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Ex Vivo Assay of Trace Nicotine Using a Voltammetric Modified Biosensor

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Abstract : *In vivo* nicotine is associated with Alzheimer's, Parkinson's and lung cancer. Diagnostic assays of these diseases depend on very low analytical detection limits. In this study, a sensitive analytical method was examined using a voltammetric graphite pencil electrode (GPE) and a modified carbon nanotube paste electrode (CNE). The optimum analytical conditions for both electrodes were compared using square wave anodic stripping voltammetry (SW) and cyclic voltammetry (CV) obtaining 400 sec accumulation time and oxidation peak. Under optimum parameters, the stripping working range of GPE was 5.0-40.0 μg/L, CNE: 0.1-0.8 and 5-50 μg/L. Quantification limits were 5.0 μg/L for GPE and 0.1 μg/L for CNE, while detection limits were 0.6 μg/L for GPE and 0.07 μg/L for CNE. A standard deviation of 10.0 μg/L was observed for 0.064 GPE and 0.095 CNE (n = 12) using 400 sec accumulation time. The results obtained can be applied to non-treated urine and ex vivo biological diagnostics.

Keywords : nicotine ion, diagnosis, in vivo, stripping,

1. INTRODUCTION

Plant tobacco smoking is efficiently absorbed into the lungs and brain [1]. Major component of nicotine is responsible for an enormous number of premature deaths worldwide per year [2]. *In vivo* conditions, trace nicotine are associated with Alzheimer's disease, Parkinson's disease [3], memory malfunction [4], lung cancer [5], genotoxic and various other diseases. In *in vivo* urine or blood serum, detection of these diseases requires very low analytical detection limits (DLs). Along this line, various analytical methods for nicotine assay have been developed. These methods include liquid chromatographic mass spectrometry [6], high performance liquid chromatography [7], reversed phase HPLC with the electrochemical method [8], gas chromatography mass spectrometry [9], and solid phase extraction gas chromatographic mass spectrometry [10]. However, the need for complicated fabrication and other

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analytical detection systems remain. Some techniques are also not applicable to direct, real-time biological assay. Voltammetric detection systems that have been recently developed are simple [11,12] and usable in ubiquitous trace assay. Nonetheless, these methods are rarely used in the medicinal approach to nicotine analysis. These electrochemical working electrode techniques are dependent on specialized materials including graphite [13], carbon nanotube [14] and other modification techniques, such as ultra levels of 2,4,6 trinitrotoluene analysis [15], hepatitis B virus detection [16] and analytical redox interactions [17,18,19]. Moreover, renewable graphite pencil writing devices have been available for many years [20,21] and applicable to electrochemical diagnostics. In this experiment, carbon nanotube [22] and graphite pencil working electrodes were developed for medical applications. Under optimum conditions, the CNE was shown to be more sensitive than GPE, and both sensors were found to be suitable for use in medical diagnosis and pharmacological assay, specifically ex vivo application.

2. Experimental procedure

2.1. Experimental systems, reagents, and procedure

The experimental measurements were carried out using the voltammetric system Bioelectronics-1, which was first installed at the authors' institute. The new version is a computerized handheld battery type with a ±2.4 V potential range, a 2 mA current range, and a 10-pico A measuring current. It comes in a 5"x4"x1" cellular phone size and has a USB port interface with a PC. The instrument can be used for bio-assay and sensor techniques as well as for individual and laboratory applications. Nicotine ($C_{10}H_{14}N_2$, MW: 162.23) was prepared with sigma aldrich. The GPE was prepared from Noki pencil Model 2000, and the CNE was used with multi-walled carbon nanotubes (by catalytic CVD; outside diameter: 8 nm; inside diameter: 2-5 nm; length: 0.5-200 nm; Nanostructured & Amorphous Materials, Inc., USA). These were purified overnight prior to use via magnetic stirring in a 2 M nitric acid solution and were washed using triple-distilled pure water. A three-electrode system was used to monitor the voltammetric signal. The working electrode was made of a paste composed of CNT; mineral oil ratio of 50:50). Ag/AgCl/KCl was used as a reference electrode, and a platinum wire was used as the auxiliary electrode. Glassy carbon electrodes (BAS stationary voltammetric electrodes MF-2012 3.0 mm in diameter) were used to compare the electrodes. The three-electrode system was immersed in a solution of 10 mL 0.1 M H_3PO_4, and the other parameters were maintained at optimal conditions. All the experiments were performed at room temperature, without oxygen removal. Under this condition, the peak potential was determined via cyclic scanning.

