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Voltammetric Assay of Antibiotics for Modified Carbon Nanotube Sensor

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Abstract : A investigation of electrochemical analysis of antibiotics Neomycin ($C_{23}H_{46}N_6O_{13}$) was searched using electrochemical square wave (SW) stripping and cyclic voltammetry (CV) using working sensor of the modified carbon nanotube combination electrodes, optimum diagnostic parameters were searched by anodic stripping, final conditions were attained to working range of 1.0-14.0 ng/L, detection limit (S/N) was found to be 0.6 ng/L. The developed method was discovered to be fitting in quality control in the food, pharmaceutical and other manufacturing sectors.

Keywords : paste electrode, DNA, carbon nanotube, voltammetry, Neomycin.

1. Introduction

Neomycin is a widely used broad spectrum, water soluble aminoglycoside antibiotic, produced during the fermentation of Streptomyces fradie[1]. It has been used as a lot of antibiotics for animal and human disease, it could remain in body systems and causes immune reactions to antibiotics in human body or food systems. Recently analytical determination methods are developed such as capillary electrophoresis[2], an improved liquid chromatographic method[3,4,5,6], direct UV detection[7], Faradaic impedance spectroscopy[8], liquid chromatography with pulsed electrochemical detection[9], HPLC MS/MS[10], TLC[11],thin layer chromatography fluorescence detection[12], LC tandem mass spectrometry hydrophilic interaction chromatography[13], Ion association method[14] and an acoustic wave biosensor[15]. However there spectric photo systems are complicated and separation pre treatment techniques are demand. Herein simple and sensitive stripping voltammetric [16] handmade electrode[17] were used in this study. A working electrode was made by coating DNA on the nanotube structure. Carbon nanotube are large surface, stable mechanical and electric properties, which derive from the special properties of

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cylindrical symmetry. Moreover double helix structure of DNA molecules [18] has been immobilized on a carbon nanotube surface, which biosensor can detect low concentration[19,20]. It could apply to detection of neomycin in ointment.

2. Experimental

2.1. Apparatus and Procedure

The voltammetric systems were used in this study with an electrochemical instrumentation 1100 potentiostat from CH Instruments, Inc. These systems were equipped with a faraday cage, for environmental noise exclusion. A three-electrode cell was used to monitor the voltammetric signal. The three electrodes were prepared manually. The reference electrode was an Ag/AgCl electrode (saturated KCl), and the auxiliary electrode was a platinum wire. The DNA immobilized carbon nanotube working electrode (DCNT) was made by mixing 40 % carbon nanotube (15-40 nm in diameter for the CVD method obtained from Nanotech in Korea) powder with 40 % DNA (Double-stranded calf thymus DNA (dsDNA)) and 20 % mineral oil. Other reagents were obtained from Aldrich. This mixture was homogenized in a mortar for 30 min. The paste was inserted in a 3 mm plastic syringe needle, and a copper wire was connected to the electric system. CNT working electrode was prepared by mixing 50 % carbon nanotube powder with 50 % mineral oil. All experimental solutions were prepared from double distilled water with 18 M Ohm Cm^{-1} (MilliRO, Millipore) followed by ion exchange (MilliQ). The Neomycin ($C_{23}H_{46}N_6O_{13}\cdot 2\frac{1}{2}\ H_2SO_4$ MW 614.67) standard was prepared using Aldrich and diluted within the required time. The electrolyte solution was a 0.1 M phosphate buffer solution served as the supporting electrolyte. All the systems were performed in dissolved oxygen, and an electrode cleaning time was not necessary for every measurement. First tested were several electrolyte solutions such as acids and bases, all having 0.1 M strength. The phosphoric acid solution was found to be the most suitable medium. DNA immobilization was performed through a cyclic scan with an initial potential of +1.0 V, a switching potential of –1.0 V, and a scan rate of 100 mVs^{-1} with a tan cyclic repeat to stabilize the electrode surface.

