT

The study discussed in this work was developed to determine trace caffeine using square wave anodic stripping voltammetry (SWASV). We found that at the optimized conditions and for a 1.4 V anodic peak current, the voltammetric concentration effects were comparable to that of common electrodes, and that the carbon fiber microelectrode (CFME) is more sensitive than other electrodes. With the CFME, two working ranges of 0.5-10 and 20-300 mgL⁻¹ were obtained. The relative standard deviation of 50.0 mgL⁻¹ was found to have been 0.01 % (n = 12) at the optimum conditions, and the detection limit (S/N) attained was 45 ugL⁻¹ (2.3×10⁻⁸ M). The developed method was applied to the determination of the caffeine content of popular drinks and in vivo organs at real.

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INTRODUCTION

Coffee being one of the world's most popular drinks, control of its quality is very important for the beverage industry. The quality of coffee can be measured by determining its caffeine (1,3,7-trimethylxanthine) content. Thus, a sensitive and fast yet low-cost analytical technique is greatly necessary for the determination of the amount of caffeine in coffee and other beverages, which can also be used to measure the caffeine content of various foods and drugs. Thus, various such analytical techniques have been devised, including high-performance liquid chromatography with the UV method (Koch et al., 1999; Camargo and Toledo, 1999), capillary HPLC electro-spraying mass spectrometry (Robins and Guido, 1997), capillary gas chromatography (Pagliarussi et al., 2002), and the GC/MS method (Tsuda et al., 2000). These methods however, are tedious, complicated and time-consuming, and require expensive instruments. In contrast, electrochemical voltammetric techniques such as potentiometry, polarography and the stripping method are easy to use for experimentation yet also low-cost (Suw et al., 2002; Maali et al., 2000; Wang and Lu, 2000; Lin et al., 2005). Square wave anodic stripping voltammetric (SWASV) systems depend on the working electrode system, though. Recently various modified

working electrodes are developed, such as the dropping mercury electrode (Prasad and Arora, 2003; Azar and Ramazani, 2002; Kowalska et al., 2002; Barra and Santos, 2001; Bradshaw et al., 2002), the mercury film modified electrode (Wu et al., 1997; Zen et al., 1995), the glassy carbon electrode (Ojani et al., 2002; Marchiando et al., 2003; Abo et al., 2000; Abreu et al., 2002; Fijalek et al., 1998), the continuous flow method (Wang et al., 2000; Richter et al., 2002; Fernandez et al., 2000) and the carbon paste electrode (Wang et al., 2001; Maali and Wang, 2001; Raoof et al., 2001; Suw, 2005; Oni et al., 2002; Wang et al., 1998).

These methods are very useful, albeit insensitive, such that they cannot be used to determine the amount of caffeine in coffee and tea, biological foods, and pharmaceuticals, especially when there is only a low concentration of caffeine present and several kinds of electro-active species interfere. Only a few studies that discuss electrochemical caffeine analysis were found (Spataru et al., 2002; Pizzariello et al., 1999), but the methods described therein were not discussed in detail; the detectable concentration ranges were very limited (0.6 mgL⁻¹ (Andrea et al., 1999), 2.2 uM (Jyh and Yuan, 1997)

and 1.9~2.5 mgL⁻¹ (Fiona and Yuliya, 2005)); and the response times were very long. In the study described herein, various conventional types of electrodes are compared and the responsiveness of each is tested. The carbon fiber microelectrode (CFME) method was found to be more sensitive than other methods. Thus, at the optimized conditions, more sensitive detection limits were obtained with the CFME method than with the other conventional methods, and the CFME method was found to be applicable to any assay of food and pharmaceutical analysis.

EXPERIMENTAL SECTION

Apparatus

All the voltammetric measurements were performed using a Bas 100 BW (from Bioanalytical Systems, Inc.) equipped with a faraday cage (Model C2) and a pre-amplifier with a low-current module. The three-electrode system was used to monitor the SWASV signal. The various 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.

