ORIGINAL ARTICLE

Electrochemical Sensor based on Carbon Fiber Microelectrode Modified Reduced Graphene Oxide/Polypyrrole/Titanium Dioxide for Dopamine Detection

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ABSTRACT

Introduction: Dopamine (DA) is a crucial neurotransmitter in the nervous system that plays a vital role in the function of the cardiovascular system, kidneys, and hormones. Reduced levels of DA have been associated with various neurological disorders such as Alzheimer's, Parkinson's, and schizophrenia. This study employed an electrochemical sensor based on carbon fiber microelectrode (CFME) to detect DA. **Materials and Methods:** To enhance the sensitivity of the sensor, the CFME was modified with composite materials of reduced graphene oxide (rGO), polypyrrole (PPy), and titanium dioxide (TiO_2) using the electrodeposition method. **Results:** The results of electrochemical measurements indicated that the electrochemical sensor detection limit for DA was 29.9 µM with linear range 1–3 mM and the sensitivity was 32.93 µA/mM. **Conclusion:** Therefore, the rGO/PPy/TiO_2 composite has the potential to improve the electrode's sensitivity for DA detection. The sensors can be further developed for biological testing and measurement.
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Keywords: Electrochemical sensor, Dopamine, Composites, Electrodeposition, Microelectrode

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INTRODUCTION

Dopamine (DA) is a vital neurotransmitter influencing various physiological functions like metabolism, cardiovascular regulation, and hormonal balance (1). Dysregulation of DA levels can lead to severe disorders such as Parkinson's and depression (2–4). Traditional methods like enzyme-linked immunosorbent assay (ELISA) (5), high-performance liquid chromatography (HPLC) (6–8), and fluorometry (9) for measuring DA suffer from limitations in speed, cost, and sensitivity (10,11). Electrochemical methods like cyclic voltammetry (CV) offer real-time detection with high sensitivity, making them preferable for DA detection because it offers real-time detection with a response time of less than 2 seconds (12) and superior detection capabilities at low concentrations of DA (4). DA detection is complicated by similar compounds like ascorbic acid (AA) and uric acid (UA) (13). The existence of these interferences reduces the electrode's detection capacity (10). To overcome this issue, electrode sensors such as glassy carbon electrodes (GCE) (1,11), carbon paste electrodes (CPE) (14), screen printed electrodes (15), and carbon fiber microelectrodes (CFME) have been developed, utilizing various materials to modify the electrode's surface or to form the electrode itself. These materials include carbon nanotubes (CNT) (16), carbon nanofibers (CNF) (17), graphite (16), boron-doped diamond (18), graphene oxide (GO) (10), and reduced graphene oxide (19,20). Electrode sensors like carbon fiber microelectrodes (CFME) offer advantages such as biocompatibility and rapid measurements. CFMEs are

commonly modified to enhance performance, with materials like carbon nanotubes and graphene oxide. These modifications improve sensitivity and selectivity for DA detection, making CFMEs a popular choice in biosensing applications.

In this research, an electrochemical sensor based on reduced graphene oxide (rGO), polypyrrole (PPy), and titanium dioxide (TiO2) composite on carbon fiber microelectrode (CFME) was developed to detect DA. The electrochemical performance of the sensor was tested using cyclic voltammetry (CV), while the morphological and structural structure characteristics of the electrodes were observed using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR), respectively.

MATERIALS AND METHODS

Materials

The graphite powder, sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), potassium permanganate ($KMnO_4$), 30 wt% hydrogen peroxide ($H2O_2$), hydrochloric acid (HCl), 0.1 M monomer pyrrole, and DA were purchased from Sigma-Aldrich. Ammonium persulfate (APS) were purchased from Merck. TiO_2 powder were purchased from local market in Indonesia and phosphate buffer saline (PBS) pH 7.0 were self-made. The chemicals were analytical grade.

