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ELECTROCHEMICAL DETECTION OF ZANAMIVIR USING GOLD AND GOLD-MODIFIED BORON DOPED DIAMOND ELECTRODE

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Abstract

Zanamivir is a neuraminidase inhibitor approved by WHO for treatment and prophylaxis of influenza virus A and B. Quantitative analysis of zanamivir is important to monitor its content in medicine and aquatic environment. This study addressed the electrochemical detection of zanamivir using gold and gold-modified boron doped diamond (BDD) electrodes. The measurement was conducted using cyclic voltammetry method. Logarithmic curve was consistently observed from measurement of zanamivir in wide range of concentration on three electrodes. Linear calibration curve of zanamivir was obtained in the concentration range of 1 x 10^{-5} - 1 x 10^{-4} mol/L (r^2 = 0.969) on gold electrode, 5 x 10^{-6} – 1 x 10^{-4} mol/L (r^2 = 0.990) on Au-BDD electrode, and 1 x 10^{-6} - 1 x 10^{-4} mol/L (r^2 = 0.998) on AuNPs-BDD. Meanwhile, the detection limit estimated to be 1.19 x 10^{-6}, 1.49 x 10^{-5}, and 2.29 x 10^{-6} mol/L, respectively for gold, Au-BDD, and AuNPs-BDD electrode. The precision of zanamivir measurements on three electrodes was good with %RSD < 2.5 %.

Keywords: Diamond, Electrochemical, Gold, Voltammetry, Zanamivir.

INTRODUCTION

Zanamivir (Figure 1) is a neuraminidase inhibitor approved by WHO for treatment and prophylaxis of influenza virus A and B. This compound prevents the release and spread of the newly formed virion through blocking the active site of neuraminidase. Zanamivir therapy was reported to be more effective for influenza B treatment compare to oseltamivir (former neuraminidase inhibitor) [1]. The use of zanamivir was rose as oseltamivir resistant virus was reported [2]. Examination of zanamivir content in commercial drugs should be conducted in order to ensure that the patients get the appropriate treatment dosage. Improper dosages lead to resistance effect of the viruses. Furthermore, the concentration of zanamivir in the aquatic environment also important to be monitored due to rising content by human

excresions and improper disposal [3]. Undesired high levels of antivirals in the aquatic environment which contact with the viruses are potentially cause resistance.

Several analysis methods was reported for zanamivir determination, such as LC-MS [4,5] and detection using mercury drop electrode [6]. These techniques are effective for zanamivir measurement, otherwise LC-MS needs several types of chemicals and sophisticated instrument. Meanwhile, detection using mercury drop electrode was avoided because of safety reason. Due to this reasons, a alternative method for zanamivir measurement need to be developed.

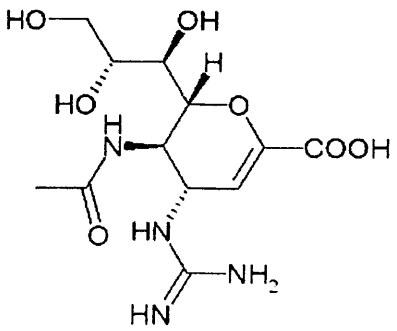

Figure 1 Molecular structure of Zanamivir.

Electrochemical method for zanamivir measurement is prospective to be developed since it is simple, fast, and reagentless. In this work, electrochemical measurement of zanamivir using gold and gold modified boron doped diamond electrode (BDD) was developed. BDD was used as working electrode due to itslow background currents, stability, and wide potential window in aqueous solution [7]. Meanwhile, gold used for BDD modification to enhanced its performace in zanamivir sensing.

MATERIALS AND METHODS

Materials

Zanamivir (Tokyo Chemical Industry Co. Ltd.), $KAuCl_4.4H_2O$ (Wako Chemicals), allylamine (Tokyo Chemical Industry Co. Ltd.), $Na_3C_6H_5O_7$, K_2HPO_4, KH_2PO_4, H_2SO_4, $C_3H_9BO_3$, hydrogen, oxygen, methane, silicon wafer, diamond powder, 2-propanol, Gold electrode, Ag/AgCl as reference electrode, platinum as counter electrode, and high purity water with maximum conductivity of 18 MΩ obtained from Simply-Lab water system (DIRECT-Q 3 UV, Millipore).