Preparation of the working electrode, the reagents and the experimental procedure

The paste electrode was prepared by mixing 70 % graphite powder with 20 % mineral oil. This mixture was homogenized in a mortar for 30 mins. The mixed paste was inserted in a plastic syringe needle with a diameter of 3 mm, and a copper wire was connected to the electrical system (Wang et al., 2001; Oni et al., 2002). Then the CFME was prepared with a 7 μ m-diameter, 15 mm-long carbon fiber attached to a copper wire via silver paint (Jose et al., 2001; Zhang et al., 1999; Huebra et al., 2001). This fiber was inserted into a polyethylene tube with a 0.3 mm diameter. The electrode was sealed by heating and cleaned by sonication for 10 mins, first in acetone, then in nitric acid (1:1), and finally, in double-distilled water (Fiona and Yuliya, 2005; Jose et al., 2001; Zhang et al., 1999; Huebra et al., 2001; Gerhardt and Hoffman, 2001). Other pure metals such as gold, platinum and copper electrodes were used. All the experimental solutions were prepared with double-distilled water (18 M Ω ·cm⁻¹). The caffeine standard stock solution was obtained from Aldrich and diluted within the required time. The 0.1 M phosphate buffer solution served as the supporting electrolyte. The electrical measurements were performed in dissolved oxygen, after which the electrode cell system was the contents of a 10 mL (pH 6.4) 0.1 M-(NH₄)₂HPO₄ and 0.1 M-H₃PO₄ buffer solution. The caffeine was scanned at 1.90~-1.60 V. A cyclic voltammogram was recorded at 100 mVs⁻¹ scan rates. We

found a caffeine peak current of anodic 1.4 V (Spataru et al., 2002) in the cyclic voltammograms, and we also found that electrode cleaning 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 a 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

First, cyclic voltammograms of 100~600 mgL⁻¹ of caffeine in a phosphate buffer solution (pH 6.4) with a conventional glassy carbon electrode were tested. When the potential ranged from 1.6~-0.60 V (versus Ag/AgCl) in the first cyclic voltammograms of 100 mgL⁻¹, we obtained a reversible oxidation and reduction peak. Then at the increased concentrations, the oxidation peaks (1.4 V) slowly increased, whereas the reduction peaks continually decreased. We thus used the oxidation signals, after which we tested the sensitivity of various types of electrodes, all of which were commonly usable, while their responsiveness at different values appeared.

Comparison of the sensitivity and working ranges of the electrodes

In Figure 1A, the sensitivity of common electrodes are compared at a fixed concentration of 50 mgL⁻¹ at the optimized conditions for the same electrolyte cell systems; raw voltammograms for the glassy carbon, gold, carbon paste, carbon fiber, platinum and copper electrodes are shown; and identical measurement scales are presented. Figure 1A shows that the copper and platinum electrodes did not respond, 1.4V peak signals for the other electrodes slowly appeared, and the carbon fiber electrodes sharply appeared. At these conditions, the concentration effects were examined. Figure 1B shows the calibrated working ranges during which time the platinum, copper and carbon paste electrodes slowly increased while the carbon fiber, gold and glassy carbon electrodes linearly increased. The carbon fibers were more sensitive than the others, though. Therefore, at the more concentrated states, each detectable working range was examined at optimum conditions. First, the metal copper electrodes appeared at the 10~110 mgL⁻¹ range, and their peak signals started at high concentrations and their peak width was broad, such that they cannot be used for caffeine analysis. Then the metal gold electrodes were examined, the signals of which appeared in the range of 5~80 mgL⁻¹ caffeine; their peak widths very sharply appeared; and wide detectable ranges were obtained,