Synthesis of Graphene Oxide (GO)

The synthesis of graphene oxide (GO) was carried out using a modified Hummer method (21). Initially, 2 g of graphite powder was mixed with 180 mL of H_2SO_4 and 20 mL of H_3PO_4 at 20℃, then 6 g of $KMnO_4$ was slowly added, causing an exothermic reaction and raising the temperature to 35℃. After 2 hours of stirring, the solution underwent 1-hour ultrasonication and was then stirred until reaching room temperature. Subsequently, 400 mL of deionized (DI) water and 10 mL of 30 wt% H_2O_2 were added while maintaining the temperature. The solution was filtered, washed with HCl, then with DI water and ethanol alternately to remove residual metal ions and acid. Finally, the brown precipitate was dried at 70℃ for 24 hours.

Preparation of rGO/PPy/TiO_2 Composite

The polymerization method was utilized to synthesize the rGO/PPy/TiO_2 composite. A solution containing 0.1 M pyrrole monomer dissolved in 60 mL of DI water was prepared. To this, 0.03 g of TiO_2 powder and 0.1 g of GO were added. Subsequently, a solution containing 4 g of APS in 35 mL of DI water was added dropwise into the pyrrole monomer/GO/TiO_2 solution. The mixture was stirred for 8 hours at 0-5℃ using a magnetic stirrer. After filtration, the precipitate was washed alternately with DI water and ethanol three times. Finally, the obtained precipitate was dried in an oven at 70℃ for 30 hours (22).

Fabrication of rGO/PPy/TiO_2/CFME

The fabrication of bare CFME followed standard procedures. A 0.9 mm inside diameter glass capillary tube was divided into two parts with wax, into which a carbon fiber was inserted. One end of the carbon fiber was connected to a copper wire and secured with epoxy glue. For deposition of the rGO/PPy/TiO_2 composite onto bare CFME, the composite was dissolved in ethanol (0.5×10^{-2} g in 10 mL ethanol) and electrodeposited using cyclic voltammetry. A three-electrode system was utilized, with CFME as the working electrode, Ag/AgCl as the reference electrode, and platinum wire as the counter electrode. Deposition occurred at potentials ranging from 0 V to +0.8 V with a scan rate of 200 mV/s for 10 cycles (22).

Characterization

FTIR analysis were done in the range of 400–4000 cm^{-1} to identify functional groups on the rGO/PPy/TiO_2 composite surface. SEM was performed on Zeiss (EHT 15.00 kV and 20.00 kV, magnitude 1000X) to examine the morphologies of GO sheets, rGO/PPy/TiO_2 composite, and CFME surface. Electrochemical measurements utilized Emstat4s LR hardware and PSTrace 5.9 software. CV scanning from -0.3 V to +0.6 V at a scan rate of 0.1 V/s (1) was employed to observe the electrochemical response of dopamine in pH 7.0 PBS solution, both on bare CFME and rGO/PPy/TiO_2/CFME, at room temperature.

RESULT

Morphological and Structural Characterization

The SEM analysis depicted in Fig. 1A reveals the wavy and interconnected structure of graphene oxide. Previous studies by Aliyev et al. (23) and Farah et al. (24) have attributed this wavy surface to an oxidation process taking place during the transformation from graphite oxide to graphene. This chemical change induces the formation of cracks within the carbon structure, facilitating oxidation in those regions. Fig. 1B illustrating a collection of TiO_2 nanoparticles adorned on sheets of rGO. SEM characterization was also conducted on two samples: bare CFME (C) and rGO/PPy/TiO_2/CFME (D), in order to examine their morphological structures. In Fig. 1C, the bare CFME displays a straight bar-like shape with a smooth surface, indicating the absence of any contamination. Conversely, Fig. 1D showcases the CFME after modification, transformed into rGO/PPy/TiO_2/CFME. A notable difference is observed compared to the bare CFME, as small particles are attached to the carbon fibers, resulting in a roughened surface. This

observation confirms the successful electrodeposition process employed for CFME modification.

