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Detection of dopamine in the pharmacy with a carbon nanotube paste electrode using voltammetry

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Abstract

A simply prepared DNA immobilized on a carbon nanotube paste electrode (CNTPE) was utilized to monitor dopamine ion concentration using the cyclic voltammetry (CV) and square-wave (SW) stripping voltammetry methods. The optimum analytical conditions were sought. The result obtained was a very low detection limit compared to other common voltammetry methods. The optimal parameters were found to be as follows: 3.5 pH, 0.48 V SW amplitude, 71 Hz frequency, 5 s accumulation time, 0.01 V increment potential, and −1.3 V (anodic-●-) and 1.2 V (cathodic-○-) accumulation potentials. Given these conditions, the linear working range was observed to be within 0.01–0.11 μg L^{-1} (SW anodic and CV). The analytical detection limit was determined to be SW anodic and CV: 4.0 μg L^{-1} (2.1×10^{-11} mol L^{-1}) dopamin, and the relative standard deviation at the dopamine concentration of SW anodic 0.05 μg L^{-1} was 0.02% ($n = 15$) at the optimum conditions.

Keywords: Dopamin; Carbon nano tube; DNA; Paste electrode; Voltammetry

1. Introduction

Trace dopamine assay is an important neurotransmitter in the mammalian central nervous system [1]. It can cause Parkinson's disease and other similar diseases [2,3]. Thus, various commonly usable analytical methods for dopamine and its analogs have been developed in the past. Some examples of these methods are the rapid liquid chromatography/tandem mass spectrometry (LC-MS/MS) [4], the chromatography method [5–8], and the capillary electrophoresis mass spectrometry method [9]. These methods are very sensitive. All these techniques, however, require a compressing system, temperature controlling systems, separation systems, and other spectrophotometric or electric detection systems. Recently, there has been an increasing demand for more sensitive and simpler analytical methods. Square-wave voltammetry techniques are very useful and popular for trace analysis [10–12] since these techniques are compact, efficient, and sensitive [13,14]. Various voltammetry solutions have been found to have a low detection limit required for neurotransmitter dopamine analysis, depending on the working electrode systems. For example, DL-homocysteine self-assembled gold electrode attained detection limits of 5×10^{-7} mol L^{-1} [15], phthalocyanine polymer modified electrode, 9×10^{-8} M [16]; thiolactic acid self-assembled gold electrode, 3.0×10^{-6} M [2]; poly (aminobenzoic acid) modified electrode, 2.0×10^{-8} M [3]; highly boron-doped diamond thin-film electrode, 50×10^{-9} [17]; poly (sulfosalicylic acid) modified glassy carbon electrode, 5.0×10^{-9} M [18]; nickel phthalocyanine polymer modified electrode, 9.0×10^{-8} M [1]; and covalent modified carbon electrode, 9.0×10^{-9} [19]. All these methods achieved a very low detection limit. In this study, however, more sensitive and simpler analytical methods were prepared using DNA immobilized on a carbon nanotube paste electrode [20] for dopamine analysis.

In biology, DNA immobilization is a very useful method for analysis [21]. Various researches have been conducted on DNA immobilization and the CNT function [22,23]. A huge, cylindrical surface area and graphene sheet CNT have special properties [24], such as high electrical conductivity [25], chemical stability, and catalyst support [26], which are useful for an analytical biosensor. The developed methods were combined with the properties of DNA and CNT, which are less expensive and simpler, paving the way for their successful application to the lowering of the detection limit of dopamine,

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which has been found useful in analyzing other dopamine ion concentrations.