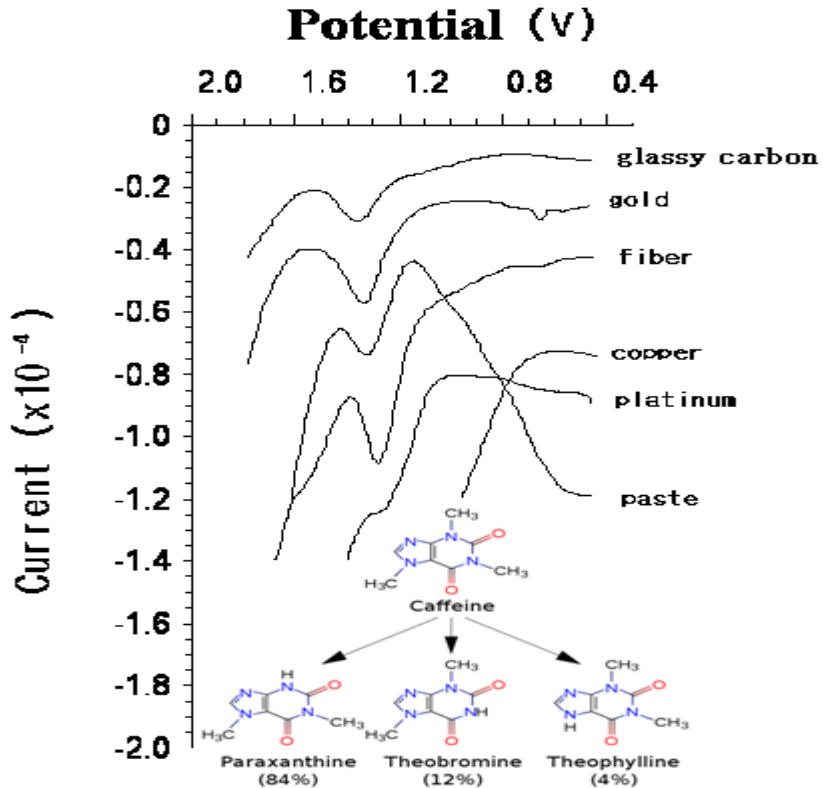


Figure 1A: Electrode comparison for the SWASV peak currents in a 50-mgL⁻¹ caffeine spike.

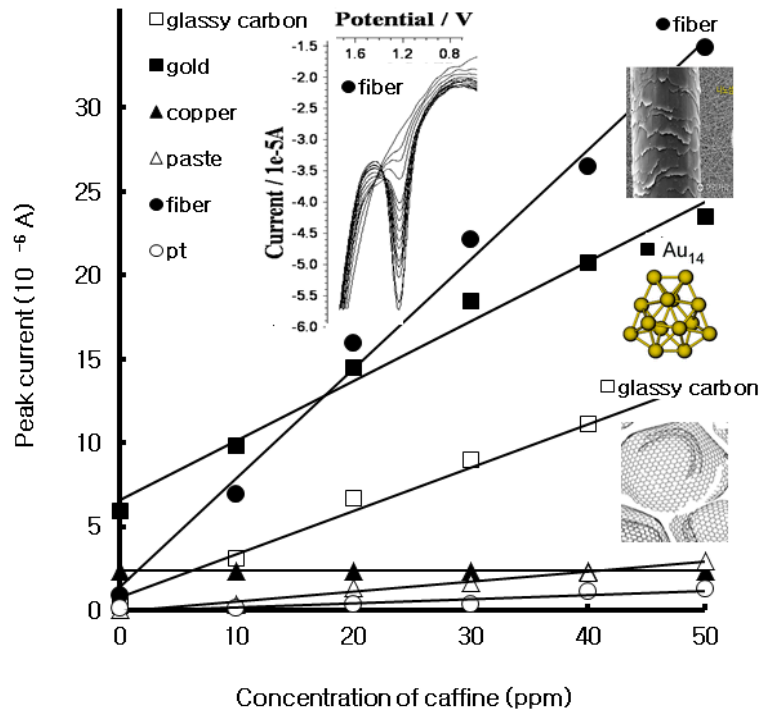


Figure 1B: Concentration effects of 0~50-mgL⁻¹ caffeine in a 0.1M (NH₄)₂H₂PO₄ + 0.1M H₃PO₄ electrolyte buffer solution (pH 6.4, deposition time 130 s, -0.4 V accumulation potential, 15 Hz frequency, 13 mV SW step potential, and 25 mV amplitude). The other parameters were at optimized conditions.

