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Article

In-Vivo Glucose Assay on the Living Fish Brain Molecule and Human Urine by Using of Bismuth-Immobilized Carbon Nanotube Paste Electrode

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Abstract: Background/Objective: *In-vivo* diabetes detection of glucose were sought using square-wave anodic stripping voltammetry (SW), with bismuth-immobilized carbon nanotube paste electrode (BCE). **Methods:** The optimum analytical results indicated sensitive peak signals on the BCE. The raw voltammogram was approached within the 1 mgL⁻¹-14 mgL⁻¹ and 10 ugL⁻¹-140 ugL⁻¹, detection limits with preconcentration times of 100 and 200 sec. **Results:** The relative standard deviation was 0.02 % (n = 15) of 10.0 mgL⁻¹ under optimum conditions. The analytical detection limit (S/N) was attained at 8 ugL⁻¹. The handmade microsensor was directly used *in vivo* on the living fish brain and human urine. **Conclusion:** The method was applied at real time *in vivo*, without requiring any pretreatment and other ionic electrolyte solutions. It can be used for medicinal and other materials requiring biological-fluid detection in real time. This study was designed to be suitable for real-time unmanned remote diagnosis and therapeutic drug injection into the body, micro-needle long-term administration, wearable artificial skin tattoo sensor, and real-time control. In addition, the glasses monitor was designed to be suitable for multitasking and multi-user control.

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Introduction

Among the *in-vivo* metabolites, glucose is the main substrate for organic energy [1,2]. Muscle sympathetic nerve activity [3] and body cells that generate energy [4] and that contain metabolites are associated with the diabetes & vascular disease, cell growth [5], and physiological fluids [6], and are fuel-cyclic-efficient. Sensitive analytical detection techniques have become an object of considerable interest in biological *in-vivo* or *in-vitro* systems [7], such as clinical applications for selective capillary electrophoretic methods [8], high-performance liquid chromatography with refractive index detection [9], and *in-vivo* near-infrared (NIR) noninvasive blood glucose assay [10]. Spectrophotometrics are in demand for pretreated sample preparation and electric detection systems, but better, simpler, faster, and inexpensive electrochemical techniques have been developed, such as those involving the use of the photopolymeric membrane amperometric biosensor [11], copper-based alloy electrodes [12], nickel copper alloy electrodes [13], graphite epoxy screen printable biocomposite sensor [14], ferrocene monocarboxylic-acid composite sol-gel glass electrode [15], and other modified sensors [16,17]. Most of these devices, however, are used only under laboratory conditions and are not usable for *in-vivo* direct assay. In this study, a simpler, more sensitive, and inexpensive square-wave anodic stripping voltammetry (SWASV) method was sought. The objective of the study was to determine the effects of bismuth immobilized onto a carbon nanotube (CNT) structure. CNT can serve as a metal semiconductor and is also called an "electrochemical capacitor" [18]. It can perform electron transfer with biomolecules, which have large cylindrical surface areas. Thus, a number of investigations of CNT for catalyst support and electronic equipment have been

conducted [19]. In this study, CNT was used for electrical support. Moreover, the bismuth-immobilized [20] techniques have already been employed for electrochemical measurements of metal and other biomolecules [21]. As such, CNT-based bismuth [22] paste was prepared for the trace detection of glucose. This method is very simple and can detect low concentration ranges within fast accumulation times. This experiment did not need complicated equipment but only a small electric circuit. It also had a very short accumulation time of only 350 sec and easily detected the metabolites. It can thus be used to detect biological *in-vivo* and other materials requiring glucose analysis.

Experiment

Apparatus and Reagents

All voltammetric measurements were carried out using a CHI660A Instruments electrochemical workstation (CH Instruments Inc., Cordova, TN, USA). A three-electrode system was used to monitor the SWASV signal. BCE was used as the working electrode, with saturated Ag/AgCl as the reference electrode (3 molL⁻¹KCl). Platinum wire was used as the auxiliary electrode. The working electrode was made of paste, which is a mixture of CNT (Nanostructured & Amorphous Materials, Inc.), bismuth standard (Aldrich 1000 ppm), and mineral oil, in the ratio of 60:30:20. A small amount of mixed paste was inserted into a plastic-needle-type capillary tube with a 1.5 mm diameter and 5 cm length, using a 0.5-m-diameter copper wire connected to the measurement system. All the systems were subjected to room temperature (24±2°C). The reagent solution was prepared from doubly distilled water (18 MOhm.cm⁻¹). The conventional paste electrode was prepared by mixing 70% graphite powder with 20% mineral oil. This mixture was homogenized in a mortar for 30 min. The mixed paste was inserted into a plastic syringe needle with a diameter of 3.0 mm, and a copper wire was connected to the electric system.

