Polydopamine-functionalized graphene nanoplatelet smart conducting electrode for bio-sensing applications

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Abstract The development of a novel polydopamine (PDA)-functionalized graphene nanoplatelets (GNPs)-based disposable sensor is described. The sensor was fabricated by drop-coating PDA@GNPs in polyethylene glycol (PEG) and poly(3,4-ethylenedioxythiophene (PEDOT):poly(styrenesulfonate) (PSS) aqueous suspension onto the working area of a screen-printed electrode (SPE). The final sensor, designated as PDA@GNPs/PPP/SPE, was characterized by scanning electron microscopy (SEM), Raman spectroscopy, Faradaic electrochemical impedance spectroscopy (FEIS) and cyclic voltammetry (CV). Mediated detection of hydrogen peroxide (H$_2$O$_2$) via the redox properties of PDA was achieved. It showed excellent selectivity and sensitivity towards H$_2$O$_2$ with a limit of detection and sensitivity of 0.55 mM (S/N = 3) and 3.0 mA mM$^{-1}$ cm$^{-2}$, respectively. Thereafter, glucose oxidase (GOx) was immobilized onto the electrode to develop GOx/PDA@GNPs/PPP/SPE sensor. The glucose biosensor exhibited a limit of detection of 0.25 mM (S/N = 3) and a sensitivity of 0.51 mA mM$^{-1}$ cm$^{-2}$; thus, proving its potential suitability for bio-sensing applications.

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1. Introduction

Graphene and its derivatives are widely used as surface modifiers for electrodes including glassy carbon and screen-printed electrodes during the preparation of electrochemical bio-(chemo) sensors (Kanyong et al., 2016a; Ratinac et al., 2011). Electrodeposition, drop-casting and physical adsorption are the most commonly employed methods for the modification...
of these electrode surfaces (Ratinac et al., 2011; Cinti et al., 2015). Several graphene-based electroanalytical methods have been developed for the individual and simultaneous detection of molecules such as ascorbic acid, uric acid, acetaminophen and dopamine. The use of graphene-based electrodes for simultaneous analysis has been useful in solving some of the main drawbacks associated with the use of traditional electrodes, namely, low reproducibility due to electrode fouling and poor selectivity arising from overlapping voltammetric peaks (Kanyong et al., 2016b, 2016c, 2015, 2013; Chen et al., 2013; Huang et al., 2011). Another possible route for resolving overlapping voltammetric peaks is the use of redox mediators. Typically, the use of mediators such cobalt phthalocyanine and ferrocene has enabled the detection of hydrogen peroxide (H₂O₂) at low potentials (Kanyong et al., 2012, 2016d; Rabti et al., 2016) and, the subsequent development of sensitive and selective sensors and biosensors (Kanyong et al., 2013, 2015).

Polydopamine (PDA) films can be formed on various materials such as glass, metals and silica. The deposition of PDA films particularly from aqueous solution constitutes a new material system. The deposition of PDA (Mrowczynski et al., 2014; Luo et al., 2013); thus, these substrates consisting of polyethylene glycol (PEG) and poly(3,4-ethylenedioxythiophene) (PEDOT):poly(styrenesulfonate) (PSS) in dioxythiophene (PEDOT):poly(3,4-ethylenedioxythiophene) (PEDOT):poly(4-styrenesulfonate) (PSS), potassium hexacyanoferrate ([Fe(CN)₆]⁴⁻/³⁻), phosphate buffered saline (PBS) tablets and dopamine hydrochloride were purchased from Sigma-Aldrich, USA. Graphene nanoplatelets (thickness of 2–10 nm) were purchased from the American Society Material, LLC (Product No.: GNNP0051), USA. Polyethylene glycol 6000 (Product No.: 29577) was purchased from BDH Chemicals Ltd (Poole, England). All other chemicals were of analytical grade and used without further purification.

2.2. Procedures

2.2.1. Preparation of PDA@GNPs hybrid nanocomposite

The PDA@GNPs nanocomposite was prepared by using a modified version of a protocol previously reported elsewhere (Feng et al., 2016). Briefly, graphene nanoplatelets (GNPs, 50.0 mg) were dispersed in 5.0 mM dopamine hydrochloride aqueous solution (dopamine hydrochloride was dissolved in 10.0 mL of 5.0 mM Tris-buffer, pH 7.5). The suspension was then stirred on a VWR® Rocking Platform Shaker for 48 h at room temperature. The resulting black product was centrifuged at 5000 rpm for 30 min to separate the sediment and supernatant. The sediment was washed several times with deionized H₂O, dried by freeze-drying and named as PDA@GNPs.

