

Dopamine Sensor Based on a Boron-Doped Diamond Electrode Modified with a Polyaniline/Au Nanocomposites in the Presence of Ascorbic Acid

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A selective dopamine (DA) sensor was developed using gold nanoparticles (Au-NPs) dispersed overoxidized-polyaniline (PANI_{ox}) based on a boron-doped diamond (BDD) thin-film electrode. The concentration of the DA was determined using voltammetry as a non-enzymatic sensor. BDD thin film has a high signal-to-noise ratio, a long-term stability, a high sensitivity, and a good reproducibility. PANI nanocomposites were directly synthesized on the BDD electrode and overoxidized using 0.5 M H₂SO₄ solution. The overoxidized PANI film enhances selectivity and sensitivity toward DA. The Au-NPs were dispersed on the PANI nanocomposite by electrochemical deposition. The nanometer-sized Au-NPs favor the sensing of DA in the presence of ascorbic acid (AA). The combination of the PANI with the Au-NPs and the BDD electrode can create synergetic effects for the performance of the biosensor, such as a fast response time, a lower detection limit, a wider linear range, enhanced selectivity, and higher sensitivity for the determination of DA.

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Introduction

The neurotransmitter dopamine (DA) plays a significant physiological role as an extracellular chemical messenger for the central nervous, renal, and hormonal systems.^{1,2} A deficiency of DA can result in some neurological disorders such as schizophrenia and Parkinson's disease.³ Rapid and accurate detection of DA is important for the diagnosis of many diseases. Dopamine is easily oxidizable, so electrochemical techniques are preferred to determine the presence of DA. However, direct quantification of the DA concentration at conventional electrodes by an electrochemical method is difficult because of the following issues: (a) a fouling effect on the surface of the electrodes due to the accumulation of oxidized product, (b) the homogeneous catalytic oxidation of ascorbic acid (AA) by the oxidized DA, (c) interference due to the coexistence of AA in the biological fluids and (d) the fact that the concentration of AA can be relatively higher than that of DA in these samples. These properties resulted in a rather poor selectivity and sensitivity for DA detection because both were oxidized more or less at the same potential.^{4,5} Hence, both sensitivity and selectivity are very important factors in developing a DA sensor. Some approaches have been proposed for modifying the electrode surface by utilizing various materials or techniques, such as ion-exchange polymers,⁶⁻⁸ inorganic materials,⁹ carbon materials,⁶ and self-assembled monolayers.^{5,10}

Boron-doped diamond (BDD) film has attracted considerable interest for use as active electrodes due to its superior chemical, physical, mechanical inertness and unique optical properties.

According to the doping condition, semiconducting or quasi-metallic qualities lead to high stability, high sensitivity, and a low detection limit.¹¹⁻¹³ The BDD electrodes enhance the signal-to-noise ratio, owing to its relatively low background current. The large potential window also opens up several important applications that cannot be accomplished using conventional electrode materials.

Polyaniline (PANI) nanocomposites have proved to be an excellent candidate for electrode material for various biosensors because of their beneficial features including a large surface area, homogeneity, unique redox properties, a high electrical conductivity, and a strong adherence to the electrode surfaces.^{14,15} In addition, the nanostructured PANI can be used as a suitable host matrix for other functional materials. The sensitivity of the polymer can be increased by increasing the volume porosity (effective volume) of the film. In particular, the combination of the nanostructured conducting polymers with catalytic nanoparticles, especially noble metal nanoparticles, was found to create synergetic effects in various electrochemical sensors, since the effective mediation of the electron transfer between the catalytic metal nanoparticles and the electrode substrate is accomplished.¹⁶ Although gold is a poor catalyst in bulk form, nanometer-sized gold nanoparticles (Au-NPs) can be very promising for practical applications by virtue of their superior peculiarities, which can facilitate the transfer of electrons and can improve the analytical sensitivity.^{17,18}

In this research, we attempted to develop a non-enzymatic sensor based on the BDD electrode with an Au-NPs dispersed PANI layer for the determination of DA. The PANI layer was directly synthesized on a BDD thin-film electrode and catalytic Au-NPs were dispersed on the surface of this nanostructured PANI using an electrochemical deposition. The nanostructured electrode exhibited good selectivity, high sensitivity, and a wide