Experimental Procedure

Several electrolyte solutions of acid, base, and buffer (all in 0.1 M) were initially examined in search for a possible supporting electrolyte. Ammonium phosphoric-acid solution was found to be a suitable electrolyte, yielding the best peak separation from the background currents. The effect of the phosphoric-acid concentration was examined within the range of 0.01-0.3 M. In the 0.1M solution, the pH of 4.69 was found to be sensitive. Under this condition, the cyclic peak potential and peak sensitivity were examined using BCE and the common-type CNT paste electrode.

Results and Discussion

Cyclic Voltammetric Property of BCE

The unit mgL⁻¹ indicated noise signals on the graphite electrode, but the definite peak current on BCE was found to be around 0.1 V. As shown in the bigger graph in the same figure, the currents from BCE grew regularly and visually with 8.78, 12.07, 16.18x10⁻⁶A, and close to a direct proportional graph, whereas the currents from the graphite electrode grew differently from BCE. The peak currents on the graphite electrode grew only slightly at Figure 1(A). Although the graph does not show this, the CNT that was modified through the previous method was also tested for mercury and was electric-metal-immobilized, but the BCE current was more gradual than the other peaks. Through this experiment, BCE was determined to be an effective electrode for the detection of glucose, Figure 1(B) is shown cyclic peak at brain cell of fish. Using BCE, more sensitive SW optimization was performed.

