Electrochemical Properties of π - π Stacking Interacted Chemically Reduced Graphene Oxide-Ferrocene Hybrid Modified Electrode: An Efficient Electrochemical Biosensor Application

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Abstract— **In the present study, chemically reduced graphene oxide (CRGO) with ferrocene (Fc) was fabricated through π - π stacking interaction. Fc-CRGO was characterized by UV-Vissible and Raman spectra. Fc-CRGO Nonohybrid modified glassy carbon electrode (GCE) by electrochemical drop cast method. The effective Fc-CRGO Nanohybrid are studied by Cyclic voltammetry. Electrochemical properties of an Fc-CRGO/GC modified electrode by π - π stacking interactions are studied and the electron transfer rate values are calculated. Furthermore, the fabricated GCE shows excellent mediation of Ascorbic acid (AA) based on Fc/Fc^+ used as electron-transfer mediator for oxidation of AA, using Amperometry determination for AA suggesting specific properties of Fc-CRGO/GC are studied. Fc-CRGO/GC modified electrode shows the linear response in the range of 199 μM and 310 mM, with a very low detection limit of 0.0027 M. Also the Fc-CRGO/GC modified electrode displayed good selectivity, sensitivity and stability with appreciable consistency and high precision.**

Keywords- Chemically reduced graphene oxide; ferrocene; Ascorbic acid; cyclic voltammetry

I. INTRODUCTION

Ascorbic acid (AA or vitamin C) is water soluble and naturally occurring vitamin act as the important antioxidant in animals and humans. AA is an essential nutrient due to its participation in several metabolic reactions such as enhancing iron uptake in human intestinal cells [1]. AA is widely used to prevent scurvy, common cold, mental illness, Hepatic and cancer diseases. For diagnostic and food safety applications [2], a simple and rapid detection method of AA under complex conditions is needed for biomedical chemistry, diagnostics and pathological research. AA analytical quantification was reported such as enzymatic [3], chromatographic [4], Fluorimetry [5] and Electrochemical methods. Among them, electrochemical sensing has proven to be an inexpensive simple and green analytical method. The use of nanoparticles modified electrodes provide a promising route for electrochemical AA sensor fabrication. Recently, Graphene anchored Metal oxides, Metal sulfides, Metal nanoparticles [6], Multi-walled carbon nanotubes composite [7] and nitrogen doped graphene have been reported as advanced electrode materials for AA electrochemical sensing [8].Graphene (Gr) has emerged as an interesting material because of its unique electronic properties and large accessible surface area It shows the fast electron-transfer kinetics and "further signal amplification in electrochemical detection" [9]. Ferrocene (Fc) is a well known electron mediator due to its reversibility, regeneration at low potential difference, and generation of stable redox states [10]. There are very limited reports about the nanohybrid of Fc or its derivatives with graphene. The non-covalent hybrid of Fc with Chemically Reduced Graphene oxide (CRGO) was used to determine AA and this method was not been reported. Graphene possesses huge open π-electron system. The formation of nanohybrid of Fc with CRGO by π – π stacking interaction is possible. Therefore, we studied the interaction between Fc and CRGO and their electron transfer behaviors. The nonohybrid showed high stability and greatly enhanced electron transfer property. It was applied to biosensing application for Ascorbic Acid determination.

II. EXPERIMENTAL

A. Synthesis of Chemically Reduced Graphene oxide

Graphite oxide (GO) was synthesized from graphite powder by using a modified Hummers method [11]. The synthesized graphite oxide was dispersed in double distilled water (1 mg/ml) with sonication for 1 h to get homogeneous dispersion. The reducing agent hydrazine hydrate (3 mg/ml) was added drop wise to the graphene oxide solution. The mixture was refluxed 12 h at 95 °C in an oil bath the block colored solution was filtered and dried in hot air oven at 60 °C for 6 h to get chemically reduced graphene oxide.

B. *Preparation of Fc hybridized CRGO (Fc-CRGO)*

250 mg of CRGO was dispersed in 50 ml of N,N dimethylformamide (DMF) by sonication for 30 min. 800 mg of Fc dissolved in 100 ml DMF. Then, Fc solution was poured into CRGO solution. The mixture was magnetically stirred for 48 h at 25 °C. The hybrid (Fc-CRGO) was collected by filtration and washed thoroughly by centrifuge 500 rpm and was dried in a hot air oven at 60 0C for 6 h. Above procedure was followed for the Fc-GO preparation.

C. *Fabrication of different modified electrodes*

The Glassy carbon electrode (GCE) was polished on 1.0, 0.3 and 0.05 µm alumina powder until a mirror-like surface is obtaining. It was rinsed with water thoroughly and dried. 2 mg of Fc-CRGO hybrid was dispersed 1ml of ethanol in 0.5% Nafion solution and mixed by ultrasonic agitation for 30 min. 10 µL of Fc-CRGO was drop casted on the electrode surface and allowed to dry to obtain Fc-CRGO/GC modified electrode. Similarly Fc-Go/GCE modified electrode was fabricated.