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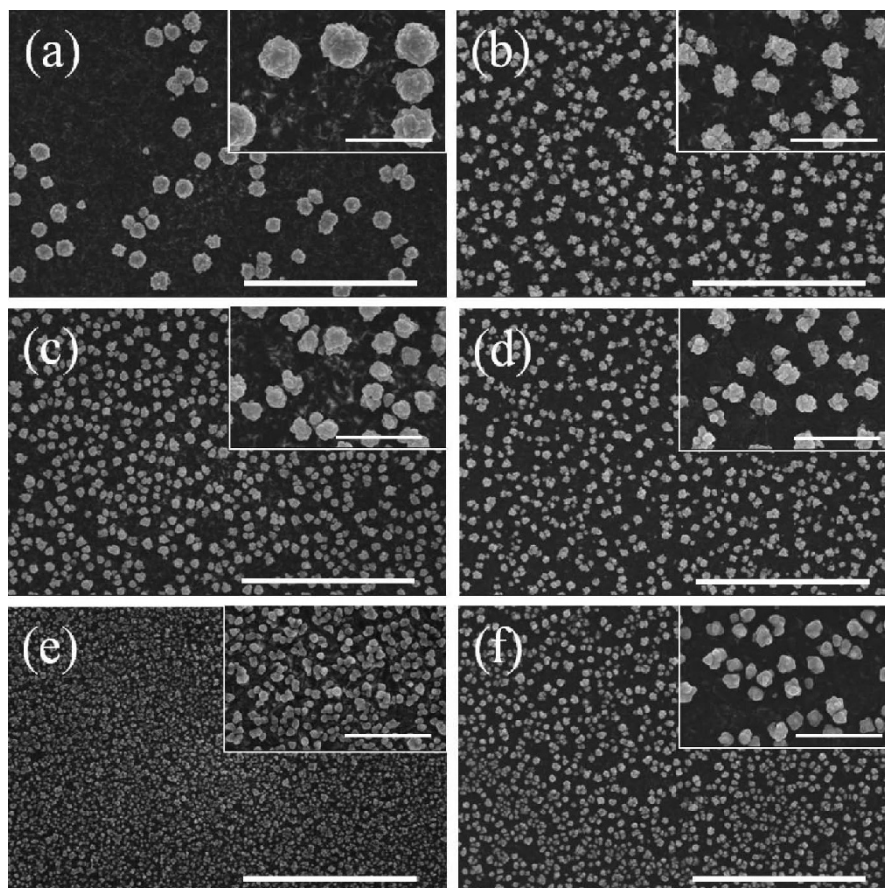


Fig. 1 SEM images of Au-nanostructures electrodeposited at various potentials: (a) -0.1 , (b) -0.2 , (c) -0.3 , (d) -0.4 , (e) -0.5 , and (f) -0.6 V on the modified BDD electrode. Scale bar, $5 \mu\text{m}$ (insert bar, $1 \mu\text{m}$).

linear range for electrochemical detection of DA in the presence of AA.

Experimental

Reagents and chemicals

All of the stock solutions were dissolved in a 0.05 M phosphate buffer solution (PBS) at $\text{pH } 7.0$. Dopamine hydrochloride and L-ascorbic acid, aniline, and gold(III) chloride trihydrate (HAuCl_4) were obtained from Sigma-Aldrich Chemical (St. Louis, USA). Diluted dopamine and ascorbic acid were prepared from stock solutions immediately before use. Potassium hexacyanoferrate(III) ($\text{K}_3\text{Fe}(\text{CN})_6$) and sulfuric acid were obtained from Samchun Chemicals (Gyeonggi-do, Korea). Nano diamond powder with an average individual particle size of 3.2 nm was purchased from JinGanfYuan New Material Development Co., Ltd. (Guangdong, China). Poly diallyldimethyl ammonium chloride (PDDA; M_w , $400000 - 500000$), and poly sodium 4-styrene sulfonate (PSS; M_w , 70000) were obtained from Duksan Pure Chemicals (Gyeonggi-do, Korea).