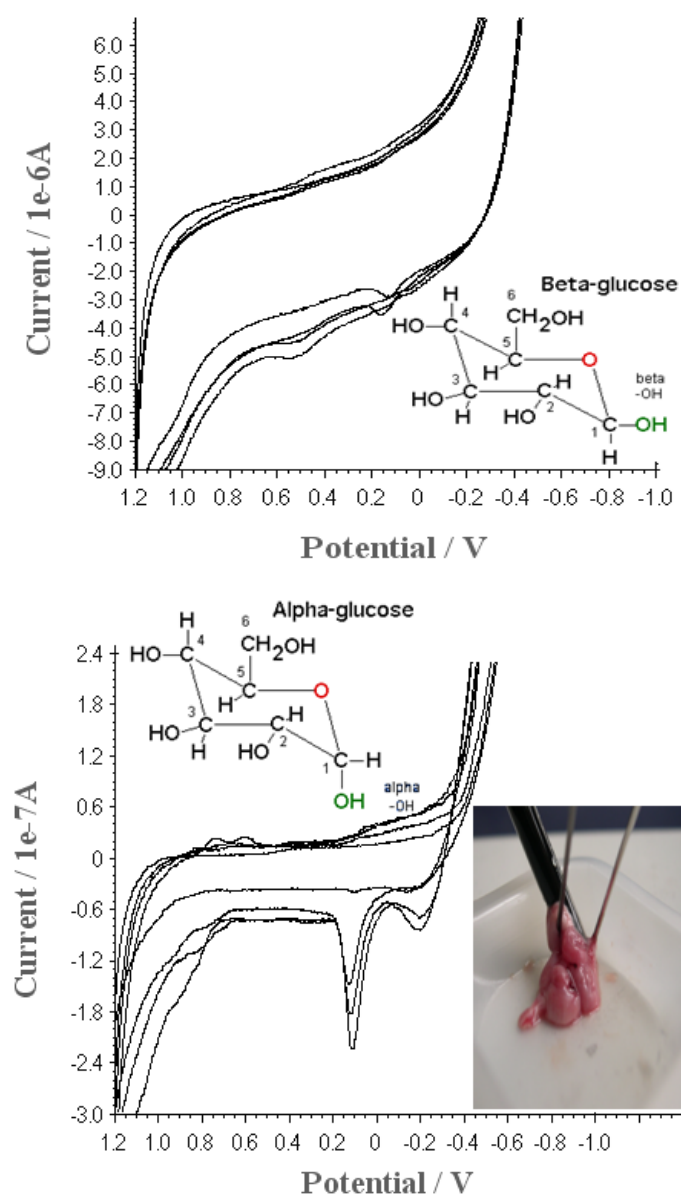
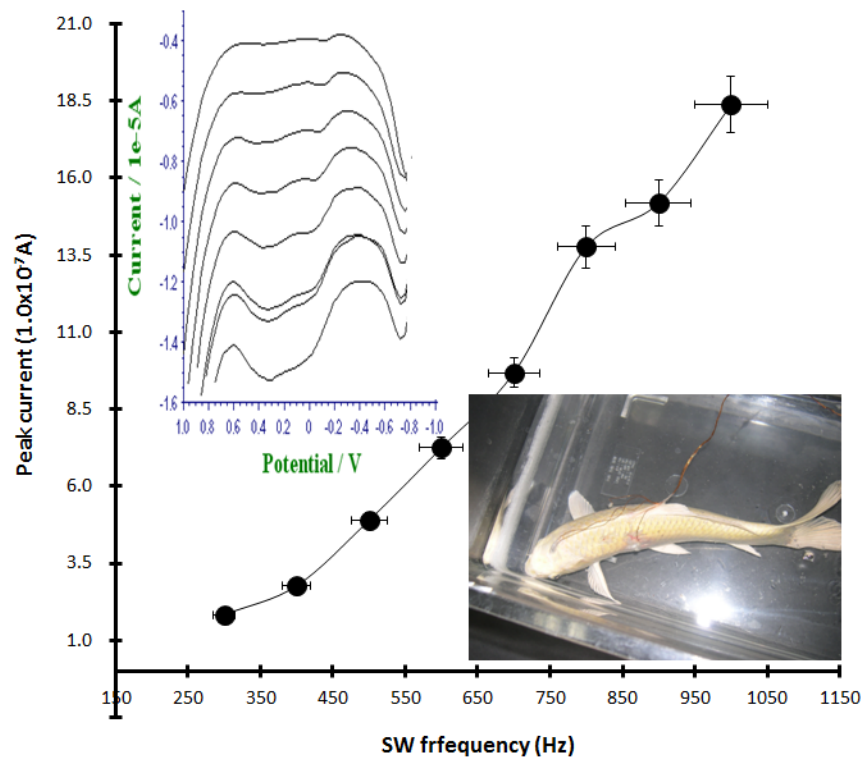
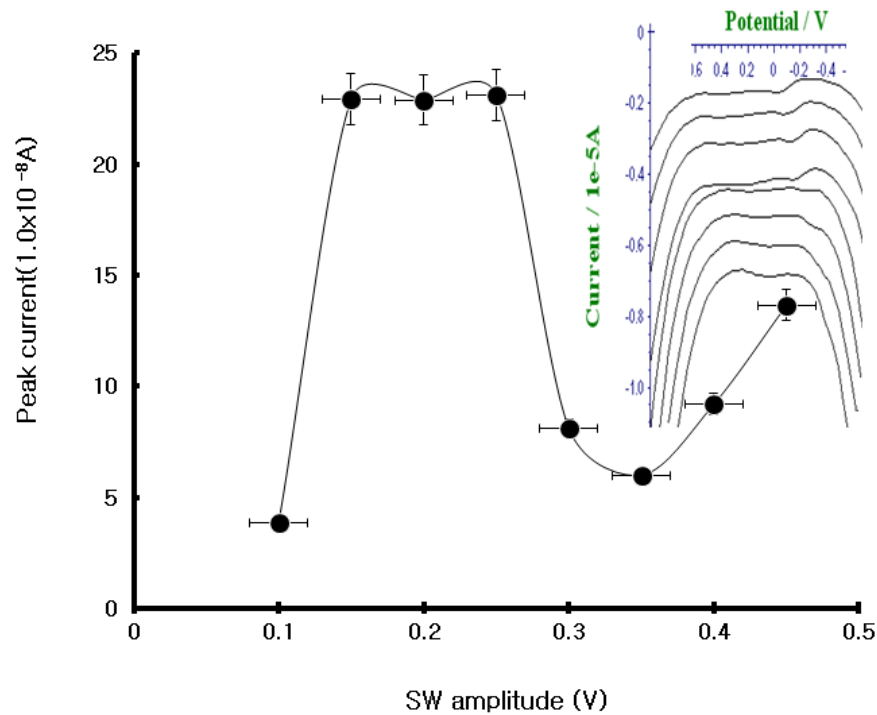


