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FULL PAPER

In situ oxygenous functionalization of graphite electrode for enhanced affinity towards charged species and reduced graphene oxide mediator

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The weak affinity of bare carbon based electrodes for biological molecules or charged species is a major drawback for their direct application in analytical electrochemistry. We observed that the surfaces of graphite rod and glassy carbon (GC) ring electrodes can be modified by oxygenated functional groups through controlled electrochemical oxidation in aqueous media. Study of cyclic voltammetry, surface conductivity, X ray photoelectron spectroscopy (XPS), Raman spectroscopy, infrared spectroscopy (IR) and powder X-ray diffraction (PXRD) spectroscopy confirmed the chemical change. In electrodynamic study, the modified GC ring in a rotating ring-disk electrode (RRDE) assembly showed better electron transfer efficiency than the virgin electrode when evaluated by $Ru(bpy)_3^{2+/3+}$ couple. In comparison to virgin electrode, the modified graphite rod exhibited better affinity toward polyvinylpyrrolidone protected reduced graphene oxide (PVP-rGO) mediated to glucose oxidase enzyme (GOx), and represents direct attachment of mediators to the oxygenated electrode surface. These observations imply that a small extent oxygenated layer mounting the carbon electrode surface can significantly increase its activity. The present evidences indicate the possibility of oxygenous functionalization of carbon based electrode for areas like electro kinetic study and biosensing, where a strong analyte-electrode interaction is supposed to promote the utility.

1. Introduction

Carbon electrodes such as graphite rod, glassy carbon, HOPG etc. are highly conductive, but their inert nature renders the capability of binding biological analyte or enzyme such as GOx.[1,2] This demerit confines their utility for analytical application. To solve this problem, numerous research works has been focused on the modification of electrode surfaces.[3,4] Up to date most of these modifications are based on the physical attachment of some catalytic ingredients like metal or metal oxide (/sulfide/arsenide and so on) nanoparticles, enzymes, DNA etc. in the pure form or as hybrids with carbon nanotube (CNT), graphene oxide (GO) or reduced graphene oxide (rGO). However, to deposit these ingredients successfully on the graphitic surfaces, some gluing materials like conductive polymers, ionic liquids and organic compounds with polar/substitutable terminals are commonly employed.[5-11] The use of a gluing layer is almost unavoidable as it can establish either a direct linkage or indirectly a mediator transmitted electrical communication between the catalytic materials / enzyme and electrode surface. As the pure carbon substrates are ordinarily inert and charge neutral, the physical attachments they can make with catalytic ingredients or even with the gluing material are relatively weak. This major disadvantage has confined the use of carbon electrodes nowadays, and there is a need to develop any method for increasing the activity of carbon electrode surfaces. In our recent reports, we observed that the oxygenated form of the thinnest carbon allotrope, the graphene oxide (GO) exhibit significant electrostatic attraction for both heavy metal ions and proton.[12,13] These observations motivated us to consider the possibility of surface oxidation of carbon electrode for generating a polar layer, which might show strong attraction for oppositely charged species. Our hypothesis includes that an oxygenated surface can adopt the carbon electrodes for the areas, where a strong electrode-analyte (or gluing materials) interaction is essential. For this purpose, we choose the electrochemical oxidation as the best way because: i) the surface roughness and capacitance that usually rises during oxidation can be carefully minimized by tuning both the applied potential and treatment time; ii) compared with chemical treatment, electrochemical process might generate a perfectly oxidized, but uncontaminated surface; iii) The target area on the electrode surface for oxidation can be controlled easily by immerging the respected area only under the electrolytic solution. Herein, we report the controlled electrochemical oxidation of graphite rod and glassy carbon ring to avail modified surfaces with optimized oxygenated functional sites and minimized capacitance. The modified glassy carbon ring showed higher collection efficiency (N%) for oxidized species in RRDE experiment, evaluated by $Ru(bpy)_3^{2+}$ solutions. In addition, the modified graphite rod exhibited better attachment to mildly reduced PVP-rGO, which was mediated to GOx enzyme beforehand.

Surface oxidation of carbon electrodes is a cognizant issue, which is almost unavoidable in all types of electrochemical analysis. A lot of literature has been reported for understanding the structure of these oxidized carbon surfaces.[14-16] These modified surfaces attribute some unwanted effects, such as adsorption, electron transfer kinetics and electrocatalysis during electrochemical processes.[17] Therefore, many efforts have been assigned to minimize the extent of oxidation. In reverse to this negative expect, for the first time our finding demonstrates the utility of anodized electrode generated by controlled oxidation.