3. Results and Discussion

3.1. Cyclic voltammetric peak potential in GPE and CNE

In Fig. 1, the voltammetric redox potential was examined using cyclic scanning at a wide potential, a -1.0 V initial potential, and a 1.0 V switching potential in a 0.1 M H_3PO_4 electrolyte solution. Both electrodes were scanned in the same cell systems using a blank solution that appeared in the form of a simple linear curve. Thus, 10 μg/L of nicotine was spiked and obtained for a 0.2 V oxidation peak (←) with the CNE. Subsequently, 20 and 30 μg/L were spiked and the peak current increased from 0.8 to $1.6x10^{-4}$ A. On the other hand, 40 μg/L was

spiked at the GPE and exhibited a 0.2 V oxidation peak reaching $7.5x10^{-4}$ A. Under these conditions, the CNE is shown to be more sensitive than the GPE peak current and obtained to −0.15 V reduction small peak. Therefore, better sensitive stripping conditions were examined.

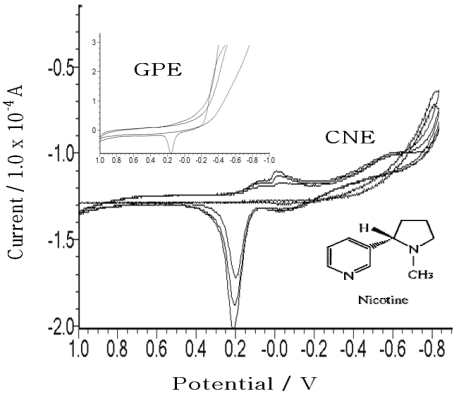
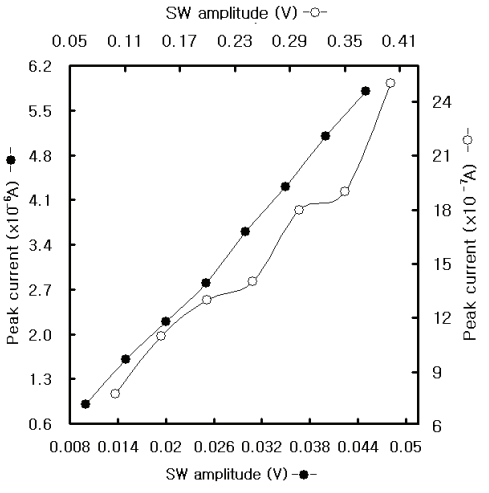


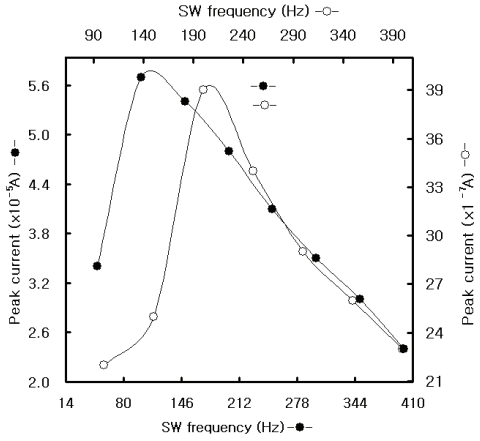
Fig. 1. Comparison of the reaction potential in the common-type GPE for 0-μg/L and 40-μg/L nicotine spikes and 0, 10, 20, and 30 μg/L spikes at the CNE, a -1.0 V initial potential, a 1.0 V switching potential, and a 0.1 V/s scan rate, using a 0.1 M H_3PO_4 electrolyte solution.