Figure 1. Cyclic effects of BCE. (A) Various concentration effects of 0-, 10-, 20-, and 30-mg L^{-1} glucose using cyclic voltammogram (CV) as graphite common pencil electrode. (B) BCE at a 0.5 V scan rate, in a 0.1M ammonium phosphate electrolyte solution and extracted brain of fish.

SW Optimization for BCE

Figure 2(A) illustrates the voltammetric peak current in a 1-mg L^{-1} glucose concentration as a function of varying square-wave amplitudes. The amplitude range increased very quickly from 0.1 to 0.15 V while the 0.15-0.25 V peak remained very high. The peak, however, again rapidly decreased to 8.11×10^{-8} A then once again increased to 0.35-0.45 V. At this amplitude, a glucose peak ratio of 23.12×10^{-5} A : 3.85×10^{-5} A appeared. Here, 0.2 V was fixed. Using this result, the square-wave frequencies for Figure 2(B) were tested at fish brain cell real, and the results within the range gradually increased, except at 800-900 Hz, where the peak decreased from 13.76 to 15.19×10^{-7} A. The peak current range was 1.83 - 18.37×10^{-7} . As such, the optimum frequency was 1000 Hz. Under these conditions, Figure 2(C) shows the results that exhibit the square-wave increment potential. The range was 9.63 - 18.33×10^{-7} A, and the highest peak was 0.04 V. The square-wave accumulation time test was also carried out. Figure 2(D) shows the accumulation time test consequence. The test was conducted for 30 sec. Up to 350 sec, the peak current rose, but at 400 sec, the peak current decreased to 13.58 . At 300-350 sec, the peak current went up rapidly from 10.75 to 16.64×10^{-5} A. The range of increase was

1.17-16.64x10⁻⁵A. Therefore, the best peak current was shown to be at 350 sec, by 16.64x10⁻⁵A. The pH was also tested (data not shown). The peak current was highly movable upward and downward. It was tested eight times, at 2.89, 3.33, 4.06, 4.69, 5.11, 6, 6.61, and 7.19 pH. From pH 3.33 to 4.69, the peak current grew steadily, but it rapidly declined at pH 5.11 by 0.6161x10⁻⁶. pH 4.69 was found to be suitable by 2.257x10⁻⁶. As such, through this procedure, the optimum conditions of 0.1 V amplitude, 300 Hz frequency, 0.04 V increment potential, 350 sec accumulation time, and 4.69 pH were arrived at. Using these conditions, analytical linear ranges and statistic applications were performed.