In RRDE experiments, rotation oriented sequential propagation of oxidized species from the disk electrode to the ring electrode due to hydrodynamic effect is a classical phenomenon for studying electrodynamics and redox reaction mechanism.[18,19] The capturing efficiency of ring electrodes for oxidized species is usually lower than those produced at the disk oxidizer. As a result, the overall ion collection efficiency is measured in terms of proximity between the measured value and the theoretical value of N%. It is reported that N% is invariant of the rotation speed or operating potential. However, it depends on the geometry and structure of the electrodes. Therefore, as the experimental evidences confirm about the chemical change at GC ring top, we considered that the improved interaction between oxidized species and negatively charged functional sites might reveal improved value for N%. And as per our hypothesis, compared with pure GC ring electrode, the modified ring disk electrode reflected better N% for Ru^{3+} ion.

Nowadays, graphene based materials are frequently being used for biosensor application. The tiny GO and rGO fragments exhibit ionic and electronic conductivity, respectively.[12,13] Therefore, they can support conductive bridging between the electrode and the active core of some enzyme by penetrating the nonconductive protein or lipid shell surrounding the core.[20,21] But to consider its attachment on carbon electrode, a mediator is needed.[22] However, we found that PVP-rGO can be attached directly on the oxygenated graphite electrode. We justified the attachment of GOx enzyme to the modified electrode surface, and glucose detection as a model representation for direct electrode mediator attachment.

2. Experimental

All chemicals were purchased from Wako pure chemical co. Japan, and was used without further purification. The water used for all experiments was ultra pure (18.2 MΩ).

2.1 Electrode modification.

Graphite rod (10.1 cm long and 0.42 cm diameter) and RRDE made from GC (disk with 4.6 mm diameter, ring with 0.45 mm width and a gap of 0.35 mm between ring and disk) were purchased from Wako and BAS Ltd, respectively. Before modification, the surfaces of the electrodes were polished mechanically with 1 μm and 0.5 μm alumina slurry followed by successive ultrasonication in deionized water, methanol and hexane. For electrochemical modification, two graphite rods were immersed in doubly distilled water, and were connected to a 10V DC voltage source (Matsusada precision inc., P4K36-1). The treatment time was regulated from 5 minutes to 12 hours. Electrode connected to the positive terminal of DC source was justified by cyclic voltammogram (CV) after some randomly selected treatment duration (5, 10, 30, 90 minutes and 12 hours). The extent of oxidized spots at the surface increases with treatment time, and the increased capacitance results diffused signal in CV. To fit the electrode with a narrow CV, but significant amount of oxygenated functional sites, a treatment time of 10 minutes was choose for optimization. An untreated rod and that one treated for 10 minutes were labelled as virgin graphite (VG) and modified graphite (MG), respectively and were used for glucose sensing experiment. In a similar way to modify the RRDE, the ring electrode and a platinum wire were connected to the DC voltage source through the positive and negative terminal, respectively. A virgin RRDE (VR) and another RRDE containing the treated ring electrode (MR) was used for electrodynamic study.

The modified electrodes MG and MR were washed with copious amount of distilled water. The electrode VG and MG were characterized by cyclic voltammetry, surface conductivity, Raman, IR, PXRD and XPS spectroscopy. Electrode VR and MR were characterized by cyclic voltammetry only. When unused, MG and MR were stored in deionized water to prevent adsorption of impurities to the negatively charged surfaces.

A micro Raman spectrometer (NRS-3100, Jasco, Japan) with a 532 nm excitation source at room temperature was used to study the Raman spectroscopy. For XPS, a Thermo Scientific (Sigma Probe) kits equipped with a monochromatized X-ray source (Al Kα, hν = 1486.6 eV) was used. A discharge source (He I, hν = 21.2 eV) was used for Ultraviolet Photoelectron Spectroscopy (UPS) in the same instrument. The graphite rod surface was measured directly. For measurement of GO and rGO, a Pt substrate (in GO / Pt film) was used. All measurements were done in a vacuum better than 10^{-7} Pa. Emitted electrons were detected by a hemispherical energy analyzer equipped with six channeltrons. FTIR spectra were recorded in a JASCO FT-IR-410 instrument using KBr disk. Powder X-ray diffraction (XRD) studies were performed on a Rigaku X-ray diffractometer RAD-2A with a 2.0 kW Cu Ka X-ray. The conductivities along the surfaces were measured by two electrode system (Ivium Compactstat) with a distance of 2 cm within the electrodes.