3.2. Stripping voltammetric optimizations for GPE and CNE

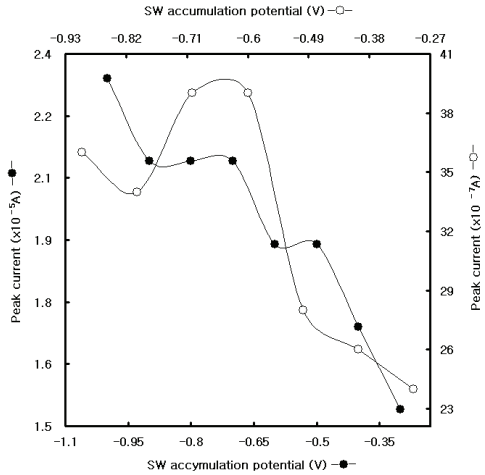
The GPE and CNE were compared to find the optimum conditions. Fig. 2 (A) shows the peak current in a 15 mg/L nicotine as a function of the square wave amplitude 0.01-0.045 V variations. When 0.01 V amplitude was given, the stripping peak current of GPE reached $0.9x10^{-6}$ A. When 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, and 0.045 V were given, the stripping peak current of GPE reached 1.6, 2.2, 2.8, 3.6, 4.3, 5.1, and $5.8x10^{-6}$ A individually. Under these ranges, 0.045 V was chosen as the optimum, where the peak width was sharp and sensitive. Under this condition, the stripping peak current of CNE was examined with 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, and 0.45 V, and their peak currents were 7.8, 11, 13, 14, 18, 19, and $25x10^{-7}$ A, respectively. In the end, 0.4 V was chosen. Fig. 2 (B) shows varying square wave frequencies variation for 50, 100, 150, 200, 250, 300, 350, and 400 Hz. The peak current of GPE reached 3.4 5.7, 5.4, 4.8, 4.1, 3.5, 3 and $2.4x10^{-6}$A, respectively. 100 Hz was chosen as the optimum frequency. Furthermore, the CNE for 100, 150, 200, 250, 300, 350, and 400 Hz were revealed as 7.8, 25, 39, 34, 29, 26 and $23x10^{-7}$A, respectively. 200 Hz was sensitive and had a narrow peak width. Using these conditions, stripping deposition potentials were examined. Fig. 2 (C) shows their anodic effects. Varying from -1.0 to -0.3 V GPE (-○-), the peak current varied from 1.53 to $2.38x10^{-5}$ A, and CNE (-●-) peak potential was varied from -0.9 to -0.3 V, while peak current was 2.4-40.2$x10^{-7}$A. Consequently, -0;7 V was chosen. Under these parameters, Fig. 2 (D) shows varying square wave incremental potentials for the range of 0.05-0.042 V, yielding 0.005 V GPE, 0.04 V CNE obtained. Under these parameters, the usable working ranges were examined.



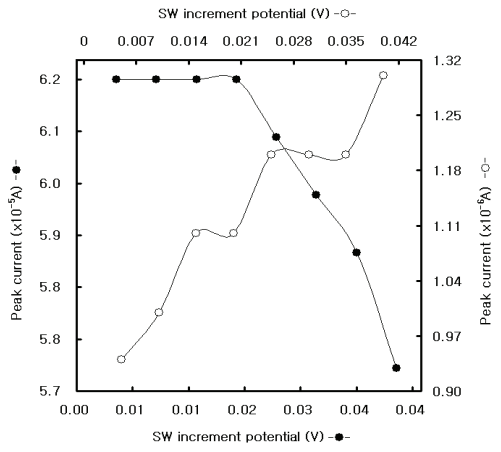
(A)



(B)



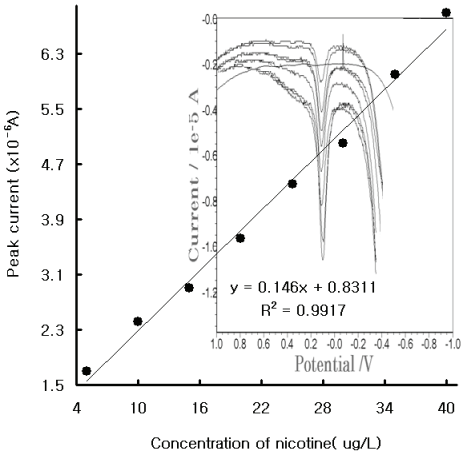
(C)



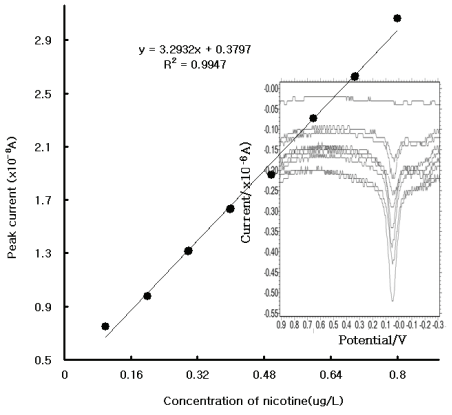
(D)

Fig. 2. SW comparison of GPE (-●-) and CNE (-○-). (A): SW amplitude variation of 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04 and 0.045 V. (B): SW frequency variation of 50, 100, 150, 200, 250, 300, 350 and 400 Hz. (C): SW accumulation potential variations of -1.0, -0.8, -0.7, -0.6, -0.5, -0.4 and -0.3 V. (D): SW incremental potential variations of 0.05, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035 and 0.04 V.