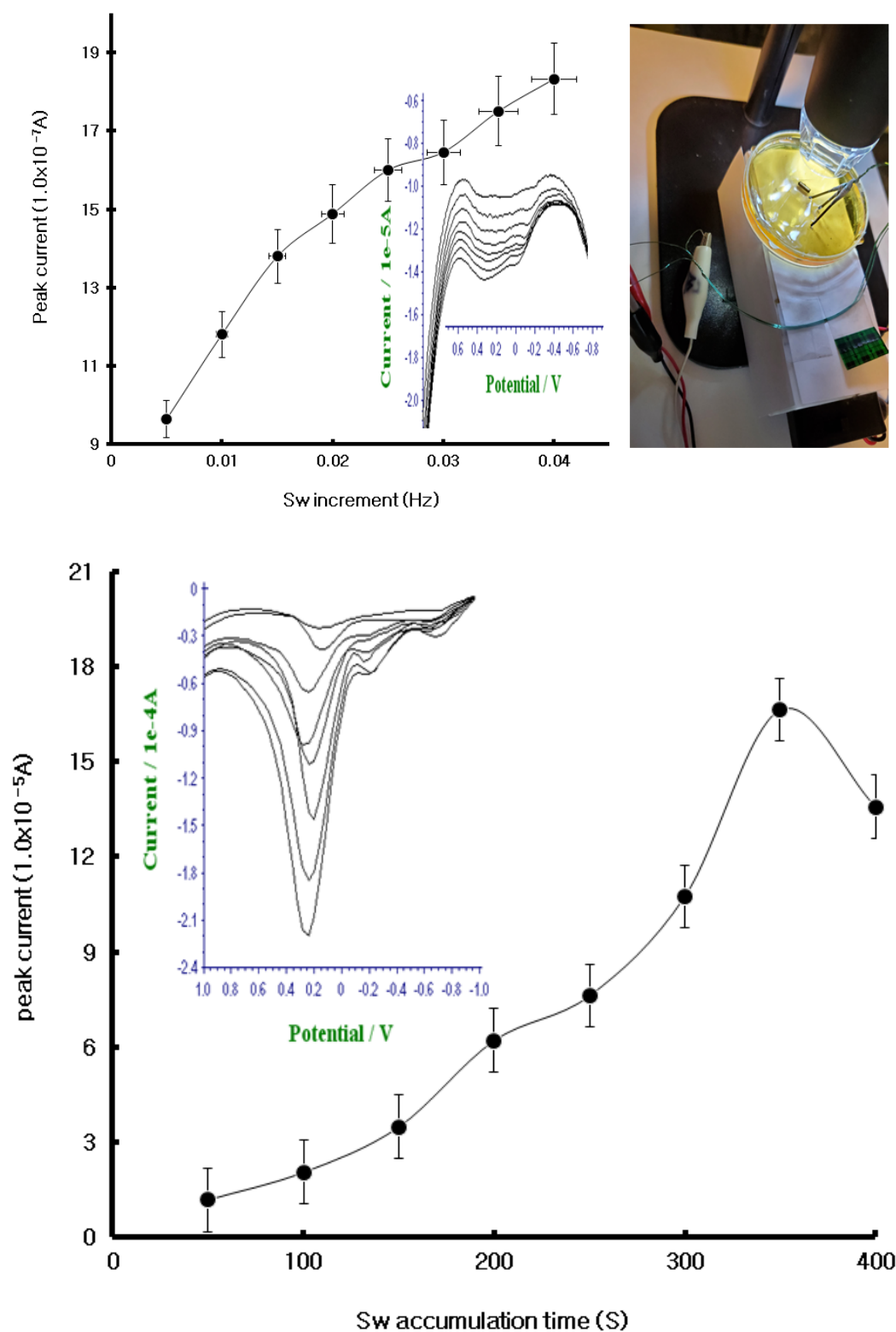
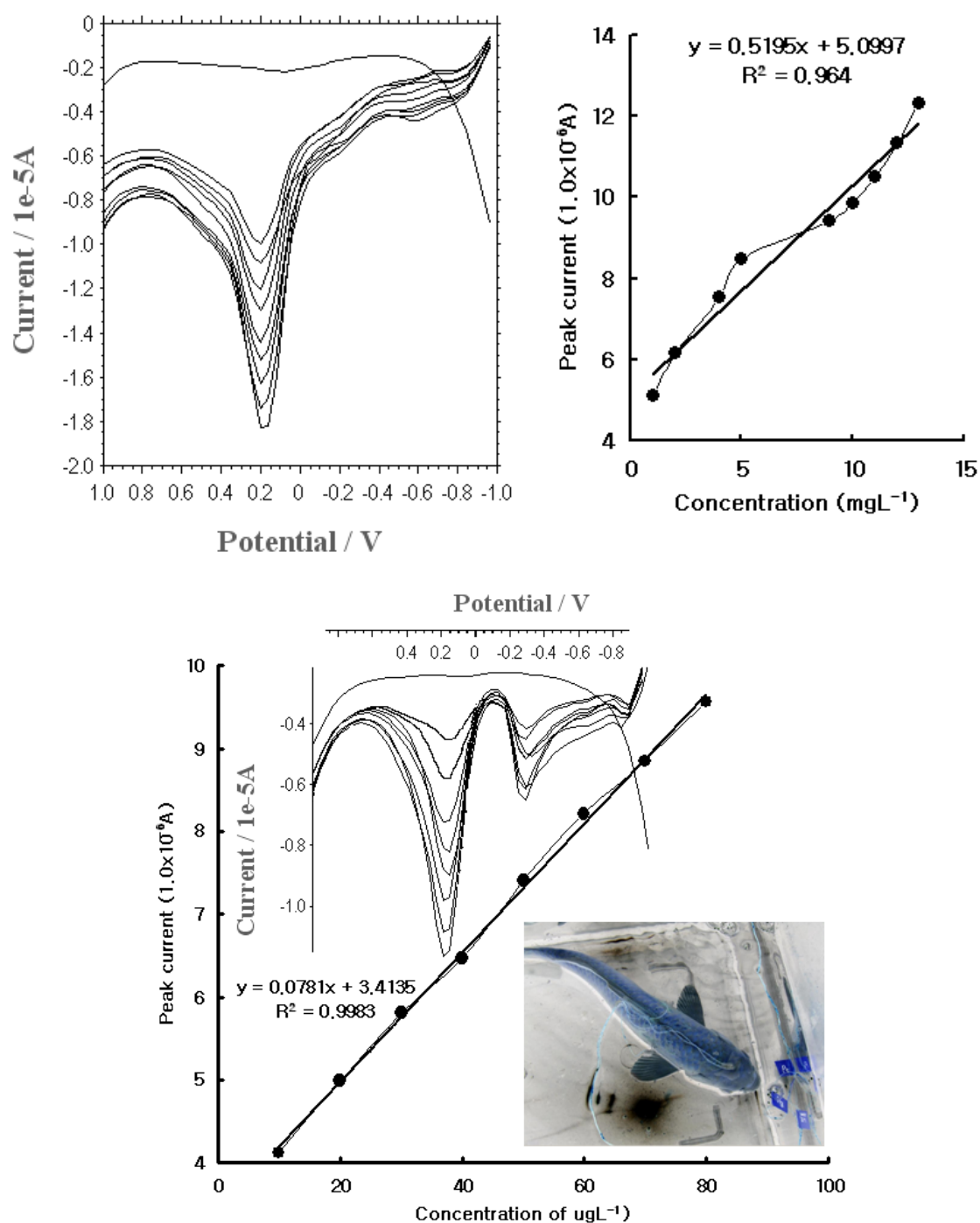