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Methods

Preparation of Working Electrode

Boron doped diamond (B/C atomic ratio of 1:1000) were deposited on Si (100) wafers in microwave plasma-assisted chemical vapor deposition (MPCVD) system (ASTeX Corp.). BDD was electrochemically polarized at 3 V (vs. SSCE) in 0.1 mol/L H_2SO_4 for 20 min to obtain oxygen terminated BDD. Au-BDD was prepared through electrochemical deposition in equal volume of 2 mM $HAuCl_4.4H_2O$ and H_2SO_4 0.5 mM mixture at 0.2 V (vs. SSCE) for 100 min, detail preparation are describe elsewhere [8]. Meanwhile, AuNPs-BDD was obtained by photochemical reaction using allylamine followed by chemical reaction with AuNPs. The AuNPs prepared according to Turkevich and Frens method [9, 10]. XPS were used to characterize the Au-BDD and AuNPs-BDD electrode prior to use as working electrode.

Electrochemical Measurement of Zanamivir

Electrochemical measurement of zanamivir was performed in a single compartment cell with three electrodes. A platinum wire was used as counter electrode and silver/silver chloride electrode (SSCE) used as reference electrode. Working electrode Au-BDD and AuNPs-BDD was pressed against the bottom of the electrochemical cell by an O-ring with the geometric area approximately 0.125 cm^2. Phosphate buffer (PBS) 0.1 M was used as the supporting electrolyte. Cyclic voltammetry measurements of various concentrations of zanamivir in PBS 0.1 M was performed at a scan rate of 100 mVs^{-1}. Precision of zanamivir measurement was expressed as percentage relative standard deviation (% RSD) which obtained from nine replicates analysis of three concentration level of zanamivir.

RESULTS AND DISCUSSION

Hydrogen terminated BDD with 0.1 % boron has successfully prepared by MPCVD system. Raman spectra (Figure 2a) shows an intense peak at 1332 cm^{-1} which confirmed that carbon sp^3 of diamond have already deposited on Si wafer [7] and the broad peak at 500 cm^{-1} expected to be boron doped on diamond film [11,12]. Diamond crystals with approximately 3 μm in size were covered the silica wafer (Figure 2b).

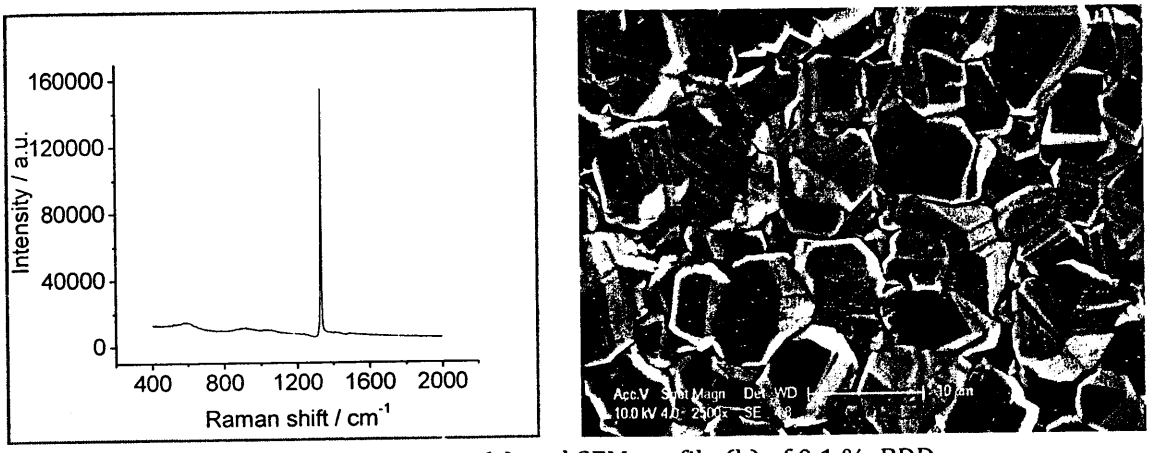


Figure 2 Raman spectra (a) and SEM profile (b) of 0.1 % BDD.