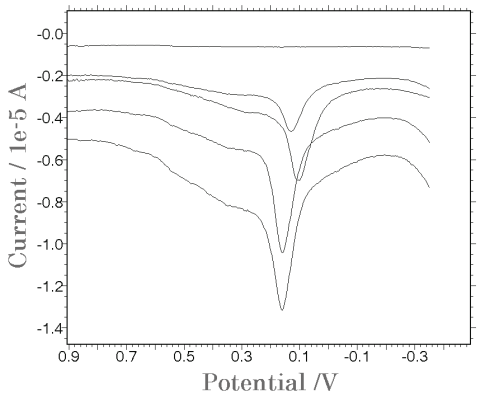
3.2. Analytical working ranges, statistics, and application



(A)



(B)



(C)

Fig. 3. (A): SW concentration effects on GPE of 5, 10, 15, 20, 25, 30, 35, and 40 μg /L, SW amplitude of 0.045 V, frequency of 100.0 Hz, accumulation potential of -0.7 V, and incremental potential of 0.005 V. For CNE (B): 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 μg/L variations. (C): Diagnostic application, the first curve represents the electrolyte blank, 1.0 ml non-smoker's urine and 5, 10 15 μg/L standard, using optimum parameters.

Fig. 3 (A) shows the SW analytical working ranges using GPE under optimized conditions from 0 to 40 μg/L variation. The electrolyte blank did not yield any signal. When 5-40 μg/L was added, the peak current increased from 1.7 to 6.9×10^{-6} A with anodic current, but no cathodic peak appeared. The width of nicotine was sensitive and narrow, and it had a linear equation of y = 0.146x + 0.8311 and statistic of R^2 = 0.9917. This can be applied to ex vivo assay. Also, Fig. 3 (B) shows the SW result using CNE at optimized conditions. The working range was 0.1-0.8 μg /L. When 0.1 μg/L was spiked, the SW working range reached 0.79×10^{-8}A. After that, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 μg/L were spiked, yielding $0.96\text{-}3.1 \times 10^{-8}$A.The linear equation was y =3.2932x+0.3797 and R^2 = 0.9947. Here, CNE was shown to be more sensitive than GPE, but both sensors were found to be applicable to biological assay. Under electrolyte blank, analytical detection limits were examined as 0.6 μg/L GPE and 0.07 μg/L CNE with a signal-to-noise ratio three times. A standard deviation of 0.064 GPE, 0.095 CNE (n = 12), 400 sec accumulation time by 10.0 μg/L constant were also used. Under optimum conditions, analytical interference effects were examined using organic and metal ions with dopamine, catechol, ephinephrine, glucose, Co, Ba, Bi, Ge, Ca, Fe and Cr ion having been added separately. These results obtained 70.00 %, 40.00 %, 24.0 %, 21.0 %, -21.43 %, -44.55 %, -27.87 %, -18.18 %, -41.67 %, 95.24 % and 0.0 % respectively. These interference effects were calibrated using the standard addition method.

The developed techniques can be used in ex vivo analysis. Fig. 3 (C) shows the SW results of the diagnostic application to urine with GPE. One ml of the non-smoker's urine was first placed in the electrolyte solution, and a small peak was obtained. Subsequently, 5, 10, and 15 μg/L of the nicotine standard were spiked. The SW voltammograms showed that peak current increased linearly and 1.307 μg/L nicotine concentration was obtained. Under CNE conditions, the smoker's urine was found to have 49.92 μg/L of nicotine (common smoker plasma level: 5-50 ng/mL [23] and hair sample: 39.0 ng/mg [24]). Thus, GPE and CNE can be applied to ex vivo analysis.

4. Conclusions

Voltammetric trace nicotine was detected using square wave stripping with common-type GPE and specialized CNE sensors. The optimal parameters of CNE obtained are as follows: 0.045 V SW amplitude, 100 Hz frequency, -0.8 V initial potential, and 0.005 V incremental potential. Moreover, the optimal GPE parameters obtained are 0.4 V SW

amplitude, 100 Hz frequency, -0.7 V initial potential, and 0.005 V incremental potential. Under these conditions, the two electrodes are able to detect micro-working ranges, with their detection limit reaching the nano range. They can also detect nicotine in a smoker's urine, indicating potential for application in other fields of biological diagnostics.

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