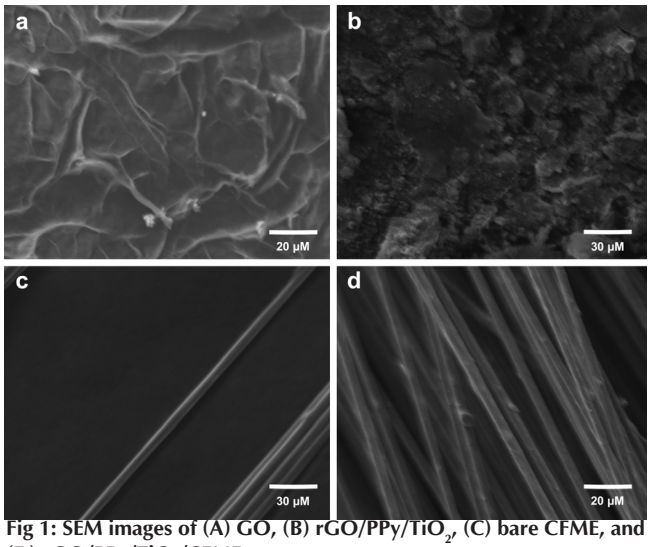

Fig 1: SEM images of (A) GO, (B) rGO/PPy/TiO_2, (C) bare CFME, and (D) rGO/PPy/TiO_2/CFME.

FTIR characterization was conducted to analyze the material composition of rGO/PPy/TiO_2 composites. In the FTIR results of the rGO/PPy/TiO_2 composite as shown in Fig. 2, several significant absorption peaks were observed. At 439 cm^{-1}, a high absorption peak indicated strain vibrations from the Ti-O bond groups. Absorption peak at 1054 cm^{-1} indicated a C-O stretching which originated from carboxyl group remaining from the reduction process. Absorption peak at 1220 cm^{-1} refers to C-N stretching. The absorption peak at 1619 cm^{-1} is due to C=C stretching of graphene from reduction process and N-H bending. The absorption peak at 2050 cm^{-1} refers to C-H bending group bonding. The C-N, N-H, and C-H group bonds corresponded to the pyrrole ring. The wide stretching of the O-H group bonds was shown by the absorption peak at 3205 cm^{-1}, indicates the presence of rGO (24,25).

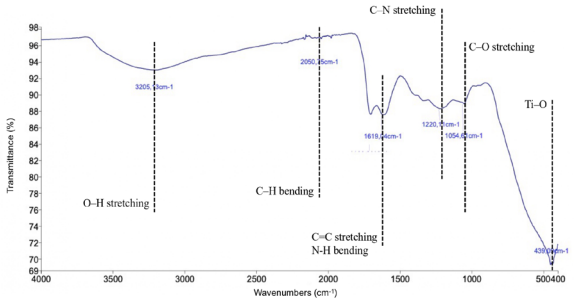

Fig 2: FTIR spectra of rGO/PPy/TiO_2.

Electrochemical Responses of DA at rGO/PPy/TiO_2/CFME

To assess the impact of composite electrodeposition on CMFE for DA detection, CV was employed. The DA concentration and scan rate were varied within a potential range of -0.3 V to +0.6 V. This specific potential range was chosen due to its ability to generate a stable voltammogram, ensuring consistency in the active surface of the electrode. By utilizing a single potential range, the electrode's active surface remains unchanged.

In Fig. 3, the electrochemical response of a 1 mM DA solution in pH 7.0 PBS (phosphate-buffered saline) was examined using a scan rate of 0.1 V/s. A reduction peak at 0.100277 V and an oxidation peak at 0.350472 V were observed on the rGO/PPy/TiO_2/CFME, shows an increasing peak for more than 60% indicating the adsorption of DA on the modified CFME surface. In contrast, the bare CFME did not exhibit any significant reduction or oxidation peaks, suggesting that this electrode was unable to detect DA when compared to the rGO/PPy/TiO_2/CFME electrode. This shows rGO/PPy/TiO_2/CFME has electrocatalytic performances for the oxidation and reduction of DA.

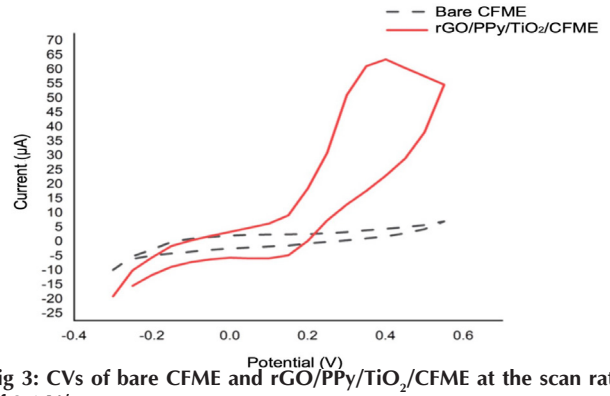

Fig 3: CVs of bare CFME and rGO/PPy/TiO_2/CFME at the scan rate of 0.1 V/s.