III. RESULT AND DISCUSSION

A. *UV-Vis and Raman Spectroscopy*

Fig:1(A) shows the Raman spectra of Fc-GO and Fc-CRGO. The spectrum exhibits the presence of D and G bands, located at 1336 cm^{-1} and 1583 cm^{-1} which are typical Raman features of FC-GO. The graphene oxide after being reduced, shows the D band at 1349 cm^{-1} and G band at 1589 cm^{-1} (curve for FC-CRGO). The intensity ratio of I$_D$/I$_G$ decreases, because of the epoxy and alkoxy functional groups gets reduced and also this up-shift and broadening may be caused by the strong - interaction between Fc and CRGO and the spectrum confirms that Fc is immobilized on the surface of CRGO.(Fig:1(B)) shows the typical UV–vis spectra of Fc, Fc-GO and Fc-CRGO nanohybrid. The Fc alone shows three different absorption peak at 273 nm, 324 nm and 439 nm due to its   electron synergism (a), Fc-GO shows its   max peak at 258 nm (b) The reduced graphene oxide with ferrocene (Fc-CRGO) composite material shows 274 nm (c) these were red-shifted compared with Fc-GO because of increase in the   conjugation and in the presence of Fc and CRGO [12]. In addition, the intensity of the absorption tail in the region 250 nm to750 nm has significantly decreased. The result suggests that the GO Nanosheets have been reduced and the electronic conjugation within the GO Nanosheet was restored upon hydrazine reduction.
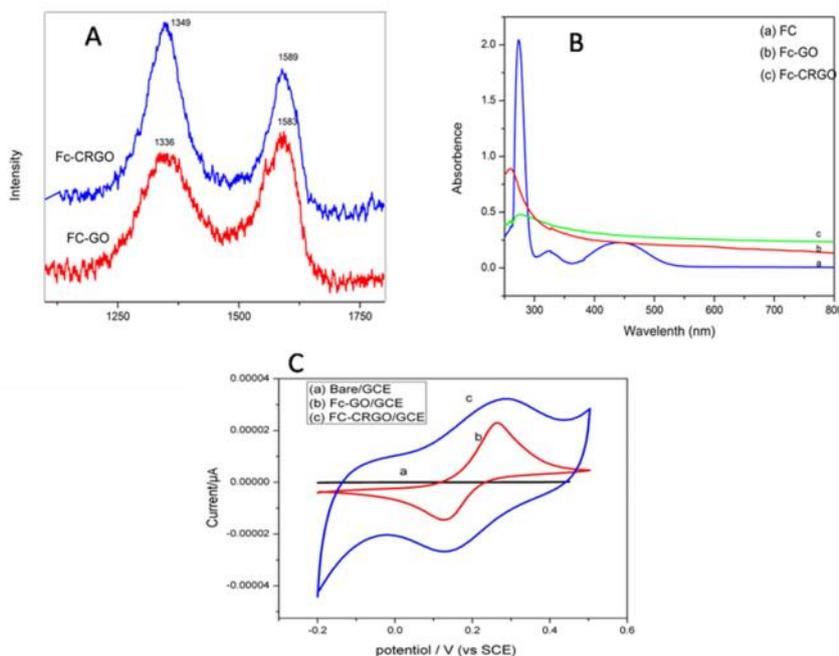

Fig:1(A) Raman Spectrum of Fc-GO and Fc-CRGO,(B) UV-Vis absorption spectra of (a) Fc, (b) Fc-GO, and (c) Fc-CRGO in (DMF) and. (C) Cyclic voltammograms of different modified electrodes (a) Bare GCE; (b) Fc-GO/GCE; (c) Fc-CRGO/GCE in 0.1 mol/L PBS (pH 7). scan rate at 50 mV/s.

B. Electrochemical Oxidation of Ascorbic Acid

The electrochemical measurements were performed on PGSTAT-12 Electrochemical workstation (AUTOLAB The Netherlands BV) the voltammetric behaviors of different modified electrodes were studied (Fig:1C). Thus Fc-GO/GCE (Fig:1C (b)) we can see a pair of well-defined redox peaks at +148 and 232 mV anodic peak current (E_{pa}) and cathodic peak current (E_{pc}) respectively. The peak–peak potential separation (Ep = Epa - Epc) is 84 mV. While at Fc- CRGO/GCE (Fig:1C (c)), a pair of strong reversible cathodic and anodic peaks can be found. This clearly indicates that the fast electron transfer kinetics take place.