Apparatus

The morphology of the electrodes was investigated using field emission scanning electron microscopy (FE-SEM) (JSM-7500F, JEOL, Japan). The operating voltage on the microscope was 15 kV . Electrochemical measurements were performed on a VersaSTAT 3 instrument (Princeton Applied Research, USA)

with a three-electrode system comprising a platinum (Pt) wire as the counter electrode, an Ag/AgCl electrode as the reference electrode, and the modified BDD electrode as the working electrode. To record square-wave voltammograms (SWVs), we used the following instrumental parameters: step potential, 4 mV ; square-wave amplitude, 25 mV ; frequency, 10 Hz ; scan rate, 40 mV/s .

Preparation of the Au-NPs/PANI_{ox} composite modified electrode

A thermally oxidized silicon (Si) wafer was densely coated with diamond nanocrystal seeds to deposit the boron-doped nanocrystalline diamond film. The doped diamond films were subsequently grown on the substrate coated with diamond seeds.¹⁹ The synthesized polycrystalline diamond showed the as-grown thickness of about $1 \mu\text{m}$.

The PANI nanostructures were deposited on the BDD electrode by the electrochemical polymerization of aniline in an electrolyte solution containing $0.2 \text{ M H}_2\text{SO}_4$ and 0.1 M aniline at $+1.5 \text{ V}$ for 720 s . Then, the electrode was washed in distilled water and dried in an oven at 80°C for 12 h . For overoxidation of PANI films, the electrode was treated by amperometry at $+0.8 \text{ V}$ for 600 s in $0.5 \text{ M H}_2\text{SO}_4$. Finally, the Au nanoparticles were electrochemically deposited on the PANI_{ox}/BDD electrode in $0.5 \text{ M H}_2\text{SO}_4 + 2.0 \text{ mM HAuCl}_4$ under a constant potential of -0.5 V for 600 s . The obtained electrode was denoted as Au-NPs/PANI_{ox}/BDD. For comparison, electrodes of two types were introduced BDD electrodes and PANI_{ox}/BDD electrodes.

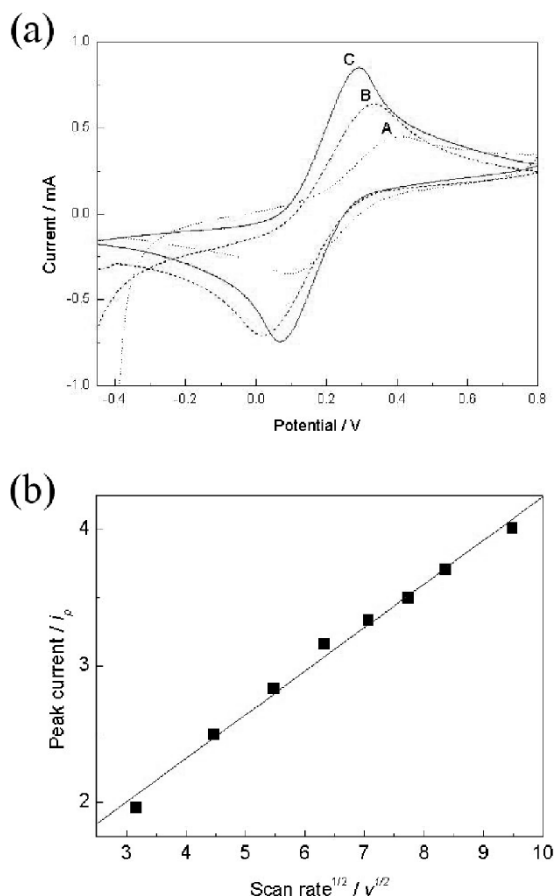


Fig. 2 (a) Cyclic voltammograms of the bare BDD electrode (A), the PANI/BDD electrode (B), and the Au-NPs/PANI/BDD electrode (C) in a solution of 10 mM $K_3Fe(CN)_6$ in 3 M KCl with a scan rate of 50 mV/s. (b) The I_p vs. $v^{1/2}$ plots for the determination of the effective surface area of the AuNPs/PANI/BDD electrode.