2.2.2. Fabrication of PDA@GNPs-based sensor

The smart conducting sensor was prepared using a simple drop-coating method. PEDOT/PSS aqueous solution was ultrasonicated and used to formulate different percentages of polyethylene glycol (PEG). This formulation is termed PEG/PEDOT/PSS. The as-prepared PDA@GNPs was then dispersed in the PEG/PEDOT/PSS aqueous solution to form PDA@GNPs/PEG/PEDOT/PSS nanocomposite. Thereafter, 2.0 μL of the resultant solution was dropped on the working area (0.1257 cm²) of the screen-printed electrode (SPE), allowed to dry in hot air oven for 1 h at 40 °C to form PDA@GNPs/PEG/PEDOT/PSS/SPE. This sensor is designated as PDA@GNPs/PPP/SPE. A schematic representation of the SPE and sensor fabrication process is illustrated in Scheme 1. The same procedure was used to fabricate PEDOT/PSS/SPE, PEG/PEDOT/PSS/SPE and GNPs/PEG/PEDOT/PSS/SPE, designated as PP/SPE, PPP/SPE and GNPs/PPP/SPE, respectively. The surfaces of all the modified
SPEs were thoroughly rinsed in PBS to remove any unbound materials. Once prepared, the sensors were stored under room temperature conditions.

Glucose oxidase (GOx) was subsequently immobilized onto the surface of the PDA@GNPs/PPP/SPE and the resulting biosensor, designated as GOx/PDA@GNPs/PPP/SPE, used to analyze glucose.

2.2.3. Sessile contact angle measurement

Contact angle measurements were carried out by the sessile drop technique; a water droplet was placed onto a flat surface of the bare SPE and PDA@GNPs/PPP/SPE, and the contact angle of the droplet with the surface measured. Reported values are the average contact angle (right and left) of 10 droplets. During the measurement time (~50 s), no change in contact angle was observed. A variation of 5° is generally considered to be sufficient to differentiate materials (Kanyong et al., 2016e).

![Scheme 1](image1.png)

**Scheme 1** Schematic representation of the SPE (left) and fabrication of the PDA@GNPs/PPP/SPE sensor.

![Fig. 1](image2.png)

**Fig. 1** (A) Cyclic voltammograms recorded at bare SPE, PP/SPE and PPP/SPEs containing different percentages of PEG at 100 mV s⁻¹ scan rate; (B) Anodic (Ipa) and cathodic (Ipc) peak currents for [Fe(CN)₆]³⁻/⁴⁻ vs. amount of PEG (%). CVs were recorded in 5.0 mM [Fe(CN)₆]³⁻/⁴⁻ in PBS (pH 7.4) containing 0.1 M KCl. P; polyethylene glycol, PP; PEDOT:PSS; 0%; bare SPE, 0%; PP/SPE.

![Scheme 2](image3.png)

**Scheme 2** A schematic representation of the mechanism of electrocatalytic enhancement of the SPE by PEDOT:PSS doped with PEG.
3. Results and discussion

3.1. Electrocatalytic study

The optimum amount of PEG in PEDOT:PSS with the highest electrocatalytic activity towards \([\text{Fe(CN)}_6]^{3/4-}\) redox couple was optimized by coating the SPE with nanocomposites containing different percentages (1, 3, 5, 10 and 15\%) of PEG in PEDOT:PSS and studying their voltammetric responses. Fig. 1A illustrates cyclic voltammograms obtained at the bare SPE and representatives of the modified electrodes while Fig. 1B shows a plot of the peak current vs. the percentages of PEG in the PEDOT:PSS aqueous suspension.

The PEDOT:PSS coated SPE shows higher electrocatalytic activity towards \([\text{Fe(CN)}_6]^{3/4-}\) in comparison with the bare SPE and on doping the PEDOT:PSS with PEG, the electrocatalytic activity is markedly increased from 1\% up to 10\% (Fig. 1A,B).

Scheme 2 represents the proposed mechanism for the enhanced electrocatalytic behavior of PEDOT:PSS doped with PEG. The electrode comprises of PEDOT:PSS polymer chains wherein the PEDOT molecules present in the core of the insulating PSS are highly conductive in nature. On being doped with PEG, the core-shell structure becomes partially linear due to the ejection of the PSS molecules. This exclusion of PSS from the surface is perhaps responsible for conformational changes in the polymer film that in turn results in increased electrocatalytic activity. The addition of ethylene glycol to aqueous suspension of PEDOT: PSS is known to decrease the columbic interactions between PEDOT and PSS molecules which in turn contribute to the reorientation of the polymer chains, resulting in improved charge carrier mobility (Wei et al., 2013). This behavior has been confirmed by Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopic studies (Kumar et al., 2015).