In order to assess the control of DA adsorption on the electrode surface, the scan rate was varied from 0.005 V/s to 0.1 V/s in a 1 mM DA solution in pH 7.0 PBS. Fig. 4 illustrates the impact of scan rate on the oxidation process of 1 mM CV on rGO/PPy/TiO_2/CFME. The redox peak current of the modified CFME exhibited a linear increase as the scan rate increased. This observation shows a diffusion process of DA on the surface of rGO/PPy/TiO_2/CFME.

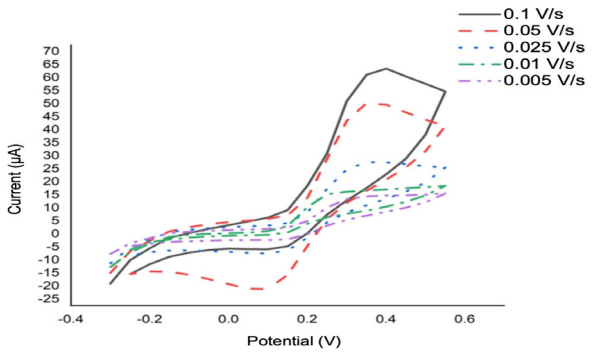

Fig 4: CVs of rGO/PPy/TiO_2/CFME at different scan rates in 1 mM DA.

In Fig. 5, the oxidation process of DA was investigated at different concentrations using a scan rate of 0.1 V/s. It is evident that the oxidation peak exhibits a

linear relationship with the total concentration of DA. However, in some cases, a noticeable reduction peak was not observed in the graphs.

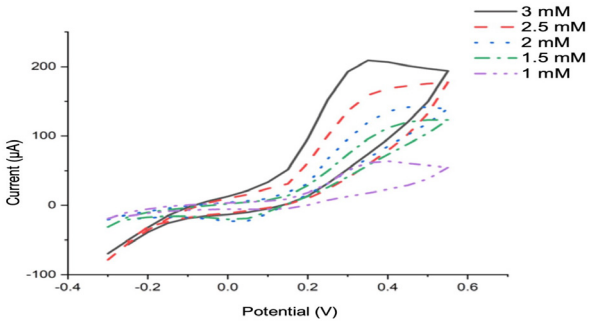

Fig 5: CVs of rGO/PPy/TiO_2/CFME at different DA concentration with scan rate 0.1 V/s.

Fig. 6 demonstrates that the anodic peak current rises proportionally with increasing DA concentrations: 1 mM, 1.5 mM, 2 mM, 2.5 mM, and 3 mM. The relationship can be described by the linear regression equation Ipa (µA) = 32.931x (µM) + 39.874, with a correlation coefficient of 0.9573. Based on this equation, the sensor exhibits a sensitivity of 32.93 µA/mM.

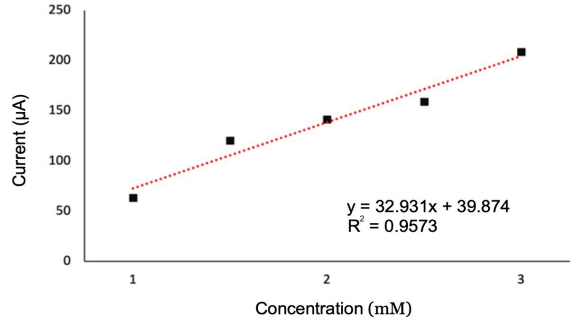

Fig 6: Plot of the peak current against square root of the DA concentration.