Results and Discussion

Figure 1 shows the SEM images of Au-NPs electrodeposited at various potentials on the BDD electrodes modified with the PANI film. The Au nanoclusters were electrochemically deposited in a solution of 2 mM $HAuCl_4$ and 0.5 M H_2SO_4 for 600 s. When a negative potential was applied until it reached -0.5 V (Figs. 1(a) – 1(e)), the Au-NPs became denser. When a greater potential than -0.6 V was applied, their density was decreased (Fig. 1(f)). From this result, we chose -0.5 V as the optimal applied potential (Fig. 1(e)). At this potential, the Au nanoclusters are homogeneously distributed with an average diameter of 80 nm on the electrode surface.

Figure 2(a) shows the cyclic voltammograms (CVs) for bare BDD (A), PANI/BDD (B), and Au-NPs/PANI/BDD (C) electrodes in a 10 mM $K_3Fe(CN)_6^{3-/4-}$ containing 3 M potassium chloride (KCl) solution. In the case of a bare BDD, one pair of waves was observed at an anodic peak (the current of 0.45 mA at a potential of 0.4 V) and a cathodic peak (-0.34 mA, 0.1 V). The peak potential separation (ΔE_p) was about 0.3 V. For the PANI/BDD, the anodic peak potential (E_{pa}) was at 0.33 V (the current of 0.64 mA) with the cathodic peak potential (E_{pc}) at 0.02 V (the current of -0.71 mA), with a peak-to-peak separation (ΔE_p) of 0.31 V. Furthermore, the Au-NPs/PANI/BDD electrode showed the enhanced oxidation peak current of 0.85 mA (E_{pa} 0.3 V) and the reduction peak current of -0.75 mA (E_{pc} 0.07 V).

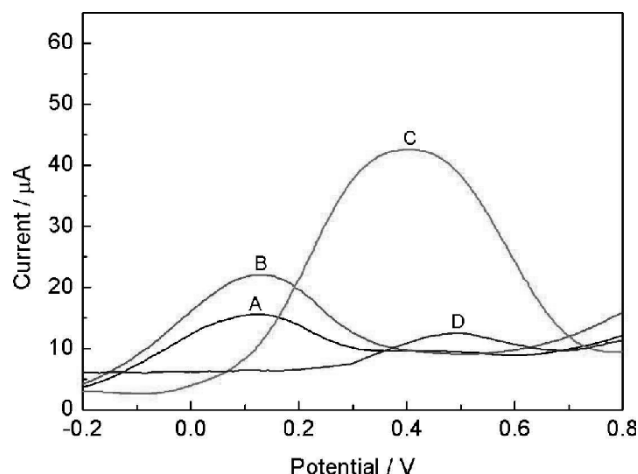


Fig. 3 Square-wave voltammograms (SWVs) for oxidation of 0.5 mM DA and 0.5 mM AA according to effect of PANI overoxidation at the PANI/BDD electrode. Oxidation peaks of DA (A) and AA (B) without overoxidation, and DA (C) and AA (D) with overoxidation.

Its ΔE_p was about 0.23 V. The smaller ΔE_p indicates faster electron-transfer kinetics.²⁰ The magnitude of the electrochemical response for these different electrodes increased in the following order: Au-NPs/PANI/BDD > PANI/BDD > BDD. The increase in the peak currents indicates the increase of the effective surface area.²¹ In addition, the well-defined diffusion-controlled reversible redox peaks suggest a favorable direct electron transfer between the electrode and the redox species.²² The remarkably enhanced signal in the Au-NPs/PANI/BDD electrode is due to the synergistic effects of the Au-NPs and the PANI layer, such effects play an important role in accelerating the electron transfer and enlarging the effective surface area.