Gold was electrochemically deposited on BDD surface. Two peaks observed at 83.2 eV and 87 eV are attributable to Au $4f^{7/2}$ and Au $4f^{5/2}$, respectively (Figure 3a). Au particles expected to be physically deposited at BDD surface without any chemical bonding since there was no significant shift of C 1s peak at Au-BDD electrode. On the other hand, modification of the BDD surface with AuNPs was initiated by photochemical reaction of BDD surface with allylamine followed by chemical reaction of amine-terminated BDD with AuNPs. Amine functional groups facilitated the chemical bonding of AuNPs with BDD surface. XPS spectra confirm that AuNPs has successfully deposited on amine-terminated BDD. XPS peaks of Au $4f^{7/2}$ and $4f^{5/2}$ was detected at binding energies of 84 and 88 eV, respectively (Figure 3b).

Figure 4 shows cyclic voltammograms (CV) of 0.1 M PBS pH 7 obtained at a potential scan rate of 100 mVs^{-1} in the absence and in the presence of Zanamivir at Au electrode. There is no new oxidation or reduction peak observed in potential range of 0 V to +1.50 V. However, the CV showed that the presence of Zanamivir leads to the decrease of oxidation and reduction peak currents of gold. As proposed in our previous report, Zanamivir was adsorp on gold electrode and reduces the direct contact between gold surface and water/oxygen [8]. The condition leads to the decrement of oxidation and reduction of gold surface.This afford the chance for Zanamivir measurement using gold and gold modified BDD.

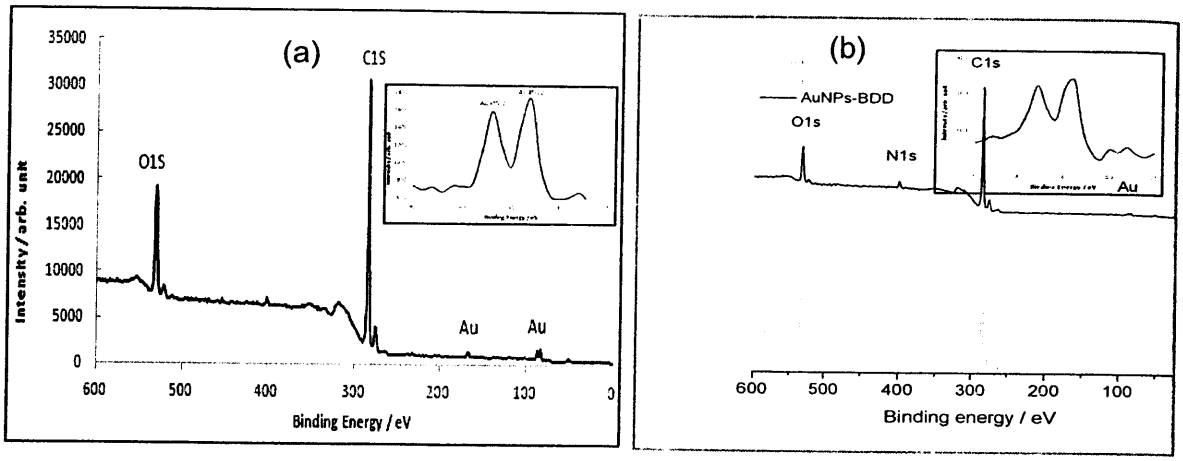


Figure 3 XPS spectrum of Au-BDD (a), oxidized BDD, allylamine modified BDD, and AuNPs modified BDD (b).