Figure 2. (A) SW anodic peak currents for the amplitude variations of 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, and 0.45 (V). (B) SW frequencies of 300, 400, 500, 600, 700, 800, 900, and 1000 (Hz). (C) SW increment potentials of 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, and 0.04 (V). (D) SW accumulation times of 50, 100, 150, 200, 250, 300, 350, and 400 (sec).

Analytical Working Ranges, Interference, Statistics, and Application

In Figure 3(A), the first curve is shown to have a 100 sec accumulation time and an electrolyte blank. The peak current clearly appeared at around 0.1 V and regularly grew henceforth. The graph is also close to a straight line because each signal gradually increased within the range of $5.11\text{--}12.33 \times 10^{-6}\text{A}$. In Figure 3(B), glucose was also detected in a lower μgL^{-1} concentration in the subsequent examination. The last concentration examination was carried out in the mgL^{-1} concentration. The deposition time was 200 sec. Microranges were recognized, which can be applied to *in-vivo* fish brain at real or *in-vitro* cells. After the completion of all the concentration tests, analogy interference tests were carried out. In Figure 3(C), the deposition time for such tests was 50 sec, and the reagents that were used were histidine, catechol, dopamine, epinaphrine, glycine, phenylalanine, and gaba, in the ratio of $5:5 \text{ mgL}^{-1}$. The reagents influenced the concentration of glucose by 100 %, -14.84 %, -58.08 %, -39.97 %, -56.81 %, -47.14 %, -61.98 %, and -52.63 %, respectively. Phenylalanine had the strongest effect on glucose. The presence of other ions was also effectively corrected using standard addition methods.