The sensor's characteristics are also evaluated by determining its detection limit, which represents its ability to detect analytes. The detection limit of the sensor after modification was determined to be 29.9 µM. This value was obtained by using the standard deviation of the calibration curve (Sy) and the slope of the calibration curve (S) in the calculation (1).

$$LoD = 3x\frac{Sy}{S}$$

DISCUSSION

CFME is widely used in biosensors, especially for neurotransmitter detection, and is fabricated by placing it in a glass capillary tube, connecting it with copper wire, and securing it with epoxy glue (26). To enhance conductivity, CFMEs are often modified using electrodeposition techniques with various materials such as carbon, polymers, and metals. PPy is a popular choice for electrode modification due to its strong electrical and electrochemical properties. Its molecular structure, characterized by cation radicals, improves electrical conductivity and thermal stability. Combining PPy with semiconductive nanofillers like TiO_2 enhances its electrochemical properties. TiO_2 offers advantages such as non-toxicity, low production costs, and a high refractive index. Incorporating rGO into PPy/TiO_2 composites further enhances their thermal, mechanical, electrical, and electrochemical properties. The rGO/PPy/TiO_2 composite exhibits high conductivity and dielectric constant, with rGO and TiO_2 enhancing charge transfer between layers. In this study, we focused on fabricating a modified CFME incorporating a composite of three materials: rGO, PPy, and TiO_2. This modified electrode was used for DA detection via CV. The rGO/PPy/TiO_2/CFME exhibited superior sensitivity and a lower detection limit compared to bare CFME and previous studies. However, precise control of the electrodeposition process for the rGO/PPy/TiO_2 composite is critical. Excessive deposition may introduce noise and interfere with analyte detection, while insufficient deposition could lead to inadequate sensitivity (27).

Compared to studies in Table I, Puthongkham et al. (28) achieved the lowest detection limit of 0.003 µM for CFME modification. This can be attributed to the utilization of nanodiamonds as the modifying material. Fang et al. (29) utilized carbon dots, achieving a detection limit of 0.02 µM and simultaneous detection of other neurotransmitters. The enhanced performance of their modified CFME can be attributed to the incorporation of carbon dots (CD) as the modifying material, which increased the electrode's surface area (from 7 µm to 9 µm after modification) and its electrocatalytic activity. Chang et al. (30) reported a detection limit of 0.01 µM with GO/CFME, showcasing a high sensitivity of 41 ± 2 µA/mM due to optimized electrodeposition parameters. The findings of this study demonstrate that employing a wider potential range leads to a higher peak anodic current. Ates et al. (27) modified CFME using poly(carbazole-co-p-tolylsulfonyl pyrrole) and reached a detection limit of 0.5 µM but with a low

Table I. Electrochemical Characterization Comparison of Bioensor

Electrode	Linear Detection	Detection Limit (µM)	Sensitivity (µA/mM)	Ref.
PCz-co-p-Tsp/ CFME	~130 µM	0.5	0.05	(27)
NDs/CFME	25 nM^{-1} µM	0.3 x 10^{-2}	29 ± 2	(28)
rGO/CD/ CFME	0.1 – 100 µM	0.02	6.5	(29)
GO/CFME	25 nM^{-1} µM	0.01	41 ± 2	(30)
PPy/ $[Fe(CN)_6]^{4-}$/ CPE	0.20–0.95 mM	38.6	-	(31)
rGO/PPy/ TiO_2/CFME	1 – 3 mM	29.9	32.93	This Work

NDS = Nanodiamonds, CFME = Carbon Fiber Microelectrode, CPE = Carbon Paste Electrode, rGO = Reduced Graphene Oxide, CD = Carbon Dots, PCz-co-p-Tsp =Poly(carbazoleco-p-tolylsulfonyl pyrrole), GO = Graphene Oxide, PPy = Polypyrrole, TiO_2 = Titanium Dioxide, $[Fe(CN)_6]^{4-}$ = Ferrocyanide

sensitivity of 0.05 µA/mM, the lowest among the four studies compared.