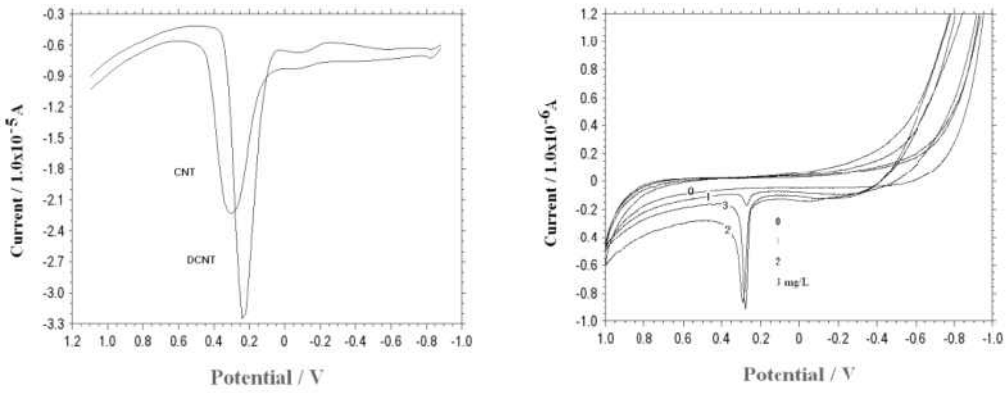
3. Results and Discussion

3.1. Sensor Comparison.

Fig. 1(A) shows the comparison between CNT and DCNT. Two electrodes were compared using SW, 3 mg/L standard at same electrolyte solution. The peak current of CNT was attained $2.25x10^{-5}$A as 3 mg/L Neomycin spike. Its DCNT was obtained $3.25X10^{-7}$A. DCNT was much more sensitive than CNT. Therefore, DCNT was chosen and used as our working electrode. With the working electrode, CV was studied ranges from 1 to 3 mg/L. Fig. 2(B) illustrates the results. The shape of the curve was narrowed at 0.3V. It became sharper as more concentration was spiked. After these experiments, optimum conditions were studied with SW using DCNT

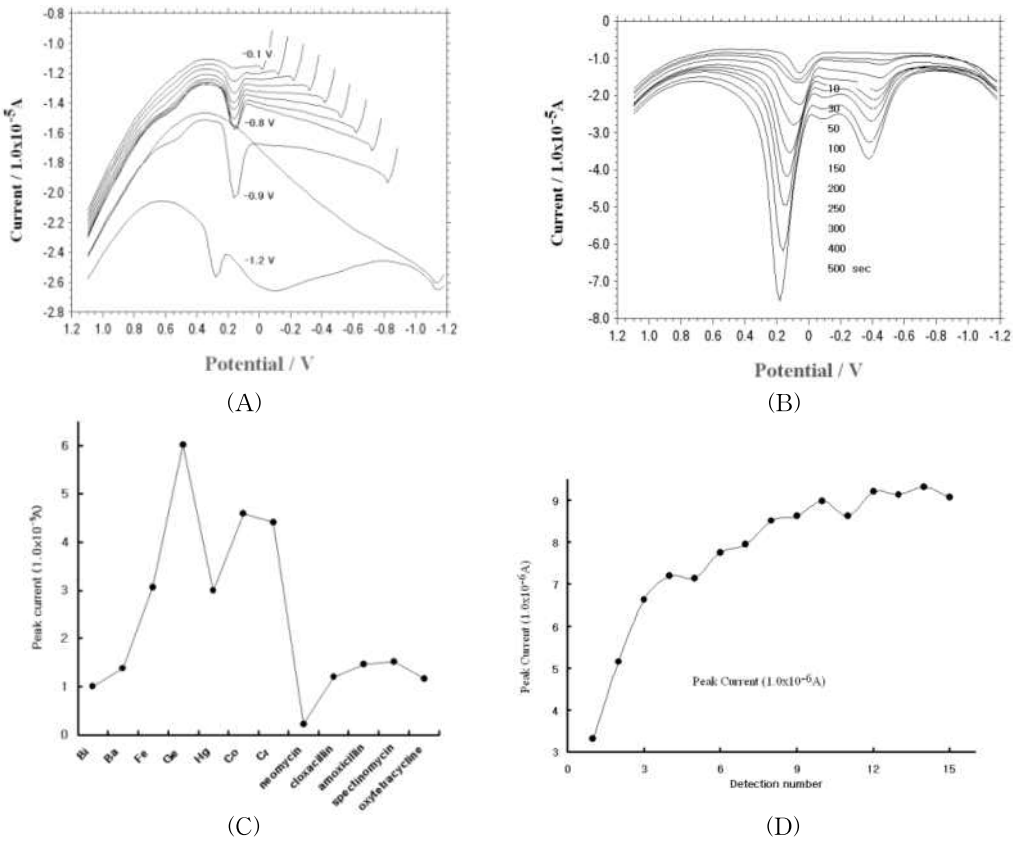
3.2. Optimizations of the Neomycin for the Square Wave Anodic Stripping Voltammetric Effect for pH, Accumulation Time, and Accumulation Potential