C. Effect of Concentration, Scan rate, and pH

In order to investigate the electrocatalytic activity of Fc-CRGO on the modified electrode, The CV of FC-CRGO/GC modified electrode Shows lowering the oxidation potential take place. generally AA oxidized in 0.25-0.4v in the case of Fc-CRGO modified electrode exist in -0.025v in 0.1M PBS (pH 7.0) containing varied concentrations of AA was investigated (Fig:2(A)) The oxidation current increased dramatically with the increasing concentration of AA, The calibration plot (Fig:2(B)) shows that the catalytic current had a linear relationship with the concentration of AA ranging from 199 µM to 196 mM (the correlative coefficient is $R^2 = 0.9993$). The effect of scan rate of electrochemical response 0.1 mM AA was investigated by CV and the results are shown in (Fig:2(C)). On increasing the scan rate from 20 to 180 mV s^{-1}, the Epa peak current increased gradually and linearly. from the calibration plot (Fig:2(D)) scan rate is linear relationship with oxidation peak current this indicates that the oxidation AA at Fc-CRGO/GCE is an adsorption-controlled process and the correlative coefficient is $R^2 = 0.999$
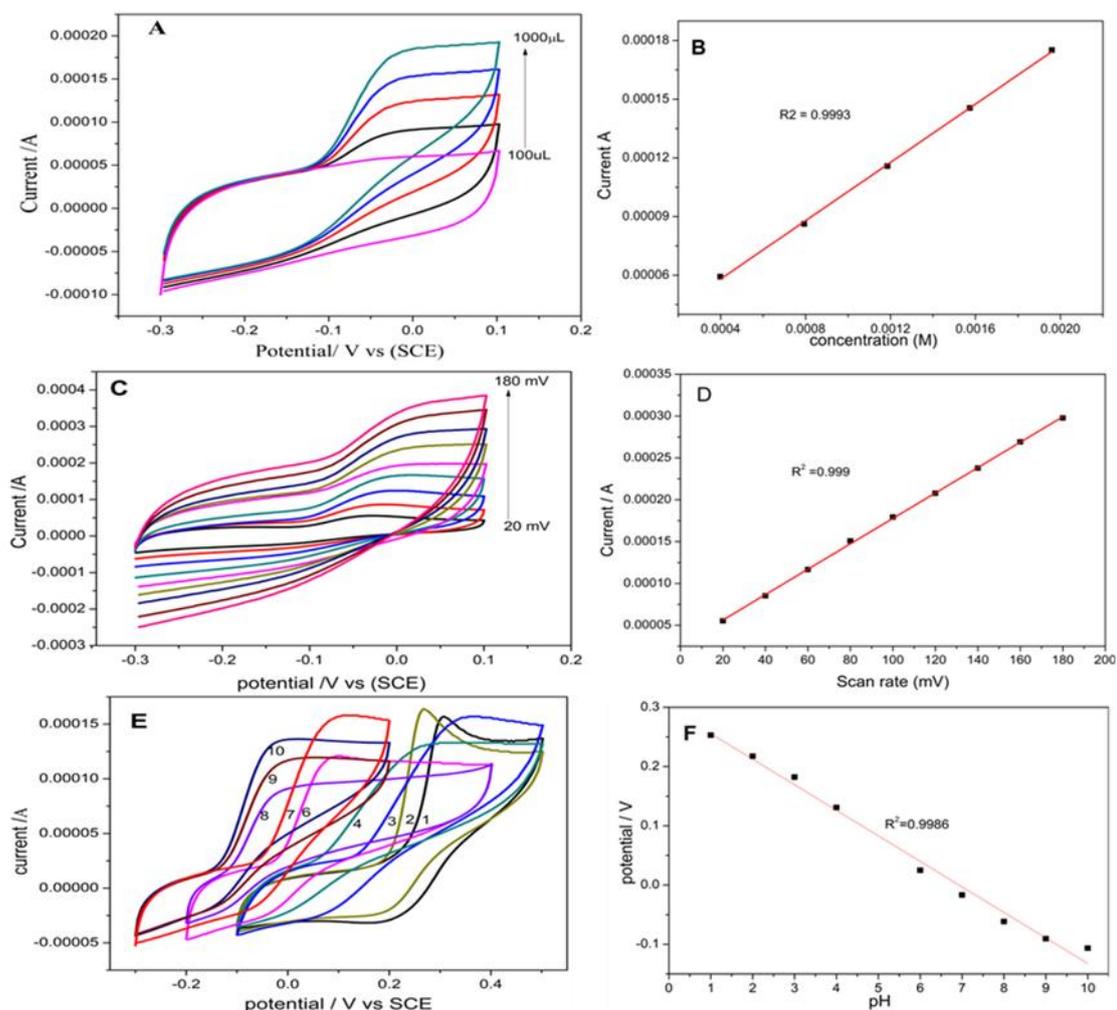

Fig:3(A) Cyclic voltammograms of 199 µM to 196 mM in 0.1M phosphate buffer solution (pH7.0) scane rate at 50 mV. (B) Calibration plot of concentration vs current. (C) different scan rates 20 to 180 mV (D)calibration plot of scan rate vs peak current.(E) CV of Fc-CRGO/GC electrode in different pH 1-10 presence of 2mM AA scan rate 50 mV (B) calibration plot of pH vs potential.