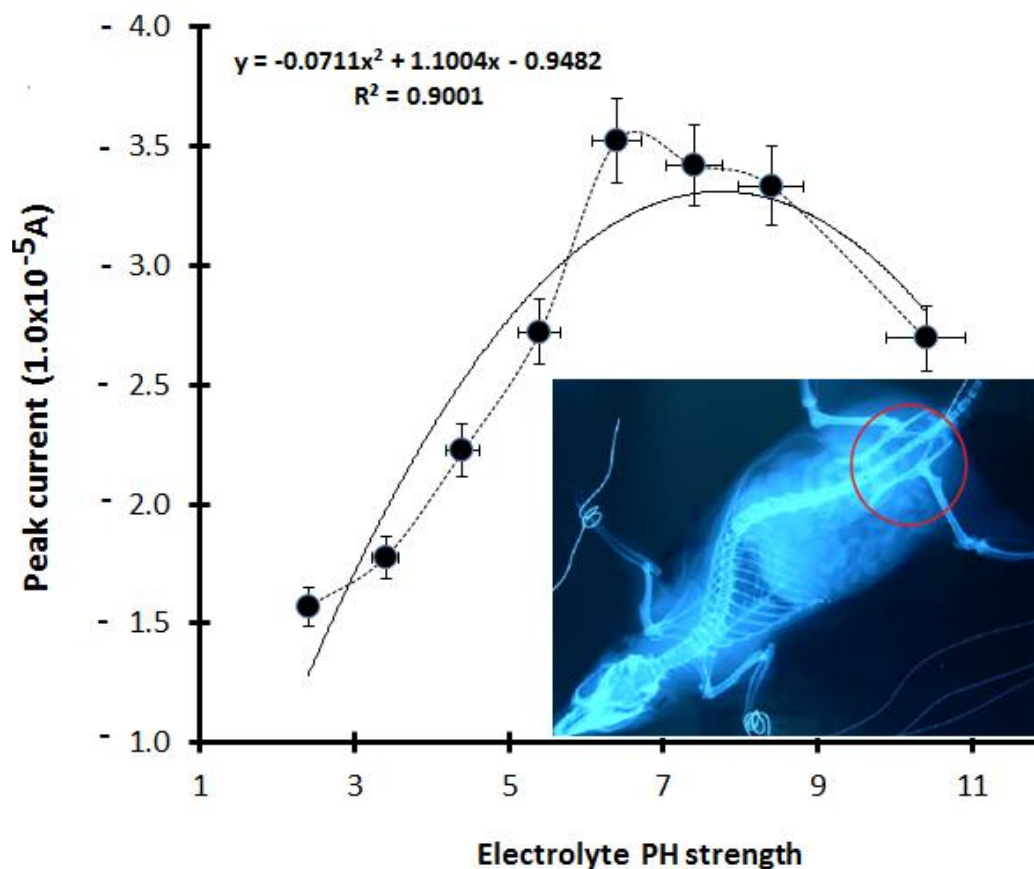


Figure 2A: Dependence of the SWASV peak current on various pH values (2.4, 3.4, 5.4, 6.4, 7.4, 8.4, 10.4 and 11.4 pH) injection at mouse bladder.

indicating their analytical utility. Next, the platinum metal electrodes were tested at the high concentration range of 4~70 mgL⁻¹, and their peak signals were poor, which makes them unusable for caffeine assay. Afterwards, conventional glassy carbon electrodes were examined. Their range was found to be sensitive and wide, format a 5~160 mgL⁻¹ spike. They appeared very sharply and their working ranges were linear, such that they were found to be usable for experimentation. Then conventional carbon paste electrodes were examined. Their working range was found to be 30~490 mgL⁻¹ and their analytical sensitivity was found to be poor. At these conditions, the carbon fiber microelectrodes were examined and their working range was determined to be 0.3~390 mgL⁻¹. In fact, exactly two ranges appeared and they had very high sensitive peaks. As such, the carbon fiber was found to be more sensitive than the other common electrodes, as a result of which its various parameters were optimized and its analytical working ranges and detection limits were examined.

Optimization of CFME using SWASV

The mole effects of phosphate buffer solutions were

examined at a variation range of 0.01~0.5 M. Their molar concentrations were changed via dilution with distilled water at the fixed caffeine concentration of 20.0 mgL⁻¹. The peak current of the optimum point was observed for the 0.1M phosphate buffer solution. At this condition, various other electrolyte solutions of potassium chloride, sodium acetate, potassium nitrate and phosphate (each 0.1 M) were examined as supporting electrolytes. The phosphate buffer was found to have had the most suitable result. Moreover, the response of the SWASV peak current for caffeine varied from 2.4 to 11.4 pH as shown in Figure 2A, photo of pH electrolyte injection into the rat bladder using 0.1M hydrochloric acid and 0.1M sodium hydroxide. The measurements were performed by micro three electrode systems at an initial potential of 0.4 V, a final potential of 1.80 V, a step potential of 13 mV, a deposition time of 150 s, and a fixed caffeine concentration of 5.0 mg L⁻¹. As shown in Figure 2A, at pH 6.4, the anodic peak current reached the maximum level. Before and after this point, both sides' peak heights slowly decreased and the peak half width broadly increased; but beyond this pH, the peak current quickly decreased. Therefore, we can use this pH level for the optimum conditions, at which time the stripping peak potentials are commonly carried out at 1.5 V. These peaks

