Graphene Oxide-Chitosan based Flexible Biosensor

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***Abstract*- The paper presents flexible graphene oxide (GO)-Chitosan composite based biosensor for electrochemical detection of dopamine (DA). Electrochemical redox based detection tool for analytes has gained popularity due to their high specificity, sensitivity and label free technique. The GO-Chitosan based electrochemical electrodes presented here for three-electrode voltammetry have Ag/AgCl and Platinum based reference and counter electrode, respectively. The cyclic voltammetry (CV) of DA with as prepared electrode show quasi-reversible behaviour with characteristic cathodic peak (I_{pc}) at +200mV and anodic peak (I_{pa}) at -400mV. The voltammogram showed stability against repetitive scan cycles while the peak current showed increase with scan rates (100-200mV/s). The sensors show steady state peak enhancement (6.05-134.05µA) with DA concentration range of 0-100µM. The fabricated electrode is suitable for measuring DA with high sensitivity. The biodegradability of the prepared electrode is suitable for eco-friendly and disposable applications, which aligns with the green technology.**

Keywords: Graphene oxide, Chitosan, Flexible biosensor, Dopamine.

I. INTRODUCTION

Wearable electrochemical biosensor have become popular for clinical analytes because of simple, fast and label free detection [1-3]. Great varieties of matrices, on which the analytes react to give a specific redox, are needed for this purpose. Majority of the matrices reported thus far are metal or metal derivatives, which are stiff, non-degradable and eventually not suitable for *in situ* measurement with wearable systems. Further, the current biosensors in practice are non-environment friendly because they require special disposal mechanism [4]. Therefore, wearable systems with degradable, eco-friendly electrode matrix with adequate flexibility and biocompatibility will be a significant advance with potential for many new applications [5].

Recently collagen, poly-L-lysine, fibronectin etc. have been used for functionalizing metal electrodes for electrochemical applications [6,7]. However, none of these materials has been using as electrode matrices. It would be excellent for the wearable electrochemical sensing, if these materials are renewable and sufficiently conductive. As electrode matrices, biomaterial needs to be reorganizes and functionalized to form a flexible conductive surface [8]. There are materials of bio-origin such as chitosan, alginate and crustacean polysaccharides, which are readily available, self-renewable, inexpensive and biocompatible. These materials are already being used as principal components of bandage, gauge and other space fillers in the biomedical applications [9]. These materials are potential treasure trove for conformable bio-electrode. However, thus far they could not be used as bioelectrodes because they either lack conductivity or have poor conductivity [10]. Introduction of conductivity in these materials could open new opportunities for obtaining flexible and disposable bio-electrodes. The work presented here addresses this issue for the first time.

Considering the excellent stability and self-renewability chitosan has been chosen to fabricate a conformable and degradable bio-electrode. Herein, the electrical conductivity of chitosan was achieved with GO functionalization. Then, GO-chitosan conductive surface was used as a working electrode for sensing DA. In this case, the Ag/AgCl and Platinum (Pt) served as reference and counter electrode, respectively. Cyclic voltammetry (CV) and Linear swipe voltammetry (LSV) were performed to measure dopamine concentration based on the dose depended enhancement of redox peak current.

II. MATERIALS AND METHOD

A. Electrode fabrication

Chitosan (high molecular weight) powder (Sigma-Aldrich Co., 3050, Spruce Street, USA) was dissolved in 2M acetic acid solution at 37^0C by continuous stirring until homogenous clean solution achieved. Then chitosan solution of 1% (w/v) was subjected to GO (Ultra Highly concentrated single layer graphene oxide solution, graphene super market , 6.2 g/l) functionalization at a ratio of 16:1 by adding four times aqueous dilution of as received GO during stirring. The homogenous GO-Chitosan composite was achieved after overnight stirring. Then GO-Chitosan composite was drop casted on graphite conductive sheets (Graphene super market, thickness 25 µm) of dimension 0.5x2 cm and allowed it to dry overnight. The dimension of drop casted GO-chitosan composite on graphene sheet after drying is 3 mm in diameter (Figure 1).

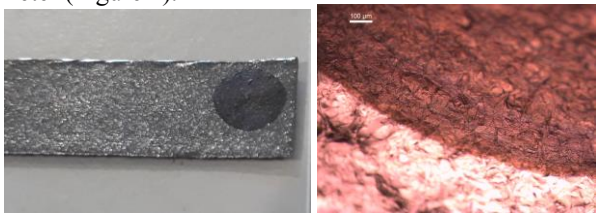

Figure 1. Optical images of the fabricated GO-Chitosan electrode on graphite sheet.