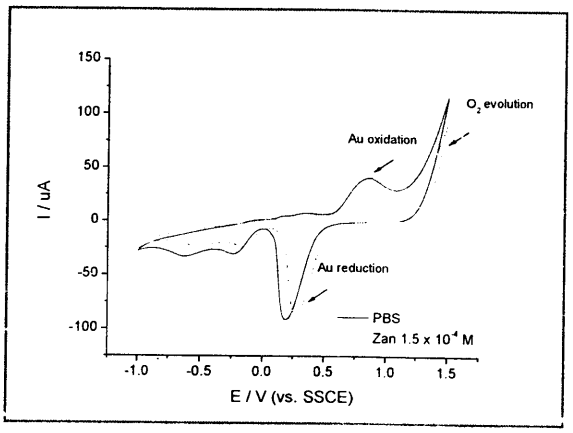
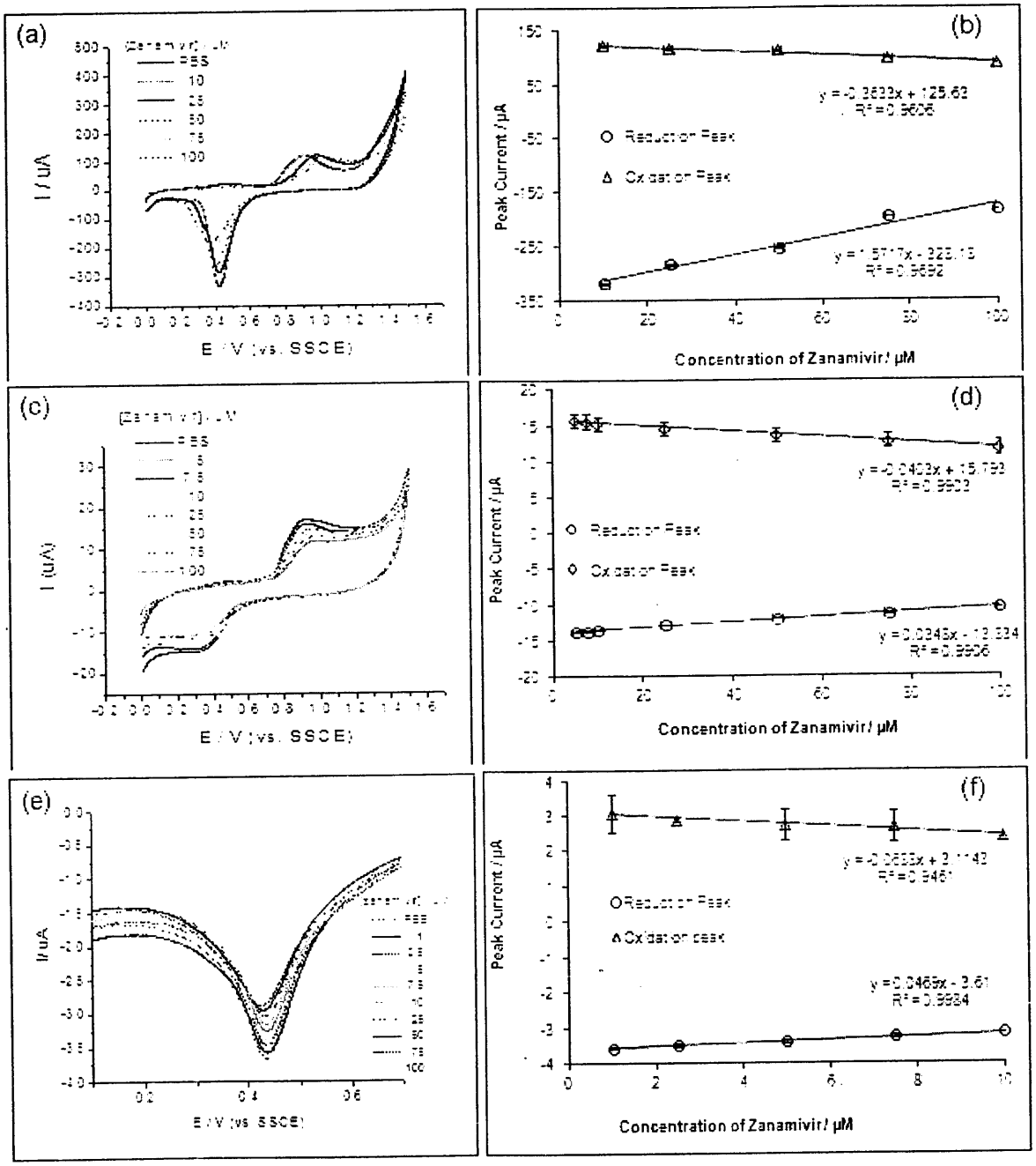


Figure 4 Cyclic voltammogram of 0.1 M PBS pH 7 in the absence and in the presence of Zanamivir at Au electrode.