The effective surface area of the Au-NPs/PANI/BDD electrode is determined using the Randles-Sevcik equation.²⁴ This equation is as follows:

$$I_p = (2.69 \times 10^5) n^{3/2} A D_0^{1/2} C_0^* v^{1/2}$$

where n the number of electrons participating in the redox reaction, v the scan rate of the potential perturbation (V/s), A the area of the electrode (cm^2), D the diffusion coefficient of the molecules in the solution (cm^2/s), C^* the concentration of the probe molecule in the bulk solution (mol/cm^3), and I_p the peak current of the redox couple (A). As one can see in this equation, the effective surface area (A) is proportional to the value of $I_p/v^{1/2}$. Cyclic voltammograms of the modified electrode were measured in a 3 M KCl solution containing 10 mM of $K_3Fe(CN)_6$ at a variety of scan rates. The relationship between the peak current (I_p) and the square root of the scan rate ($v^{1/2}$) for the modified electrodes is linear in Fig. 2(b). The Randles' slope was 0.319 (correlation coefficient, $R = 0.9959$) and the effective surface area of the modified electrode was about 1.49 cm^2 . This effective working area of a bare BDD electrode, calculated from their slopes of 0.163 ($R = 0.9981$), were 0.761 cm^2 (data not shown). These results show that the metal nanoparticles on the conducting polymer increased the effective area through the formation of a 3D structure.

Figure 3 shows the SWV response for 0.5 mM DA and 0.5 mM AA at the PANI/BDD electrode according to overoxidation of the PANI film. On the PANI/BDD electrode without overoxidation, the oxidation potential of DA (A) is

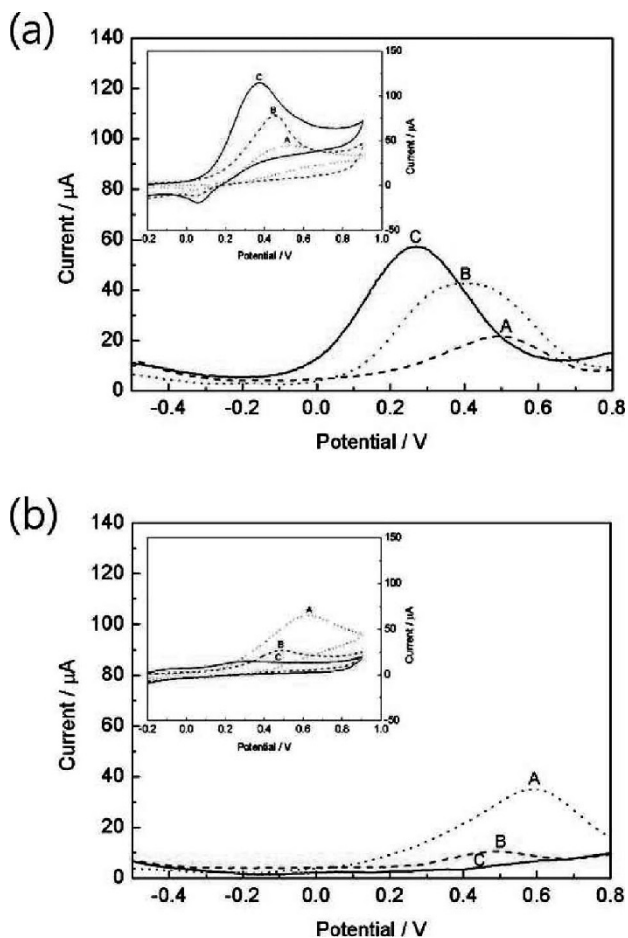


Fig. 4 Square-wave voltammograms (SWVs) and cyclic voltammograms (CVs) for oxidation of 0.5 mM DA (a) and 0.5 mM AA (b) at the BDD (A), PANI_{ox}/BDD (B) and AuNPs/PANI_{ox}/BDD (C) electrodes.

similar to that of AA (B), about 0.12 V, while their potentials were separated to 0.4 V for DA (C) and 0.5 V for AA (D) on the electrodes modified with PANI_{ox}. In addition, its oxidation peak current for DA increased. It was likely that the PANI_{ox} film has superior selectivity and sensitivity toward DA compared with AA. In 2007, Li's group reported that the overoxidized polypyrrole (PPy_{ox}) film is also favorable for DA and 5-HT in the neutral solution.¹⁹ As a result, the peak of DA was readily separated from that of AA and overlapping of the voltammetric responses of AA and DA was eliminated.