B. Morpholigical characterization

For SEM imaging GO-Chitosan composite membrane was established on a silicon substrate and a thin layer of Au sputtered on it prior to SEM imaging. Images were obtained with field emission scanning electron microscope (Hitachi S-4700) at an accelerated voltage of 10KV and 10mA current. Whereas, for optical imaging the GO-Chitosan

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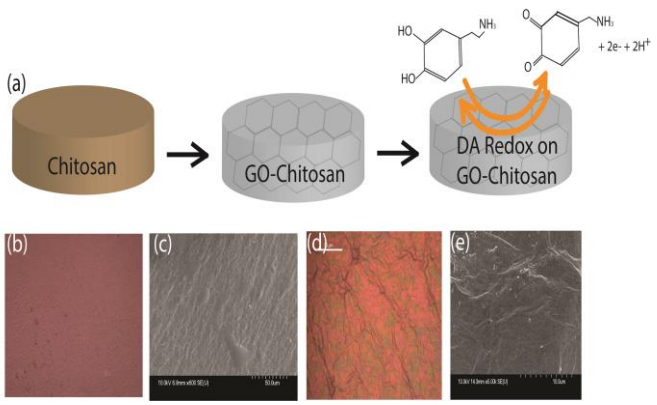

Figure 2. (a) Schematics of GO-Chitosan electrode fabrication and dopamine redox on it. Optical and SEM images of the chitosan (b-c) and GO-Chitosan (d-e) electrode, respectively

membrane was formed on glass slide and images were obtained from Nikon Eclipse LV100ND microscope connected with Leica MC170HD camera

C. *Electrochemical setup*

Electrochemical characterization were performed with a standard three-electrode setup using potentiostat (Metrohm Autolab B.V., Kanaalweg 29-G 3526 KM Utrecht, Netherland) where the fabricated GO-Chitosan membrane served as a working electrode and Ag/AgCl and platinum (Pt) served as reference and counter electrode, respectively. The reference and counter electrodes were used as independent macro electrodes. CV and LSV were measured to check the conductivity of the fabricated electrode using phosphate buffer saline (PBS) as electrolyte. All experiments were performed in triplicate using freshly prepared chip.

D. *Dopamine sensing*

Prior to the electrochemical detection of DA, the GO-Chitosan electrode was rinsed twice with a 10mM PBS (pH 7.4). Then freshly prepared DA solutions in PBS were subjected to electrochemical investigation where freshly prepared PBS without dopamine served as control. All the measurements were repeated at least three times and the error bars have been shown in the figures.

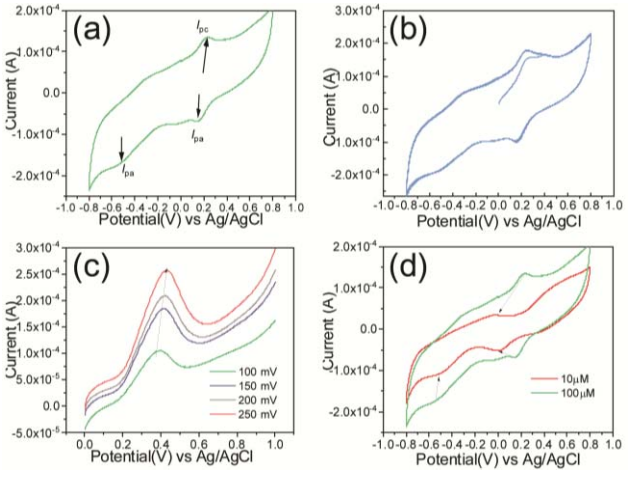

Figure 3. (a) Redox behavior of dopamine on GO-Chitosan electrode, (b) Scan cycle effect and (c) scan rate effects and (d) concentration peak shift. CV and LSV were measured using PBS (0.01 M, pH 7.4) as electrolyte at a scan rate of 150 mVs^{-1}. All the experiment was conducted at 27 ± 1°C. The experiment was repeated three times with maintaining identical condition.

III. RESULTS AND DISCUSSION

A. *Electrode fabrication*

Chitosan was modified with Graphene oxide (GO) to fabricate an all carbon electrode as shown in the Figure 2a. Chitosan is a nonconductive carbohydrate polymer which attain conductivity after being functionalized with GO [8]. GO functionalization was confirmed by both optical and SEM imaging of chitosan membranes (Figure 2b-e). The optical images of chitosan membrane (Figure 2b) and GO-Chitosan membrane (Figure 2d) showed significant differences in surface topography. Similar topographic variations was also noticed when SEM images were obtained before (Figure 2c) and after (Figure 2e) GO functionalization, confirming the GO-Chitosan electrode formation. This all carbon electrode was applied as working electrode in the three-electrode voltammetry later in this work.

B. *Electrochemical characterization and DA sensing*

For electrochemical characterization, GO-chitosan electrode was placed in PBS containing 100µM dissolved DA together with reference (Ag/AgCl) and counter (Pt) electrode. CV measurements were performed using a potentiostat with three-electrode setup (Metroohm Autolabo B.V.). The measured CV plot is shown in Figure 3a. An ideal quasi-reversible redox peak with cathodic peak (I_{pc}) at +200mV and anodic peak (I_{pa}) at -400mV was recorded from DA (Figure 3a) with an additional I_{pa} peak at +150mV. Considering quasi-reversibility the peak potential difference between I_{pc} - I_{pa} is ≥100mV and the current ratio I_{pc}/I_{pa} is ≥1 [11,12]. This indicates I_{pa} at -400mV is only attributed to the DA redox. Therefore, the additional peak could be attributed