In comparing our study with other research, key variables include the types of electrode materials used, the specific modification methods applied, and the electrochemical measurement methods employed. The differences in electrode materials significantly influence the outcomes of dopamine detection. For instance, our use of rGO enhances the surface area and conductivity, leading to improved sensitivity and selectivity. The materials chosen by other researchers, such as nanodiamonds and carbon dots, also contribute to lower detection limits and higher sensitivity due to their unique electrocatalytic properties and increased surface areas. The electrochemical measurement methods also varied. Chang et al (30) and Puthongkam et al. (28) use fast cyclic voltammetry (FSCV) for measurement. FSCV offers significant advantages over CV in terms of speed, sensitivity, background noise reduction, and applicability to dynamic and in vivo studies. These benefits make FSCV a superior choice for monitoring fast electrochemical events, such as neurotransmitter release and uptake, and for applications requiring high temporal resolution and sensitivity. Thus, this can attribute to high sensitivity and low detection limit of their study. Fang et al (29), used differential pulse voltammetry (DPV) for sensitivity measurement. DPV is particularly suitable for analytical applications where precise and accurate detection of low-concentration analytes is critical. Additionally, Chang et al (30) studied GO deposition by dip coating, drop casting, and electrodeposition for material modification and electrodeposition shows best result. Ates et al. (27) used electrocoating with polycarbazole and poly(carbazole-co-p-tolylsulfonyl pyrrole) films, while Puthongkham et al. (28) utilized nanodiamond coatings to enhance sensitivity and antifouling properties, and Fang et al. (29) employed co-deposition of carbon dots and reduced graphene oxide nanosheets.

In this study, the rGO/PPy/TiO_2/CFME exhibited a relatively high detection limit. This may be attributed to uncontrolled composite electrodeposition parameters, leading to excessive deposition and increased noise during DA detection. This is supported by the fact that rGO/PPy/TiO_2/CFME could not detect DA below a concentration of 1 mM. Nevertheless, the sensitivity of rGO/PPy/TiO_2/CFME was fairly high at 32.93 µA/mM compared to other studies, indicating its capability to detect DA effectively. This study exhibited a detection limit for DA of 29.9 µM, which is comparable to those obtained with other modified electrodes for dopamine detection. For instance, carbon paste electrodes modified with polypyrrole/ferrocyanide films achieved detection limits of 38.6 µM (31). The integration of rGO and TiO_2 into the PPy matrix significantly improves charge transfer capabilities and overall conductivity, leading to efficient electron transfer during the electrochemical detection process. Furthermore, the inclusion of TiO_2 and rGO enhances the mechanical strength and durability of the electrode, making it more robust and less prone to damage during handling and use.

Understanding the differences in electrode materials, modification methods, and electrochemical measurement methods is crucial for optimizing electrode design and improving detection capabilities. For example, using rGO enhances surface area and conductivity, improving sensitivity and selectivity. The choice of measurement methods, like FSCV or DPV can significantly impact detection speed and sensitivity. Optimizing these variables can lead to more sensitive, reliable, and faster sensors, essential for medical diagnostics and environmental monitoring. Additionally, understanding the role of different materials in reducing noise and improving signal stability can enhance long-term stability and reproducibility, aiding in the mass production of high-performance sensors.

In conclusion, rGO/PPy/TiO_2/CFME demonstrates a favorable ability to detect DA due to its high sensitivity. The electrodeposition method remains a rapid, cost-effective approach, but enhancing control over parameters is crucial for achieving lower detection limits and broader linear ranges. There is still room for improvement for the modified sensor to achieve lower detection limit with high sensitivity and selectivity. Optimizing parameters such as the electrodeposition scan rate and cycle number can further enhance reproducibility and reliability. The method of electrode fabrication can also be explored further to create micro-sized electrodes that are even smaller, uniform, and possibly nano-sized, such as array sensor. Exploring more methods so the electrode can be modified more easily and with various methods, making them suitable for mass fabrication. This knowledge can guide future research in developing advanced biosensors with lower detection limits and broader linear ranges, improving the accuracy and efficiency of neurotransmitter detection.

CONCLUSION

The synthesis of the rGO/PPy/TiO_2/CFME electrochemical sensor was successful using the electrodeposition method, resulting electrode coated with composite which has desirable structural and morphological characteristics. The sensor demonstrated the ability to detect DA, unlike the bare CFME. Furthermore, the rGO/PPy/TiO_2/CFME electrochemical sensor exhibited a reasonably high sensitivity of 32.93 µA/mM and a detection limit of 29.9 µM. However, there is room for improvement in terms of the detection limit and linear range of the electrochemical sensor. The stability, repeatability, and reproducibility of the sensor can also further analyzed.

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