Figure 4 shows SWVs and CVs for DA (0.5 mM) and AA (0.5 mM) at the three types of electrodes: *i.e.* the bare BDD (A), the PANI_{ox}/BDD (B) and the Au-NPs/PANI_{ox}/BDD (C) electrodes. As shown in Fig. 4(a), the shape and position of the oxidation peak for DA were different according to the modifications of the electrodes. In the case of the bare BDD electrode, DA was oxidized at +0.5 V. After modification of the electrodes, the oxidation peaks for dopamine were shifted only slightly to +0.4 V for PANI_{ox}/BDD electrode and +0.27 V for AuNPs/PANI_{ox}/BDD electrode, respectively. In addition, the peak currents increased in the following order: BDD < PANI_{ox}/BDD < Au-NPs/PANI_{ox}/BDD. Because as-produced BDD thin-film electrodes possess the hydrogen terminated surfaces,²³ H-terminated BDD electrodes repel the positively charged substances. It is well known that DA is positively charged with pK_a 8.9 at physiological pH.⁵ So, the DA was repelled on the

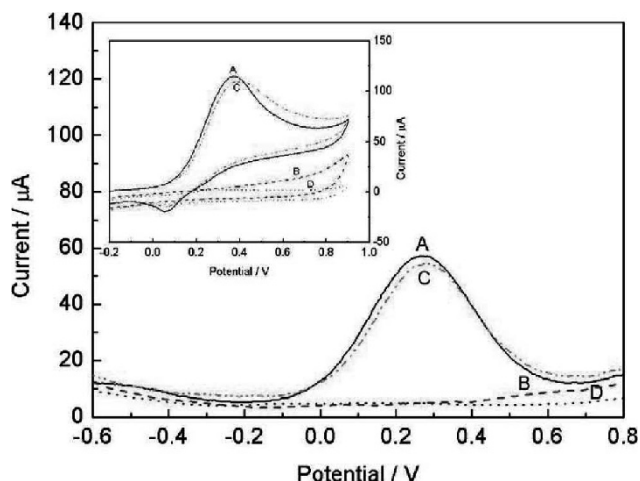


Fig. 5 Square-wave voltammograms (SWVs) and cyclic voltammograms (CVs) for oxidation of 0.5 mM DA (A), 0.5 mM AA (B), a DA and AA mixture (C) and PBS (pH 7.0) (D) at AuNPs/PANI_{ox}/BDD electrode.

BDD electrode by electrostatic repulsion, and then blocked the oxidative reaction of DA.⁹ Although the PANI_{ox} layer were favorable for DA, the Au-NPs may possess a very strong catalytic activity for DA oxidation.²⁴ Due to the synergic effect of the PANI_{ox} film and the Au-NPs, the Au-NPs/PANI_{ox}/BDD electrode greatly enhances the catalytic activity and the electric conductivity, and enlarges the effective surface area. In the case of AA in Fig. 4(b), the oxidation peaks of the BDD and the BDD/PANI_{ox} electrodes were +0.6 V and +0.48 V, respectively. There was no oxidation peak for AA at the Au-NPs/PANI_{ox}/BDD electrode. This absence was attributed to the synergic effect of PANI_{ox} film and the Au-NPs. And peak currents increased in the opposite order compared with DA: Au-NPs/PANI_{ox}/BDD < PANI_{ox}/BDD < BDD electrodes. The H-terminated BDD electrode shows the advantage for electrochemical oxidation of negatively charged compounds. The AA is negatively charged in 0.05 M PBS (pH 7.0) since the pK_a of AA is 4.17.⁵ Thus, the BDD electrode had a stronger attraction for AA in comparison to the others.