The SPE coated with PEDOT:PSS doped with 10\% PEG has been termed a smart conducting electrode. In other studies, the electrocatalytic behavior of PEDOT:PSS dipped in methanol, \(\text{H}_2\text{SO}_4\) and ionic liquid was markedly enhanced (Alemu et al., 2012; Krampa et al., 2017). In this study, the effect of PEG on the electrocatalytic behavior of PEDOT:PSS has been investigated. We observed that the addition of different percentages of PEG to PEDOT:PSS increased the electrocatalytic behavior up to 10\%; thereafter, any further increase in the percentage of PEG does not result in an increase in the electrocatalytic behavior. Consequently, 10\% PEG was chosen as the optimum amount of PEG required to be present in the PEDOT:PSS aqueous solution to give the highest electrocatalytic response.

![Cyclic voltammograms of SPE, PP/SPE, PPP/SPE, GNP/SPP/PPE and PDA@GNP/SPP/PPE in PBS (pH 7.4) at 50 mV s\(^{-1}\) scan rate.](image1)

![Nyquist plots observed for electrochemical impedance spectroscopy at bare SPE (curve a) and PDA@GNP/SPP/PPE (curve b) in PBS (pH 7.4) containing 5.0 mM \([\text{Fe(CN)}_6]^{3/4-}\) and 0.1 M KCl.](image2)
Thereafter, we incorporated PDA@GNPs into the 10% PEG/PEDOT:PSS matrix for further electrochemical studies since GNPs exhibits excellent electrochemical properties (Kavan et al., 2011). The voltammetric behavior of the bare SPE, PP/SPE, PPP/SPE, GNPs/PPP/SPE and PDA@GNPs/PPP/SPE in PBS (pH 7.4) is shown in Fig. 2A. No redox peaks were observed when the voltamograms of the bare SPE, PP/SPE, PPP/SPE and GNPs/PPP/SPE in buffer were recorded. However, a pair of well-defined reversible anodic and cathodic peak potentials located at 271.1 mV and 107.6 mV, respectively, were observed on the PDA@GNPs/PPP/SPE; this confirms the presence of redox species. The peaks can be attributed to the oxidation and reduction of quinone units present in PDA (Kanyong et al., 2016e; Mrowczynski et al., 2014). The effect of scan rate on the voltammetric behavior of the PDA in the sensor was also examined by cyclic voltammetry (Fig. 2B). At the scan rates investigated, the anodic and cathodic peak potentials remained unchanged while the anodic (Ipa) and cathodic (Ipc) peak currents increased linearly with scan rate (Fig. 2C). This suggests an electrochemical process that is a mixture of diffusion and adsorption-controlled. It also indicates a behavior consistent with surface confined voltammetry and corresponding ‘thin layer’ type voltammetry (Lee et al., 2014). Moreover, the Ipc-to-Ipa (Ipc/Ipa) was found to be close to unity, which is a criterion for a quasi-reversible electrode reaction and indicates stable redox products at the electrode surface (Krampa et al., 2017).

3.2. Electrochemical impedance analysis of PDA@GNPs/PPP/SPE

The PDA@GNPs/P/PPP/SPE interface was examined by Faradaic electrochemical impedance spectroscopy (FEIS) in the presence of 5.0 mM [Fe(CN)₆]³⁻⁴⁻. In a Nyquist plot impedance spectrum, the diameter of the semicircle at the high frequency region represents charge-transfer resistance (RCT) at the electrode surface (Kanyong et al., 2016e). Fig. 3 shows the impedance spectra of the bare SPE and PDA@GNPs/PPP/SPE. There was a significant decrease in the RCT value after coating the SPE with PDA@GNPs nanocomposite, indicating enhanced electron transfer occurring at the PDA@GNPs/PPP/SPE surface.