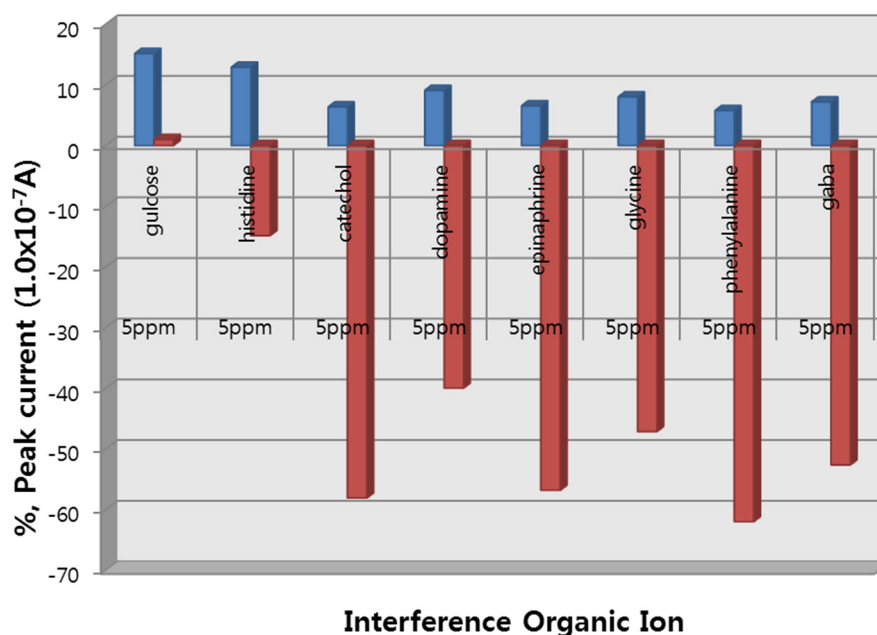


Figure 3. (A) SW voltammograms of the 1, 2, 3, 4, 6, 8, 10, 12, and 14 mgL^{-1} variations. (B) Microranges of 10, 20, 40, 60, 80, 100, 120, and 140 μgL^{-1} with 100 sec accumulation time and calibration curves, in deep brain fish at real, (C) Interference effects.

Statistics and Application

Before the experiments shown in Figure 4(A), (B), and (C) were conducted, glucose detection statistics were obtained. At first, detection was performed in 0.1M ammonium phosphoric electrolyte acid without any glucose, with a deposition time of 50 sec as blank. After that, a 1000- mgL^{-1} glucose solution of 10 mgL^{-1} was spiked, and glucose was examined 15 times, with a deposition time of 50 sec. These statistics were gathered to prove that the response of BCE was effective through RSD. The peak current signal clearly appeared at -0.1 V and grew closely and in an orderly way. According to this experiment, the RSD was 0.02. The last experiments were applications of this study. Figure 4(A) shows the human-urine result. Blank solutions are shown, which manifest that no noise signal was obtained and that glucose peak currents were obtained. First of all, human urine's peak currents were checked. The peak current of the 0.05-ml urine appeared at around 0.1 V, and whenever a 10-ppm reagent was added, the peak current was rechecked. After the addition of a reagent, the peak current check was calibrated three times. As shown by the graph, the peak currents grew one after the other. This first application experiment result shows peak currents of 1.932, 2.265, 3.005, and 3.290×10^{-6} A. Therefore, glucose can be easily detected by using a small amount of urine. The next test involved the application of plant cells for in vivo implanted sensor, as shown in Figure 4(B). This figure shows the one whose peak currents grew the most in many of the tests. The graph also grew at the same position (around 0.1 V). This experiment was carried out by putting an electrode in the plant cell and fish brain. This test can also detect glucose very precisely at between 0.1 and 0.2 V. In these two tests, glucose was very easily detected in fish and plant tissue even with a very small solution amount.