2. Experiment

2.1. Apparatus reagents and the procedure

Voltammetric systems were conducted using the CHI-660A instruments electrochemical workstation (CH Instruments Inc., Cordova TN, USA). Three-electrode cell systems were used to monitor the cyclic and square-wave stripping voltammograms. The DNACNTPE was prepared by mixing 40% nanotube graphite powder (Nanostructured and Amorphous Materials, Inc.) and 40% DNA (double-stranded and prepared from calf thymus sigma) with 20% mineral oil. The mixing ratio was examined at 1:7, 2:6, 4:4, 6:2 and 7:1 wt.% with DNA: CNT, and 4:4 exhibited good results. The mixture amount of 5 g was homogenized in a mortar for 30 min. The mixed paste of 0.1 g was then inserted into a plastic needle-type capillary tube with a 1.5 mm diameter and a 5 cm length, using a 0.5-mm-diameter copper wire connected to the measurement system. An Ag/AgCl electrode and a platinum wire served as the reference and auxiliary electrodes, respectively. All the solutions were prepared from double-distilled water (-18 M Ω cm^{-1}). A 0.1-M $NH_4H_2PO_4$ with a pH level of 3.5 served as a supporting electrolyte solution. All other reagents were of analytical grade. The three-electrode system was immersed in a stirred solution of a known amount of dopamine. Preconcentration prior to stripping was carried out at open circuit. The common parameter for CV was a scan rate of 100 mV s^{-1}, and the common parameters for the stripping voltammetry were used at optimized conditions. The voltammetric response of dopamine is dependent on the electrolyte solutions and the hydrogen ionic strength. Various types of electrolyte solutions were tested, and the phosphate solution was found to yield the best results. In the electrode comparison, the DNA immobilized paste electrode responded more sensitively than bare-type CNT electrode in CV peak high for oxidation and reduction scan. Results obtained by CNT are as follows: -3.585×10^{-7} A (ox), 5.797×10^{-7} A (re), and DNA: -6.164×10^{-7} A (ox), 9.372×10^{-7} A (re) using 20 mg L^{-1} dopamine, at the same electrolyte condition. At this time, anodic peak was broad while cathodic peak increased sharply.

3. Results and discussion

3.1. Cyclic voltammetry and electrolyte pH effects

First, the cyclic voltammetric peak potentials with a high concentration of 0–100 mg L^{-1} dopamine were studied. Fig. 1(A) shows the raw voltammograms of the background electrolytes (0.1-M $NH_4H_2PO_4$, with a pH of 3.5) at a scan rate of 100 mV s^{-1}, based on the cyclic voltammetric signals tested on the DNA-CNTPE. During the initial cathodic scan of blank and 10 mg L^{-1} dopamine concentrations, a reduction peak appeared, as well as a shoulder at 0.0 V. Then, at the next reverse scan, a small oxidation peak of 0.4 V appeared, which

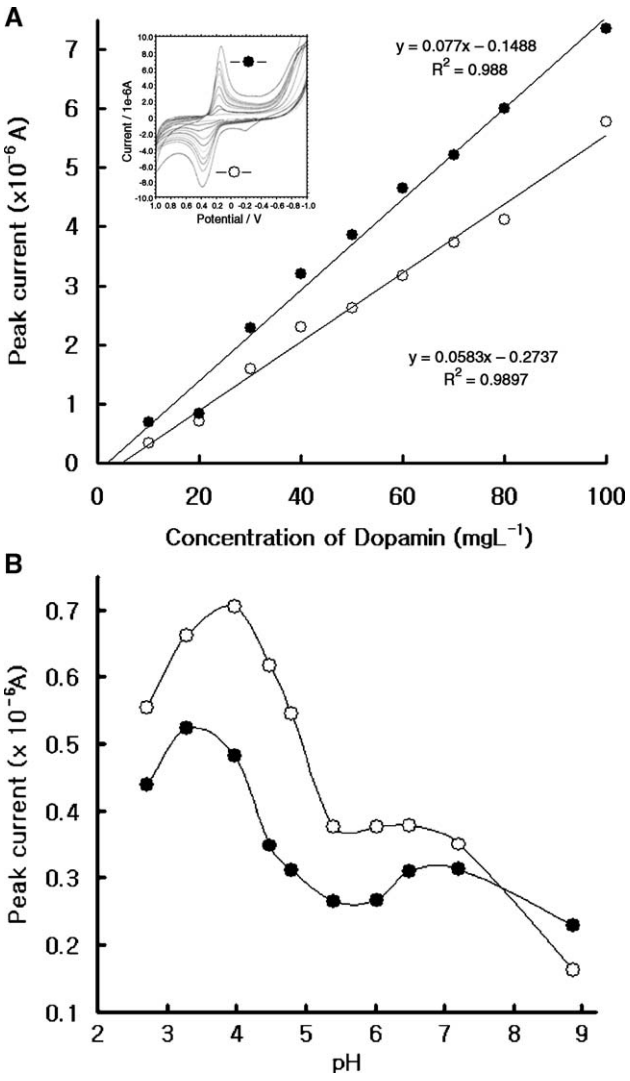

Fig. 1. (A) Cyclic voltammetric (-●-: anodic and -○-: cathodic) peak current of the various concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80 and 100 mg L^{-1} dopamin with a pH of 3.5. (B) Different pH effects of 2.7, 3.2, 4.0, 4.5, 4.8, 5.4, 6.0, 6.5, 7.2, and 8.9, with a scan rate of 100 mV s^{-1}, on 70 mg L^{-1} dopamin, initial potential of -1.1 V, and switching potential of 1.0 V. Electrolytes of the 0.1-M $NH_4H_2PO_4$ solution.