3.3. Morphological and Raman spectroscopic characterization of PDA@GNPs/PPP/SPE

The surface morphological features of the SPE and PDA@GNPs/PPP/SPE were examined by SEM and Raman spectroscopy. Fig. 4A-C shows electron micrographs of the carbon SPE and PDA@GNPs modified smart conducting SPE, respectively. The morphology of the SPE is typical for graphite materials with grains that are stacked in flakes (Fig. 4A). The heterogeneous distribution of graphene nano-platelets sheets of submicron dimension is clearly visible throughout the substrate with wrinkles on the surface.
(Fig. 4B) and large porous open surfaces (Fig. 1C). The Raman spectrum (Fig. 4D) shows characteristics D, G and 2D peaks, which are typical features of thick graphene stacks (Cancado et al., 2008). The G band is associated with the stretching of the bonds within the chains and rings while the D band can be attributed to the breathing mode of sp² atoms. It is also a characteristic of defects and substituted sites on a material (Ferrari, 2007). The 2D band is attributable to the stacking order and has been used to estimate the number of graphene layers. It can also be seen that 2D band has lower peak intensity than the G band, which is indicative of a material composed of many layers (Ferrari, 2007); a characteristic feature of graphene nanoplatelets.

### 3.4. Sessile contact angle analysis

The measurement of water contact angle on the surface of the bare SPE and PDA@GNPs/PPP/SPE was performed. The water contact angle for the bare SPE was found to be ~73.9°. However, on the PDA@GNPs/PPP/SPE the contact angle significantly decreased to ~44.3°. This increase in hydrophilicity of the modified SPE means that properties of the PDA coated GNPs can be manipulated particularly in buffer; thus, making the sensor a suitable tool for biofunctionalization, which is of great importance in a variety of applications including bio-sensing and for studying biointerfaces (Kanyong et al., 2016e). The stability of the PDA@GNPs modified SPE is crucial for bio-sensing applications. In order to investigate the stability and durability of the electrocatalytic activity of the sensor, 25 repetitive cyclic voltammograms were recorded in buffer. In general, unstable electrodes have unstable voltammograms, however, the anodic and cathodic peak currents (Ipa, Ipc) for the redox species on the PDA (shown in Fig. 2B) exhibited a standard deviation values of 1.72% and 1.39%, respectively. These standard deviation values indicate that the redox activity of the PDA is highly stable.

### 3.5. Application of PDA@GNPs/PPP/SPE to $\text{H}_2\text{O}_2$ analysis

The mediation of $\text{H}_2\text{O}_2$ oxidation and reduction was analyzed as a proof of concept for evaluating the electrocatalytic properties of PDA@GNPs/PPP/SPE for non-enzymatic detection of $\text{H}_2\text{O}_2$. Cyclic voltammograms of PDA@GNPs/PPP/SPE with and without $\text{H}_2\text{O}_2$ is shown in Fig. 5A. Both the anodic and cathodic peak currents increased when the concentration of $\text{H}_2\text{O}_2$ was increased. These results suggest that the PDA redox species catalyzed the oxidation of $\text{H}_2\text{O}_2$ to O₂ or reduction to H₂O and the GNPs-based nanocomposite efficiently promotes the direct electron transfer activity of the PDA. Therefore, the proposed mechanism for these reactions is that both the oxidation of $\text{H}_2\text{O}_2$ to O₂ and the reduction of $\text{H}_2\text{O}_2$ to...
H2O can be catalyzed by the quinone/hydroquinone moieties in PDA, which is thought to be a 2e−, 2H+ system (Kanyong et al., 2016d, 2016e).

The catalytic rate constant (K_cat) and diffusion coefficient (D) of H2O2 at the PDA@GNPs/PPP/SPE were estimated by chronoamperometry. Chronoamperometric measurements were carried out in buffer containing various concentrations of H2O2 (0.5, 1.25, 2.5 and 5.0 mM) at an applied potential of +0.2 V (Fig. 5B). The catalytic rate constant K_cat was calculated using the equation (Kanyong et al., 2016e) (Eq. (1)):

\[
\frac{i_{\text{cat}}}{i_{\text{buffer}}} = (K_{\text{cat}} \cdot C \cdot \pi)^{1/2} \cdot t^{1/2}
\]