The stripping voltammetric optimum parameters of electrolyte pH strength, accumulation time and other conditions were examined for voltammetric sensitity. Fig. 2 (A) illustrates varying square wave initial.



(A) (B)

Fig. 1. (A): The SW comparison for CNT and DCNT in 3 mg/L constant. (B): concentration effects of 0-3 mg/L CV using DCNT with optimum parameters.



(A) (B)

(C) (D)

Fig. 2. Optimization of the SW stripping voltammetric parameters for (A): accumulation potential ranges of -0.1, -0.2, -0.3, -0.4, -0.5, -0.6, -0.7, -0.8 and -0.9 V variations. (B): accumulation time variations of 10, 30, 50, 70, 100, 150, 200, 250, 300, 400, and 500s; (C): interfere by different ions; and (D) is the statistics for DCNT.

potential (V; X axes, right side) for the ranges of -0.1 ~ -0.9 V. At -0.1V, 0.3379x10^{-6}A was obtained. As the range of initial E went up, the obtained current kept rising until initial E reached -0.7V. Then, it dropped a little at -0.8V, but it increased again to 4.338X10^{-6}. For these result, -0.8V initial potential was chosen as a optimum condition. Under these conditions, Fig. 2 (B) describes how accumulation time gave an effect on detecting Neo. At first 10 seconds, it obtained 5.936x10^{-6}A. The much time was given, the bigger current was attained. Thus, the biggest current was obtained at 500 sec, and it was chosen as an optimum condition. The other conditions were as followings of -0.2V amplitude, 55 Hz frequency and 0.02 V increment potential (not shown here). Using there conditions, Fig. 2 (C) shows the interference effects by metals and organic ions. At optimum condition, analytical peak currents were studied by 10 mg/L Neo constant. 10 fold spiking was examined for 100 mg/L of Bi, 100 mg/L of Ba, 100 mg/L of Fe, 100 mg/L of Ge, 100 mg/L of Hg, 100 mg/L of Co, 100 mg/L of Cr, 100 mg/L of cloxacillin, 100 mg/L of amoxicillin, 100 mg/L of spectinomycin and 100 mg/L of oxytetracycline. They resulted in -22.69 %, 37.81 %, 121.52 %, 96.06 %, -50.17 %, 53.19 %, -3.96 %, 438.43 %, 21.70 %, 4.73 % and -23.18 % individually. Where strong effects were calibrated by the standard addition methods. Then Fig. 2 (D) is the statistics for DCNT with 10 mgL^{-1} Neo constant, SW was examined fifteen times repeating. All the Fig.s were in the permissible range of error tolerance, result can be stable and usable for analytical applications. Thus analytical working ranges were performed.

3.3. Statistics and Application

Fig. 3(A): shows the micro working ranges for 13 points using DCNT. Where linear curve was from 0.5513x10^{-6}A to 14.43x10^{-6}A (Versus blank curve) and equation was y=1.3237x-2.2873, R^3=0.976, it can be usable for pharmaceutical analysis, moreover Fig. 3(B): illustrates CV and SW effect in the low concentration. The picture of the CV results ranges from 1 ng/L to 14ng/L. No current was obtained ranges from 1ng/L to 4 ng/L. At 5 ng/L concentration, peak current was obtained at first. It increased from 0.5759X10^{5} A to 9.778X10^{5} A. Also insert curve is the results of SW examination. First curve is the blank. The width of curve started to narrow by spiking Neo. It obtained 0.5513X10^{6}A first, and finally reached to 14.43X10^{6}A at 13 ng/L add. These results shows that SW is suitable for low detection. Under there conditions, Fig.3(C) shows the application in ointment using a standard addition method. It was dissolved in 10 ml nitrate and diluted with distilled water. 2 mL unknown solution was spiked first and the peak current of 1.95210^{6} was obtained. After that, 5 mg/L, 10 mg/L and 15 mg/L standard Neo were spiked and it obtained 4.728 X10^{6}A, 6.809 X10^{6}A and 8.514X10^{6}A, respectively. It increased to scale and it shows that Neo was detected for 542 mg/L in oint solution.