was not well extracted from the background discharge. Thus, more increased concentrations of 20 to 100 mg L^{-1} dopamine were spiked. At these conditions, linear working equations of reduction $y=0.077x-0.1488$, a correlation of $R^2=0.988$ (y: current in A; x: concentration in mg L^{-1}), and oxidation $y=0.0583x-0.2737$ and $R^2=0.9897$ were obtained, so these peak potentials were used for the determination of square-wave stripping voltammetry. In Fig. 1(B), various hydrogen ionic strengths (2.7–8.9 pH) were examined using 0.1-M HCl and 0.1-M NaOH spiking, within the acid ranges of 3.5–3.8 pH. The peak current quickly increased, and the maximum peak currents of oxidation (0.70×10^{-6} A) and reduction (0.52×10^{-6} A) were obtained. Then, within the ranges of 4.0–5.4, both peaks quickly decreased, and then disappeared at high ranges. Thus, all other experiments were performed using pH 3.5 electrolyte solutions. In addition, the anodic peak current

sensitively responded, followed by the cathodic peak current. The next sections examined more sensitive methods of stripping voltammetry parameters, including CNTPE sensitivity. First, the various accumulation times were tested using a fixed concentration of 70 μg L^{-1} dopamine (not shown here). From 5 to 300 s, both the anodic and cathodic peak currents linearly decreased, and a 5 s accumulation time resulted. Thus, all other stripping voltammetry procedures were performed with a short accumulation time of 5 s.

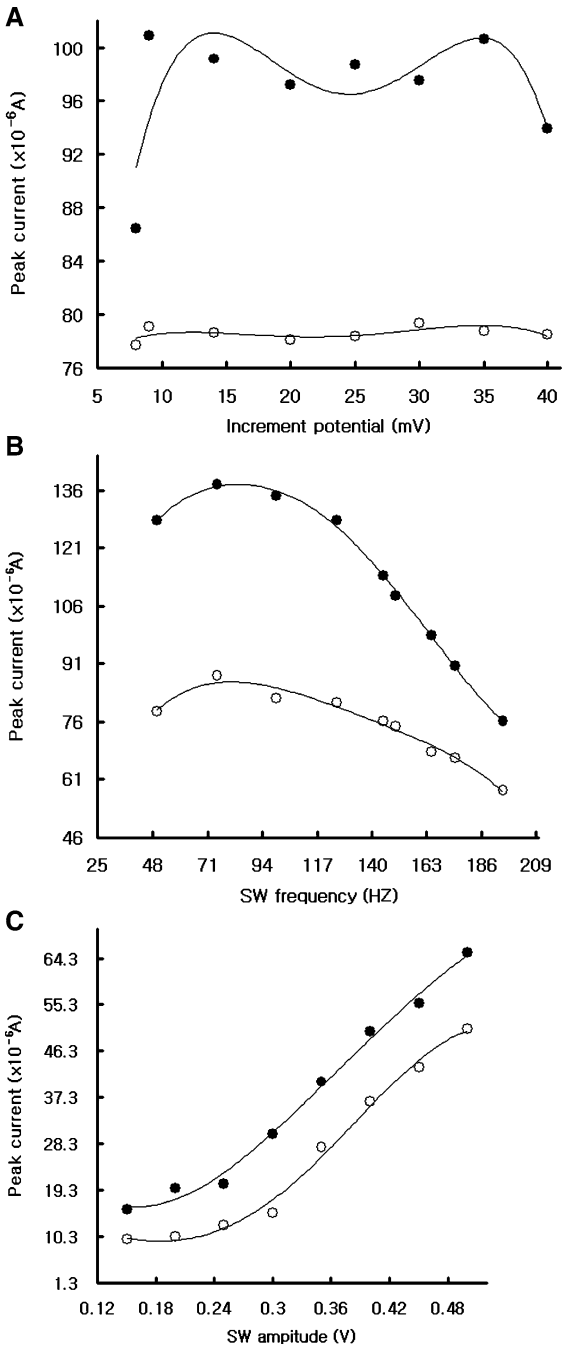

Fig. 2. (A) Square-wave (-●-: anodic and -○-: cathodic) stripping voltammetric peak current at various increment potentials of 8, 9, 14, 20, 25, 30, 35, and 40 mV. (B) Square-wave frequencies of 50, 75, 100, 125, 145, 150, 165, 175, and 195 Hz. (C) Square-wave amplitudes of 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 V in a 70 mg L^{-1} dopamine concentration, and with an accumulation time of 5 s. The other conditions in Fig. 3 were held constant.