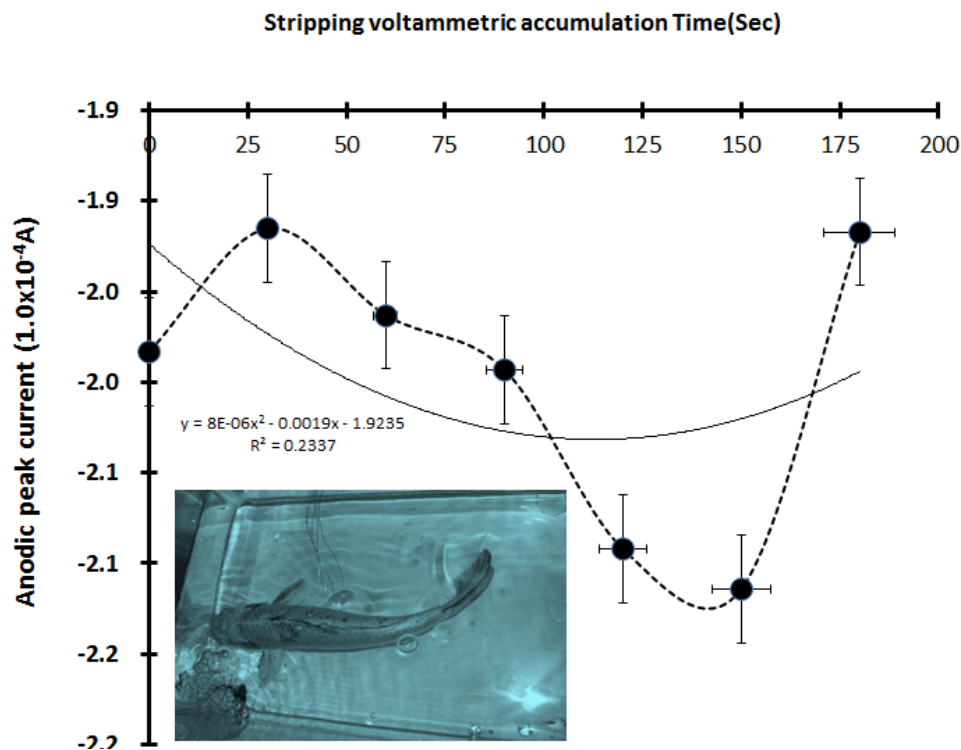


Figure 2B: The accumulation times for 0, 30, 60, 90, 120, 150 and 180 s, for the 5.0 mgL⁻¹ caffeine injection in vivo deep fish brain core using micro three electrode systems.

appeared with sharp peak widths. The cathodic peak, however, resulted in a nearly background current in the dissolved oxygen. In this condition, SW time variations were performed.

Figure 2B shows the dependence of the stripping anodic peak current on the various accumulation times from 0 to 180 s, for the 5.0 mgL⁻¹ caffeine injection in fish deep brain core in vivo micro electrode inserted systems, whereas the other experimental parameters remained the same as at the optimized conditions. In this figure, the peak current slowly decreased from 0 to 30 s then quickly increased. Thus, the optimum accumulation time was found to have been at 130 s, at which the maximum adsorption equilibrium could already be attained. Other different concentrations of caffeine were also studied, and showed short adsorption periods that yielded significant peak currents in comparison to those without accumulation. Therefore, 130 s was chosen as the optimum time in the quantitative estimations of caffeine. Under this condition, the SW initial potentials were examined. The peak currents are shown in Figure 2C. Over the -1.4~0.6 V range, the maximum peak current was obtained at -0.4 V. Other potentials, such as broad width signals, were detected in the 0.5 mL caffeine injected conditions, and there were also ratios that rose higher than the pH increments ratio. We can therefore choose the accumulation potential in the adsorptive stripping voltammetric determination of the caffeine content. Other experimental parameters were tested for the

step potential, the square wave amplitude, and the electrolyte concentrations. The results are shown in Figure 3.