Logarithmic curve was consistently observed from measurement of zanamivir in wide range of concentration on three electrodes (data not shown). Meanwhile,linear calibration curve of zanamivir was obtained in the concentration range of1 x 10^{-5} - 1 x 10^{-4}mol/L on gold electrode, 5 x 10^{-6} – 1 x 10^{-4} mol/L on Au-BDD electrode, and 1 x 10^{-6} - 1 x 10^{-5}mol/L on AuNPs-BDD. The linear calibration curve could be developed based on gold oxidation and reduction peak current. Otherwise, calibration curve developed from gold reduction peak current not always give excellent determination coefficient. Figure 5 shows the CV of 0.1 M PBS pH 7 in the presence of Zanamivir at various concentrations and calibration curve obtained from measurement on each electrode. While Au electrode shows higher sensitivity than Au-BDD and AuNPs-BDD electrode, higher determination coefficient can be achieved using Au-BDD and AuNPs-BDD electrodes. The detection limit estimated to be

1.19 x 10^{-6},1.49 x 10^{-5}, and2.29 x 10^{-6} mol/L, respectively for gold, Au-BDD, and AuNPs-BDD electrode. Furthermore, the precision of the current responses was investigated for nine consecutive measurements. The high precision of Zanamivir measurement was reflected in the relative standard deviations (RSDs), which are less than 2.5% at each electrodes. The summery of the analytical performance of Zanamivir at Au and Au-BDD was presented in Table 1.



Au (a,b), Au-BDD (c, d), and AuNPs-BDD (e, f) electrode.

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Table 1 The summary of the analytical performance of Au, Au-BDD, and AuNPs-BDD

Parameter	Electrode		
	Au	Au-BDD	AuNPs-BDD
Linearity range (M)	1×10^{-5} -1×10^{-4}	5×10^{-6} - 1×10^{-4}	1×10^{-6} - 1×10^{-5}
Determination coefficient (R^2)			
Oxidation peak	0.960	0.990	0.946
Reduction peak	0.969	0.990	0.998
Limit of Detection (M)			
Oxidation peak	1.25×10^{-5}	1.53×10^{-5}	1.89×10^{-5}
Reduction peak	1.19×10^{-6}	1.49×10^{-5}	2.29×10^{-6}
Precision of measurement n = 9 (%RSD)			
Oxidation peak	2.55	1.14	> 5
Reduction peak	0.33	0.5	2.23

CONCLUSION

Electrochemical detection of Zanamivir in 0.1 M phosphate buffer solution (PBS) has been successfully investigated at gold (Au) and gold-modified boron-doped diamond (Au-BDD and AuNPs-BDD) electrodes. Measurements of Zanamivir based on reduction peak current provide better performance than the oxidation peak. Performance of AuNPs-BDD electrode generally better than other two electrodes.

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OPTIMIZATION OF HPLC CONDITION FOR DETERMINATION OF VITAMIN A AND D_3 IN PHARMACEUTICAL PREPARATIONS

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Abstract

The purpose of the present study was to achieve the HPLC condition for the separation of vitamin A and D_3 in pharmaceutical formulations. The optimization of method was carried out by investigating the effect of HPLC conditions, namely mobile phase composition, flow rate, packing of column and analytical wavelength on peak symmetry, tailing factor, resolution and retention time reproducibility of the analytes of interest. A good simultaneous separation of vitamin A and D_3 in pharmaceutical formulations was obtained using a Chromolith® column RP-18e (100 mm x 4.6 mm) with methanol 100 % as mobile phase at flow rate of 1 ml/min. The detector was set at 265.8 nm and total analysis time was 12 min. The system suitability test demonstrated good resolution (Rs>5), tailing factor < 2, theoretical plate > 2000 and selectivity factor >1. Relative standard deviation (RSD) of retention time was 0.11% for vitamin A and 0.07% for vitamin D_3, whilst RSD of peak area was 0.09% and 0.99% for vitamin A and vitamin D_3, respectively.

Keyword : Vitamin A and D3, HPLC, Method Optimization.

INTRODUCTION

Vitamins are a group of organic compounds that are, in very small amounts, essential for normal metabolism, growth and development, and regulation of cell function. Thirteen vitamins are recognized in human nutrition which may be conveniently classified into two groups according to their solubility : as water-soluble (B-complex and C) and fat-soluble (A, D, E and K) [1].