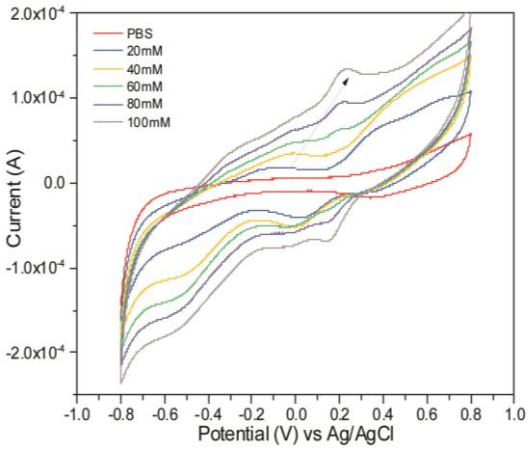

Figure 4. Cyclic vilammogrm of various concentrations of dopamine on GO-Chitosan electrode. CV was measured using PBS (0.01 M, pH 7.4) as an electrolyte at a scan rate of 150 mVs^{-1}. The experiment was repeated three times with maintaining identical condition.

to the reaction of the atmospheric oxygen with chitosan. However, considering the uniqueness and intensities, the I_{pc} at +200mV was subjected to quantitative investigations of various DA concentrations later in this experiment.

Prior to the quantitative DA sensing, the electrode stability and peaks behaviour was investigated against various scan cycles and scan rates applied during electrochemical investigations. The electrode showed stability against various scan cycles with stable peak I_{pc} as shown in the Figure 3b. However, the peaks became prominent with the increasing scan rates as indicated in the Figure 3c. Considering suitability of chitosan the scan rate of 150mV was chosen for the rest of the investigations. The potential shift behaviour of all the peaks was also noticed when CV was measured with high (10µM) and low (100µM) DA concentrations (Figure 3d) while the quasi-reversibility remained cathodic peak (I_{pc}) at +200mV and anodic peak (I_{pa}) at -400mV. The negative shift of I_{pc} and positive shift of I_{pa} was noticed when the DA concentration decreased. However, the additional I_{pa} peak at +150mV was shifting towards negative potential which indicates that this additional peak is not related with DA.

C. *Reprodicibility of electrochemical tool*

The reproducibility and sensitivity of electrochemical signals from GO-chitosan electrode was confirmed by achieving CV from various concentrations of DA from 20µM to 100µM. The CV peak intensities became prominent with increased concentrations of DA (Figure 4). The concentration dependent peak shift was reported as discussed earlier. The I_{pc} values obtained from each voltammogram is considered as sensing values of the DA concentrations here in this experiment [7]. A concentration dependent linear plot ($r^2 = 0.977$) was obtained between the current intensities I_{pc} and DA concentrations (Figure 5). The CV current peak increased exponentially with the concentrations of DA (20µM-100µM). However, the CV peak at ≤20µM DA concentrations were not clearly visible that gives like back ground current of bare GO-Chitosan electrode. Therefore, ≤20µM DA concentration is determined to be the minimum detection limit of this electrochemical investigation.

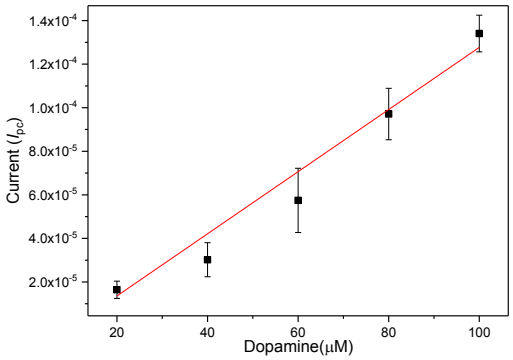

Figure 5. Changes in cathodic peak intensities (I_{pc}) corresponding to the various concentrations of DA on the GO-Chitosan electrode surface. The linear increases in current peaks (I_{pc}) in a concentration-dependent manner ($R^2 = 0.989$). Data are the mean ± standard deviation of three different experiments.

IV. CONCLUSION

A graphene oxide (GO) modified chitosan bio-electrode is reported here for the first time along with electrochemical redox sensing performance. A quasi-reversible redox was detected from dopamine (DA) when cyclic voltammetry (CV) measurement were performed. The characteristic cathodic (I_{pc}) and anodic peaks (I_{pa}) were recorded at +200mV and -400mV, respectively. The I_{pc} peak showed stability at various scan cycles while scan rate dependent peak enhancement was noticed. Therefore, the I_{pc} peak was subjected to investigate various doses of DA at a scan rate of 150mV/s. DA concentrations in the range from 20-100mM produced electrochemical signals which offer concentration dependent linearity ($r^2 = 0.977$) between the current intensities and DA concentrations. The I_{PC} peak was found to disappear when dopamine ≤20µM DA was investigated. Further miniaturization and optimization of measuring conditions in faradic cage could enhance the detection limit of this all carbon electrode. The flexibility, biodegradability and biocompatibility of presented biosensors will advance many applications, in particular with wearable detection platforms.

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