where \(i_{\text{cat}}/i_{\text{buffer}}\) are the currents obtained at the PDA@GNPs/PPP/SPE for H2O2 and buffer, \(C\) is the concentration of H2O2 and \(t\) is time in seconds, respectively. Using the slope (here 0.6954 s−1/2) of \(i_{\text{cat}}/i_{\text{buffer}}\) vs. \(t^{1/2}\) plot (insert of Fig. 5B) for 0.5 mM H2O2, a \(K_{\text{cat}}\) value of \(9.15 \times 10^4 \text{M}^{-1} \text{s}^{-1}\) was obtained, which reveals that the PDA film is suitable for developing biointerfaces for bio-sensing applications (Kanyong et al., 2016e; Rotariu et al., 2014). The slope of the linear portions of \(i\) vs. \(t^{1/2}\) plots (Fig. 5C) for the varying concentrations of H2O2 (0.5, 1.25, 2.5 and 5.0 mM) were selected and used for the construction of the \(i \cdot t^{1/2}\) vs. [H2O2] plot (Fig. 5D). The slope of \(i\cdot t^{1/2}\) vs. [H2O2] plot was used in conjunction with the Cottrell expression (Rotariu et al., 2014) (Eq. (2)):

\[
i = \left( \frac{nFAD}{\pi^{1/2}C^{1/2}} \right)
\]

to estimate the diffusion coefficient (D) for H2O2; and a value of \(\sim 2.82 \times 10^{-5} \text{cm}^2 \text{s}^{-1}\) was found.

A calibration plot was then performed for varying concentrations of H2O2 from 0.5 to 20.0 mM (Fig. 6A) using amperometry in stirred solution at an applied voltage of +0.2 V. A linear range (Fig. 6B) was recorded from 0.5 to 13.0 mM with a sensitivity of 3.0 μA mM−1 cm−2 and a calculated limit of detection of (S/N = 3) of 0.55 μM; these analytical performance characteristics are superior to similar studies reported elsewhere (Kanyong et al., 2016d; Fan et al., 2012; Ye et al., 2012).

3.6. Application of PDA@GNPs/PPP/SPE to glucose bio-sensing

This study also investigated the biofunctionalization of PDA@GNPs/PPP/SPE via the immobilization of glucose oxidase (GOx) as a model enzyme. Differential pulse voltammetric measurements show an increase in PDA oxidation current upon successive additions of standard concentrations of glucose (Fig. 6C). This behavior can be attributed to PDA mediation of glucose oxidation and can be expressed by Eq. (3):

\[
2e + H_2O_2 \rightarrow 4H^+ + 2H_2O
\]

\[
D = \frac{nFAD}{\pi^{1/2}C^{1/2}}
\]

\[
E/V
\]

\[
[Glucose]/\mu M
\]

Fig. 6 (A) Chronoamperometric responses recorded at PDA@GNPs/PPP/SPE +0.2 V in PBS (pH 7.4) for standard additions of H2O2; (B) Calibration plot for H2O2; (C) Differential pulse voltammogram (DPV) recorded for standard additions of glucose in PBS (pH 7.4) using GOx/PDA@GNPs/PPP/SPE; and (D) Calibration plot for glucose.
The electrocatalytic current generated via the reaction in Eq. (3) displayed a linear behavior in the range of 1.0 to 800.0 μM (Fig. 6D). The limit of detection was found to be 0.25 μM (based on S/N = 3) with a sensitivity of 0.51 μA μM⁻¹ cm⁻²; these analytical performance characteristics of the biosensor were superior to similar published studies which tended to have either lower limits of detection (Rabti et al., 2016; Palanisamy et al., 2014a, 2014b) and/or poor sensitivity (Palanisamy et al., 2014b; Razmi and Mohammad-Rezaei, 2013). Since the physiological concentrations of glucose in human saliva ranges from 20.0 to 240.0 μM (Siu et al., 2014), the glucose biosensor can be employed as a sensing tool for noninvasive monitoring of salivary glucose.

4. Conclusion

We have demonstrated that the functionalization of graphene nanoplatelets (GNPs) with polydopamine (PDA) allowed for the development of a smart conducting bio-(chemo)sensor. The new PDA@GNPs-based sensor was well designed and found to be suitable for the development of low-cost, disposable bio-sensors; these are clearly required for point-of-need biomedical diagnostics applications. The bio-sensor was employed for non-enzymatic and enzymatic detection of H₂O₂ and glucose, respectively. This work represents a preliminary investigation of PDA functionalized-graphene nanoplatelets preparation and, suggests that the bio-sensor is suitable for practical routine sensing applications.

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Author Contributions

P.K. conceived and designed the experiments; P.K. and F.D.K. performed the experiments; P.K. analyzed the data; G.A. A. contributed reagents/materials/analysis tools; A.Y. provided day-to-day advice and guidance to F.D.K.; P.K. wrote the paper. All the authors approved the final manuscript.

Compliance with ethical standards

The authors declare that they have no conflict of interest.

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