Working range statistics and application

At the optimized conditions, we tested the various working ranges, and we finally detected the usable analytical equations. Figure 3 shows the obtained well-defined caffeine peaks following the 130s deposition of two different concentration ranges. The first curve represents 0.5~10 mgL⁻¹ caffeine, the regression equation of $Y = -0.1687x - 25.54$ (correlation coefficient of 0.991, 13 points). $Y = \text{current, A}$ and $X = \text{caffeine concentration, mgL}^{-1}$ were obtained, and their sharp peaks increased significantly. Other caffeine heights that ranged from 20 to 300 mgL⁻¹ were obtained, and their regressions of $Y = -1.6433x - 1.0711$, correlation coefficient of 0.993, and 7 points were determined, which equations are usable for various food and drug assays. At this time, the peak widths were sharp and sensitively appeared. Under these conditions, the statistics were examined. The precision of 12 successive replicated determinations of 30.0, 150.0 and 410.0 mgL⁻¹ with 130 s accumulations were found to have been 7.915, 2.322 and 1.150% (CV), respectively, and the developed method's detection limit was estimated at 0.045 mgL⁻¹ (SN = 3) using optimum parameters. In these conditions,

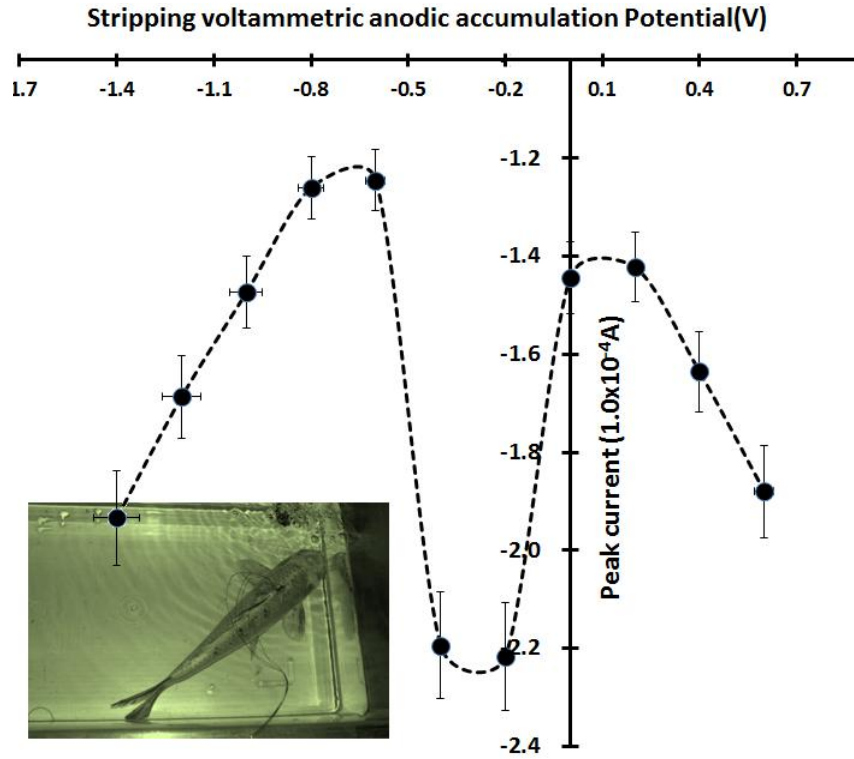


Figure 2C: The accumulation potentials for -1.4, -1.2, -1, -0.8, -0.6, -0.4, -0.2, 0, 0.2, 0.4 and 0.6 V for the 5.0 mgL^{-1} caffeine injection in in vivo deep fish brain core using micro three electrode systems.