Figure 5 shows the CV and the SWV responses to 0.5 mM DA (A), 0.5 mM AA (B), a mixture solution of 0.5 mM DA and 0.5 mM AA (C) and PBS (pH 7.0) (D) at the Au-NPs/PANI_{ox}/BDD electrode. Here, PBS was used as a control sample. From the results we can see that the oxidation peak of 0.5 mM DA was very strong at +0.27 V with a peak current of 57.5 μA. As shown in Fig. 5, the oxidation process is obviously inhibited and the current decreases dramatically with no oxidation peak in the solution of AA. In the case of a mixture solution, the peak current of DA is almost equal to that of 0.5 mM DA. This means that the current of the peak is uninfluenced by the presence of AA and the Au-NPs/PANI_{ox}/BDD electrode can selectively determine DA in the presence of AA.

Figure 6 shows the calibration plot for DA oxidation in the presence of 0.5 mM AA at the Au-NPs/PANI_{ox}/BDD electrode. Its sensitivity for DA was about 131 μA/mM ($R = 0.997$) with a linear range of $1.5 \times 10^{-7} - 5 \times 10^{-4}$ M. The detection limit of DA in the presence of 0.1 mM AA was found to be about 30 nM, with a signal-to-noise ratio of 3. As discussed above, the Au-NPs/PANI_{ox}/BDD electrode can selectively detect DA in the presence of AA. The sensor performances of the modified BDD electrodes reported in the literature for determination of

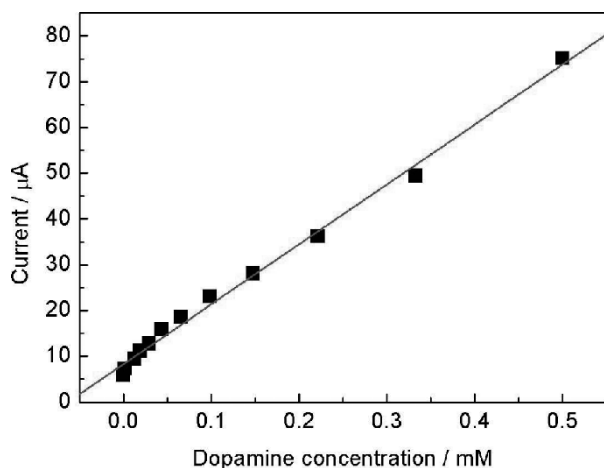


Fig. 6 Calibration plot of SWV peak current for determination of DA at the AuNPs/PANI_{ox}/BDD electrode.

Table 1 Comparison of sensing performances for determination of DA using the BDD electrodes

Electrode	Linear range/ µM	Detection limit/ µM	Sensitivity/ µA µM ⁻¹	Ref.
PDMA film-coated BDD	0.2 - 2.6	0.06	0.363	7
(Au/PAH) ₄ /(PSS/PAH) ₄ /PS multilayer-sphere modified ABDD	5 - 100	0.8	0.059	9
SAM/Au/BDD	0.01 - 10	0.001	0.00026	20
PPA/PTy-modified BDDs	Up to 80	0.05	0.02	25
AuNPs/PANI _{ox} /BDD	0.15 - 500	0.03	0.131	This work

DA are compared in Table 1.^{7,9,20,25} As shown in Table 1, many sensors had a low sensitivity, narrow linear ranges, and a high detection limit. Although the PDMA film-coated BDD electrode has a higher sensitivity than our electrode, its linear range was very narrow. Our well-designed nanostructured electrode shows better sensing performances than the other previously reported sensors.

Conclusions

We constructed a dopamine sensor based on a boron-doped polycrystalline diamond (BDD) electrode modified with gold nanoparticles dispersed in overoxidized-PANI in the presence of L-ascorbic acid. The metal nanoparticles dispersed in the conducting polymer layer were introduced for the easy surface modification of the BDD thin film and to create large effective surface areas and the effective electron transfers. Furthermore, the overoxidized PANI layer enhanced selectivity and sensitivity toward DA. The sensitivity of the fabricated DA sensor was 131 µA/mM ($R = 0.997$) with the linear range of $1.5 \times 10^{-7} - 5 \times 10^{-4}$ M in the presence of 0.1 mM AA. The detection limit was 30 nM. Consequently, the developed sensor showed a high sensitivity, a wide linear range, a low detection limit, and good selectivity.

Acknowledgements

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