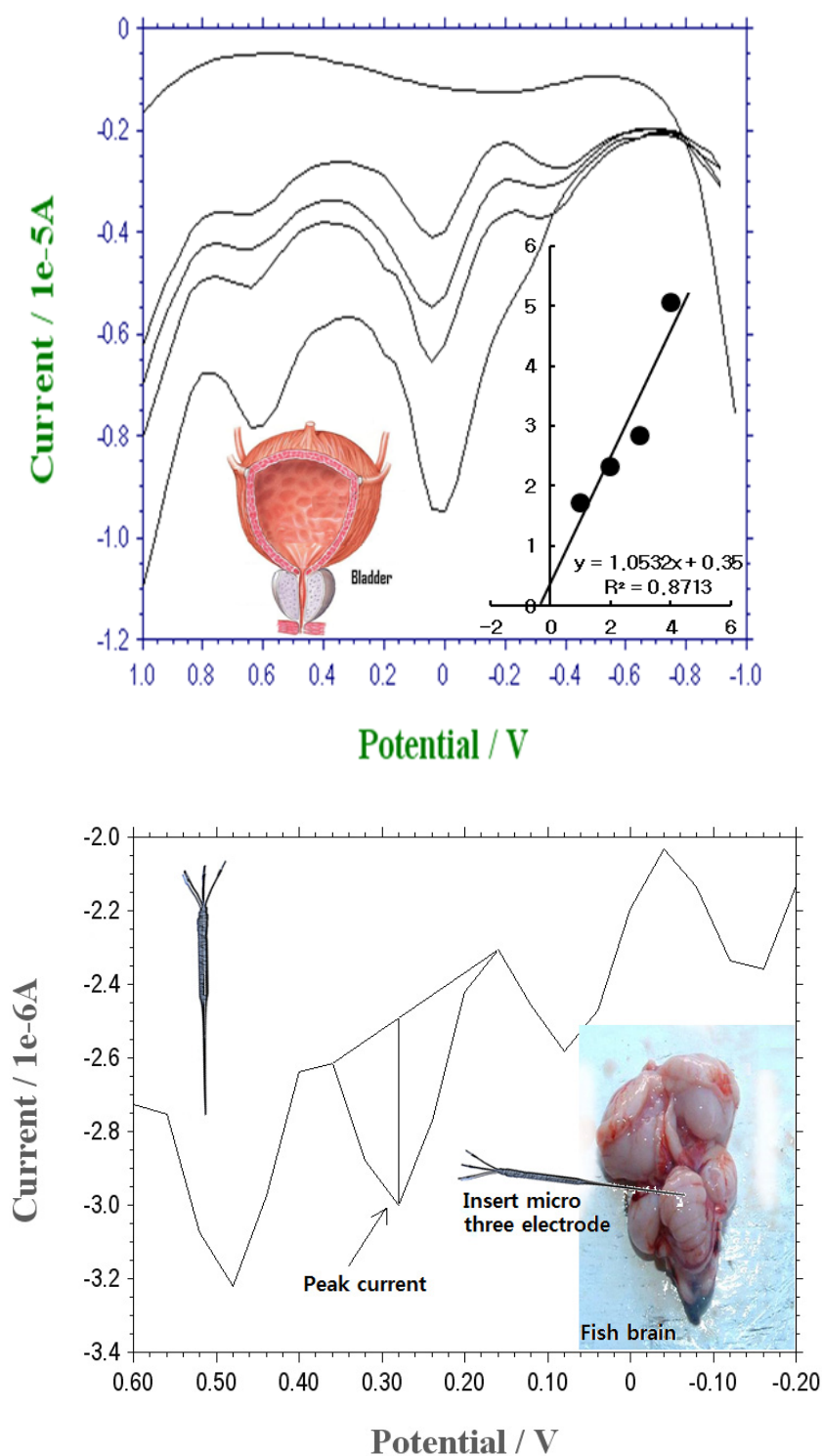


Figure 4. (A) Analytical application of human-urine spike. (B) Assay of real-time live *in-vivo* tissue.

Conclusion

The developed methods were fabricated with bismuth-immobilized carbon nanotube paste electrodes using square-wave stripping voltammetry. This method is very simple because it does not need pretreatment and diverse equipment. The test was carried out at an accumulation time of 350 sec, at an amplitude of 0.25 V, at a frequency of 1000 Hz, and at an increment potential of 0.04 V. The electrode response was linearly related to the glucose concentration, which ranged from 10-80 $\mu\text{g/L}^{-1}$

to 10-130 ngL⁻¹ and 10-90 mgL⁻¹. The peak currents reached the maximum at a phosphate buffer electrolyte solution pH of 4.8, and various interference ions were removed using standard addition methods. As the method that was used in this study had a lower detection limit, the analytical applications of human urine, fish cell, and plant tissue were examined. The proposed method can also be applied in other fields requiring glucose detection.

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Informed Consent Statement: Not applicable.

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Conflicts of Interest: Declare no conflicts of interest.

Abbreviations

SWAV: square wave anodic stripping voltammetry, **BCE:** bismuth-immobilized carbon nanotube paste electrode, **CNT:** carbon nanotube, **NIR:** near-infrared.

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