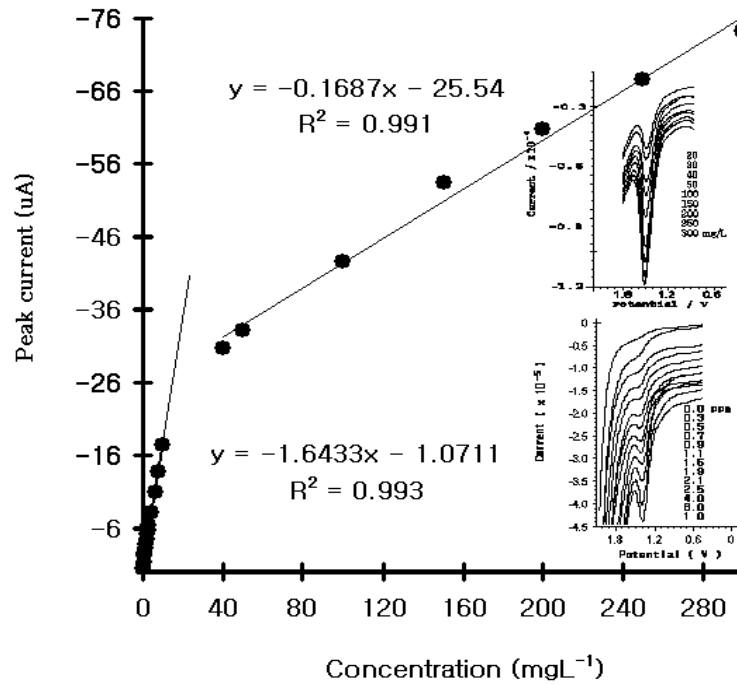


Figure 3: Square wave anodic stripping voltammetric peak current at low ($0.5, 0.7, 0.9, 1.1, 1.5, 1.9, 2.1, 2.5, 2.9, 4, 6, 8$ and 10 mgL^{-1}) and high ($20, 30, 40, 50, 100, 150, 200, 250$ and 300 mgL^{-1}) caffeine concentrations (130 s at -0.4 V , 15 Hz frequency, 13 mV step potential, and 25 mV amplitude). The other experimental parameters were the same as those in Figure 2.

various interference ions were studied by adding several other metal and analogy ions. For the determination of 10 mgL⁻¹ of caffeine, the existence of 10 mgL⁻¹ of Ni(II), Cu(II), Zn(II), Fe(II), Cd(II), Pb(II) and theophylline resulted in -8.98%, -23.4%, -15.6%, 2.96%, 3.47%, -14.4% and -10.6%. At this time, copper ions effectively interfered. The presence of a twofold excess of the ions resulted in -28.4%, -16.73%, -12.9%, -1.67%, -11.0%, -9.75% and -6.54%. At these results, only Fe(II) did not interfere, and a threefold excess of ions resulted in -100%, -7.69%, -18.2%, -15.7%, -11.0%, -1.31% and -0.39%. At this state, the Pb(II) and theophylline ions did not interfere. Here, the standard addition methods effectively corrected the other ions. Then the analytical utility of the drinking water sample was tested for the detection of known contents of 10 and 13.03 mgL⁻¹ of caffeine using D-company's products. First, a blank electrolyte solution was stripped, after which untreated raw D-company drinking water was spiked with a 1 mL sample solution. At this time, sharply sensitive peaks were obtained. The results are shown in Figure 4. Then standard solutions were spiked at 10, 20 and 30 mgL⁻¹. The results were calibrated with the standard addition methods that extrapolate the linear curve and use the X and Y intercepts. The results for the 10 mL⁻¹ and the 13.29 mgL⁻¹ caffeine samples approached ± 95% precision.

CONCLUSION

This study describes the results of testing various types of electrodes and their working ranges. Such results show that CFMEs are more sensitive than other conventional electrodes. Concentration ranges of 0.5~10 and 20~300 mgL⁻¹ in the electrolyte solutions were obtained with the use of CFME, as were the optimized conditions for the pH 6.4 ammonium phosphate solution, the accumulation time of 130 s, and the accumulation potential of 1.4 V, and the detection limit was achieved at a low concentration of 45 ugL⁻¹, which is more sensitive than previous results (Andrea et al., 1999; Jyh and Yuan, 1997). The developed method was applied to the determination of the caffeine content of water samples, and was found to be simpler, more time-saving and easier than other common methods for the determination of caffeine content. It can also be applied in other fields that require caffeine analysis.

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