The electrochemical oxidation behavior of AA at Fc-CRGO /GC modified electrode in different pH buffer media was investigated by cyclic voltammetry (Fig.(E)). The cyclic voltammetry response was

significantly different with various pH solutions. Especially on the oxidation peak current values shows that the maximum current response was obtained at pH 7.0. A plot of peak potential vs pH was found to be linear over the pH range 3.0–10 (Fig. 3F) with a regression equation that can be expressed as Epa (V) = −0.05387 pH + 1.29 (R^2 = 0.996). A slope of 53 mVpH^{-1} indicated that there are equal numbers of protons and electrons involved in electrode reaction, (Fig.3(F))

D.   Amperometric response of AA oxidation

The amperometric response of the Ascorbic Acid (AA) biosensor was investigated by adding AA to a continuous stirring PBS (pH 7) solution with the working potential of -0.025 mV. The typical current–time curve of the biosensor is shown in(Fig:3(A)). The response of current increases with increasing concentration of AA as illustrated. We used the steady-state current to plot against the concentration of AA. There is a linear relation of the current with concentration of AA between 199 µM and 310 mM (inset of (Fig:3(B)), The FC-CRGO modified GC electrode has good sensitivity and LOD= 0.0027M. and relative standard deviation (RSD) of 2.14%, indicating good reproducibility of the modified electrode.
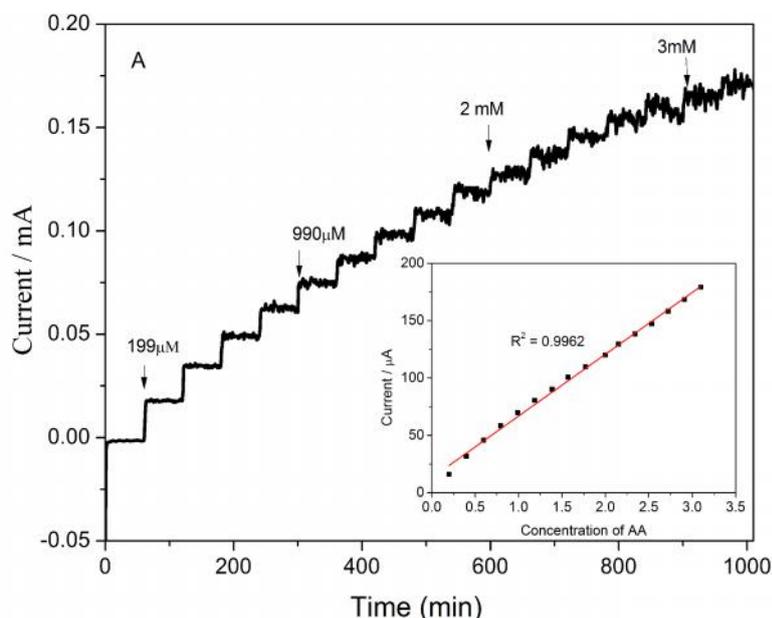

Fig:3(A) Amperometry Current vs Concentration (time) response of Fc-CRGO/GC electrode at applied potential of -0.025V by addition of 2 µM of AA in 0.1M PBS and insert calibration plot of concentration of AA vs current.

E.   Interference studies

The Fc-CRGO/GC electrode studied following interference the selectivity of electrochemical sensor for AA bio sensor several biomolecule and metal ions are tested such as dopamine, adenosine, guanosine, uric acid L-cysteine, glycine, histidin, tryptophan and Na^+,Ca^{2+},Mg^{2+},Fe^{2+} NO_3^- these substances exhibited less interference and no substantial changes appeared with the peak current. So the proposed electrode exhibited good selectivity in the electrochemical detection of AA.

IV.   CONCLUSION

The nanohybrid of Fc-CRGO was prepared by congregating Fc molecules on the nanosheets of CRGO through – stacking interaction. The nanohybrid was confirmed by raman and uv-Viss spectra. Nnohybrid modified GCE applied for AA detection. Fc-CRGO exhibited a obvious decrease in the "overvoltage". The low-potential amperometric detection for electrochemical sensing of AA was involved. The dramatically enhanced electrocatalysis and stability of Fc-CRGO would make it useful material for preparing biosensors and other bio-electrochemical devices. The FC-CRGO modified GC electrode has good sensitivity and sensitivity.

V.   Acknowledgment

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