3.2. Experimental optimization for various parameters of square-wave stripping voltammetry

In Fig. 2(A), various square-wave peak currents for the -●-: anodic and -○-: cathodic increment potentials of 8–40 mV are shown in the horizontal axis within fixed concentrations of 70 mg L^{-1} dopamine concentration. At these conditions, the accumulation time was fixed at 5 s. The anodic peak current more sensitively responded than the cathodic peak current did, while the peak width and sharps were not affected. Only the anodic peak current increased considerably. Both maximum peak currents were obtained at 35 mV. Thus, all other experiments used these potential ranges. Fig. 2(B) shows the SW frequency, at which various ranges of 50–195 Hz were examined using a fixed 70 mg L^{-1} dopamine concentration. At 75 Hz, a very high increase of anodic (137×10^{-6} A) and cathodic (87×10^{-6} A) peak currents was obtained. At this range, the anodic peak current more sensitively responded than the cathodic peak current did, while from 100 to 195 Hz, the response linearly decreased. The peak sharps and widths did not change; only the peak high changed. Thus, all experiments were performed at this 75 Hz condition. Fig. 2(C) shows the effects of SW amplitude within a range of 0.15 to 0.5 V peak current at the same conditions and at a fixed dopamine concentration of 70 mg L^{-1}. As expected, the peak current increased as the amplitude increased. Specifically, the peak current increased at a fast rate of up to about 0.5 V, then slowed down and started to level off at a range between 0.55 and 0.6 V. Thus, 0.5 V was identified as the maximum amplitude for the stripping voltammetry. At every measurement step, the electrode surface was cleaned using weighing papers, and electrode stability was obtained with statistical precisions of within 5% error in each step, with each electrode usable in one or two months.

4. Statistical results and application

In Fig. 3, at the optimum conditions, various detectable working concentration ranges were examined. After the background current was subtracted from the measured currents, only two ranges appeared as the adjusted currents were plotted. This exhibits the linear range in the high concentration range (A) of 10–100.0 mg L^{-1}. Within this range, anodic and cathodic directions sharply appeared, while anodic peaks more sensitively increased with a regression equation of $y=1.926x-1.840$ (y: current in A; x: concentration in mg L^{-1}) with $R^2=0.993$ (R^2: correlation coefficient), and cathodic reactions for $y=1.4049x-3.5807$ with $R^2=0.9951$. Then, a very sensitively low range of 0.01–0.11 μg L^{-1} (SW anodic and CV) as well as regression equations of $y=146.29x+0.1037$, $R^2=0.9954$ (SW), and $y=8.5729x+0.0054$ $R^2=0.9552$ (CV) were obtained. These equations can be used for determining the dopamine concentrations in pharmaceutical samples. The precision of the replicated 15th determination of the 0.05