4. Conclusions

DNA immobilization was successfully carried out in this paper. DNA and nanotube's unique character could help yield good results. The optimum results obtained were as follows: 4.73 pH, 500 sec accumulation time, 0.2V amplitude, 50 Hz frequency, 0.02 V increment potential and -0.8 V initial potential. Based on these condition, sensitive response was observed at nanogram levels using DCNT. These results were applied to determination of neomycin in oint. It was shown that this developed sensor could be used in the medical and pharmaceutical fields.

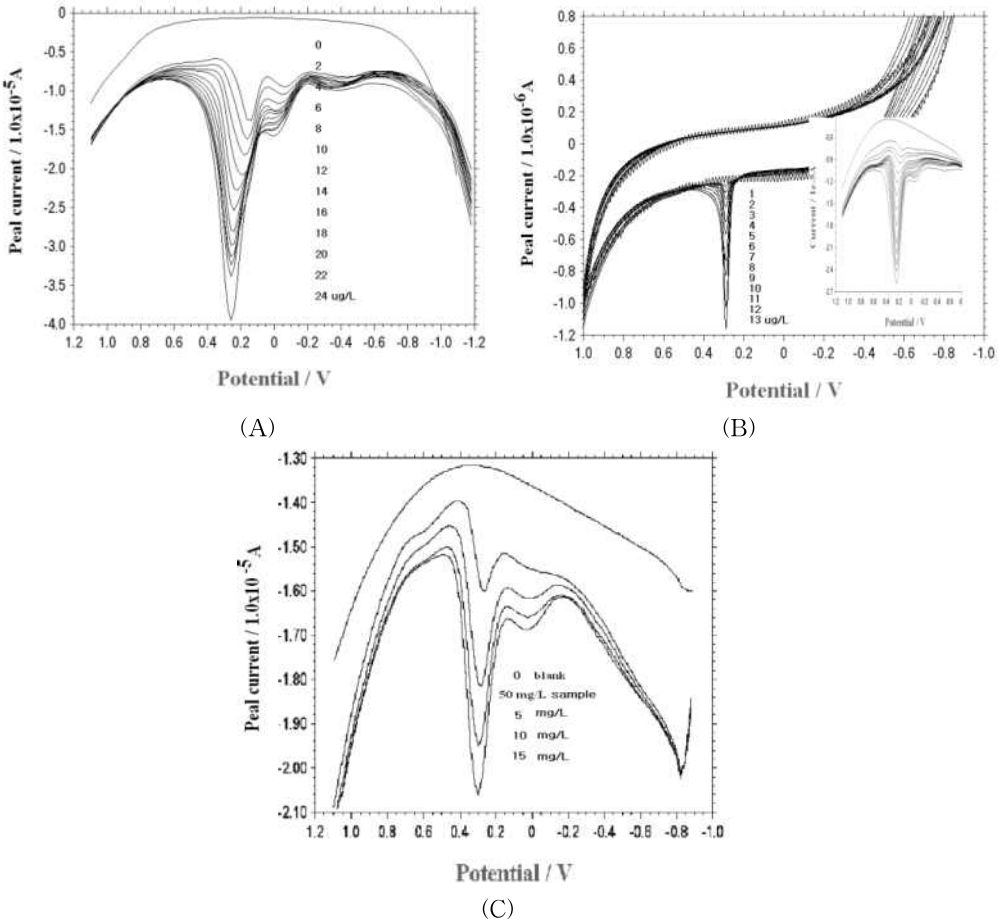


Fig. 3. (A): SW micro concentration effects for 2-24 ug/L. (B): Raw voltammograms for the SW and CV peak currents of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 ng/L based on optimum condtions. (C): Analytical application, the first curve represents an electrolyte blank solution, the second curve is a spiked 50 mg/L sample dissolved ointment, then standard spiked 5mg/L, 10mg/L, and 15mg/L. The other parameters used were the same asin Fig.2.

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