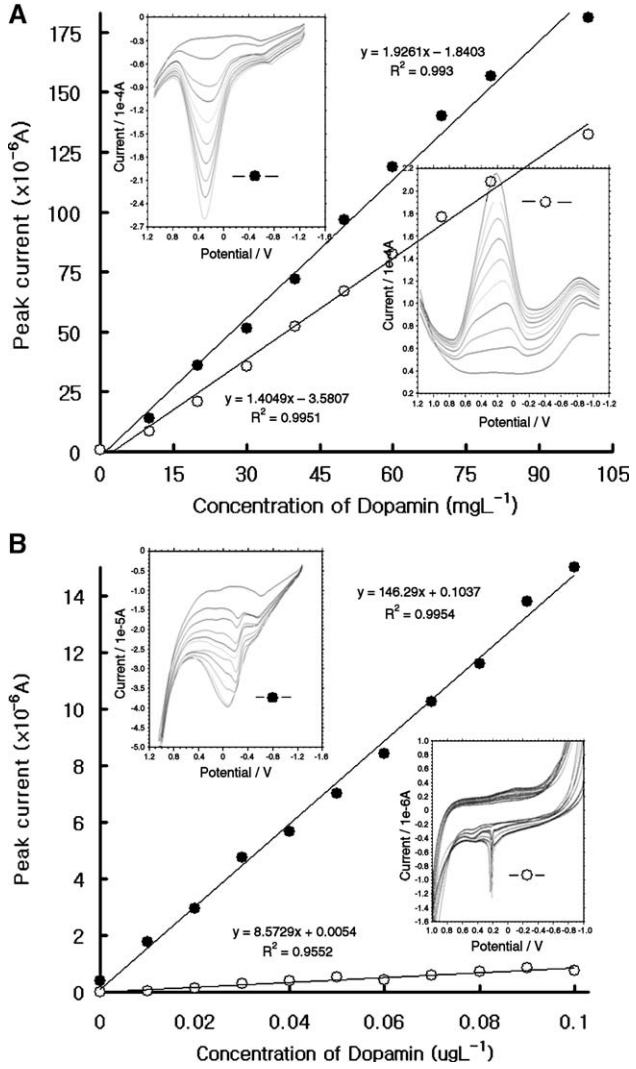

Fig. 3. Square-wave stripping voltammograms of dopamine at various concentrations: (A) 0, 10, 20, 30, 40, 50, 60, 70, 80, and 100 mg L^{-1} (anodic and cathodic); and (B) 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, and 0.11 μg L^{-1} (SW anodic and CV) at optimum conditions, in a 0.1-M $NH_4H_2PO_4$ solution with a pH of 3.5, a deposition time of 5 s at − 1.3 V anodic and 1.2 V cathodic, a frequency of 75 Hz, an increment potential of 35 mV, an amplitude of 0.5 V, and the calibration curve with the results of regression. Other parameters were set at optimum conditions.

μg L^{-1} solution yielded a relative standard deviation of 0.02%. These results are highly reproducible. An analytical detection limit of 4.0 ng L^{-1} (2.1×10^{-11} mol L^{-1}) per concentration was calculated based on the peak current from the noise characteristics of the data for $S/N=3$, at the optimum conditions. Applying the standard addition method in determining the dopamine concentration can eliminate the interference of other species. At optimum conditions, various possible interference species with metal ion and other neurotransmitter analogies were tested, with a fixed dopamine concentration of 1 mg L^{-1}. The criterion used for the presence of interference was a 5% or greater change in dopamine peak current. The 10 mg L^{-1} catechol ion did not show any interference, while 10 mg L^{-1} of epinephrine, glucose, Vitamin C, Ba(II), Ca(II), Pb(II), Cr(III), Co(II), Bi(II), Cd(II), and Cu(II) yielded 23.39%, 21.01%, 16.99%, − 12.87%, − 100%, − 100%, 195.09%, 2014.66%, 1430.42%, 956.16%, and 328.71% interference, respectively. The percentages pertain to the increase in dopamine peak current. At this time, metal ions sensitively interfered in the peak high. Other interference ions can be corrected using standard addition methods. Experimental applications were performed using a pharmaceutically known amount of 200 mg/5 mL dopamine ampule for a pre-filled syringe system (S Company Co., Korea), which was tested five times, under optimal conditions. This method yielded an amount of 198 mg ± 0.2/5 mL, with a 99% ($n=5$) recovery. More expanded applications were performed with other fixed, known concentrations, and good results were obtained. Thus, the proposed methods were approached at very low detection ranges of 2.1×10^{-11} mol L^{-1} as compared with other common voltammetry methods at 3.0×10^{-6}~5.0×10^{-9} M [15,16,2,17,18,1,19].

5. Conclusions

Optimum analytical conditions for determining dopamine ion concentration were sought using DNA-CNTPE, whose signal was shown to be more sensitive than that of the other common voltammetry methods. The experimental conditions were found to be as follows: a pH strength of 3.5 with 0.1 M $NH_4H_2PO_4$; an accumulation potential of − 1.3 V (a) and 0.8 V (c); a deposition time of 5 s; an SW frequency of 75 Hz; an SW amplitude of 0.5 V; and an increment potential of 35 mV. A low range of 0.01 to 0.11 μg L^{-1} dopamine (SW anodic and CV) was obtained. Under optimum analytical conditions, the detection limit was determined to be 4.0 ng L^{-1}, with a 0.02% relative error at a 3.0 mg L^{-1} dopamine concentration.

It can therefore be concluded that the methods used in this paper are viable for monitoring dopamine. It was also confirmed that DNA-CNTPE is much more sensitive than the conventional voltammetry.

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