2.2 Synthesis of PVP-rGO.

GO was synthesized according to our previously reported method, and was characterized by atomic force microscopy (AFM) and XPS study (supporting information).[23] The PVP-rGO was synthesized through milder condition than that reported previously.[24] In a typical method, dry GO was dispersed in water (1 mg / 5 ml) by 2 hours ultrasonication followed by discarding the aggregated carbon by centrifuging at 4000 rpm for 1 hour. This GO suspension was further diluted in water to create a 0.1 wt % solution by ultrasonication for another 30 minutes. 25.0 mL of this homogeneous dispersion was mixed with 10.0 mL of 5 mg / mL aqueous PVP solution, and the mixer was stirred at 50°C for 12 h. After cooling to room temperature, 20 μL hydrazine (98 %) and 300 μL ammonia solution (28 %) was added with vigorous shaking for 5 minutes. The vial was then kept in an oil bath (95°C) for 40 minutes. A stable black dispersion was obtained, which was filtered with a microporous nylon membrane to obtain black precipitate. It was washed 3 times by water (through successive ultrasonication and filtration) to obtain PVP-rGO. Finally, it was sonicated in water to get 0.5 wt % dispersion.

2.3 Electrochemistry

GOx powder (Aspergillus niger, MW=186,000) was stored at -20°C. For preparing mediator linked enzyme, 3 ml of 0.5 mg / ml (2.69 μM) GOx solution in 0.05 M, pH 7.0 phosphate buffer saline (PBS) at 4°C was mixed with 3 ml PVP-rGO dispersion at

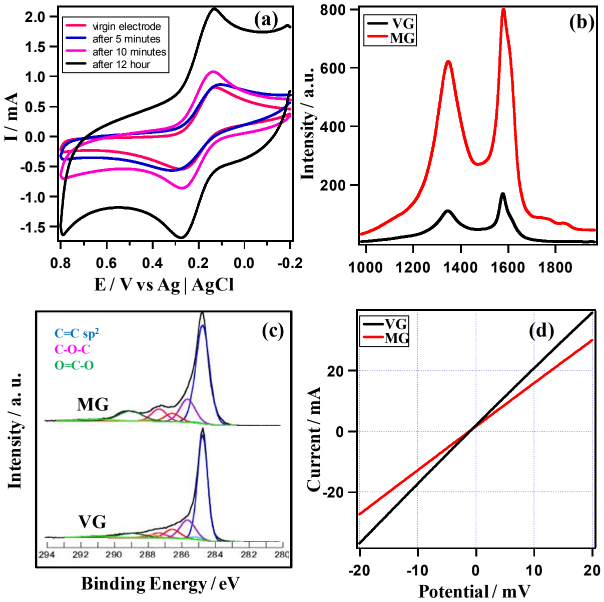

Figure 1. (a) CV of 1 mM $K_3Fe(CN)_6$ solution (100mV/sec scan rate and 0.1 M KNO_3 as supporting electrolyte) using graphite electrode with variable electrochemical treatment duration (b) Raman spectra (c) XPS spectra and (d) Current vs. applied voltage for virgin graphite rod (VG) and oxygenated rod (MG).

the same temperature. Electrodes VG and MG were washed with PBS, and was kept in 4°C by immerging in the prepared PVP-rGO-GOx-PBS solution for 12 hours. Finally they were washed by PBS, and dried under argon gas flow. D-glucose solutions were prepared in PBS (0.05 M, pH 7.0), and were bubbled by oxygen before running cyclic voltammetry. The cyclic voltammetry was performed in a cell of three electrode system, where VG and MG were used as working electrode with a Pt wire and a Ag/AgCl electrodes as counter and reference electrodes, respectively. A series of CV was recorded by varying scan rate and glucose concentration.

The RRDE experiments were operated at 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 rpm in a cell of four electrode system connected to a computer controlled bipotentiostate (ASL 701A, BAS Ltd) and an electrode rotator. The disk and ring electrodes were connected to the positive and negative terminal. A Pt wire and an Ag/AgCl electrode were used as counter and reference electrode, respectively. A solution of 0.001 M $Ru(bpy)^{2+}$ in 0.1 M KCl (supporting electrolyte) was used as the analyte for calibration.

3. Results and Discussion

The massive graphite has a very rigid structure. Therefore, it was expected that the oxidation process might occur at the outermost layer of both the rod and ring. As a result, our characterization was to justify the formation of oxygenated functional groups (-OH, -COOH, -C-O-C etc) at the surface. In addition, we compared our results with GO and rGO as these two dimensional materials perfectly represent the isolated single layers of oxidized graphite. Experimental phenomena such as XPS, Raman, IR and PXRD spectroscopy, cyclic voltammetry and surface conductivity measurement confirmed the generation of oxygenated functional groups at the surface of MG. For bulk sampling very careful attempts were considered for scratching out powders from the surface of VG and MG.

3.1 Characterization of the Electrode

In Figure 1(a), the virgin electrode shows a reversible CV for Fe^{2+} / Fe^{3+} couple. With respect to the duration of electrochemical treatment, the peak current remains almost constant, but the area under CV increases gradually with a maximum value after 12 hours. This increased area implies that the capacitance of the electrode has been increased, which is a usual phenomenon during the oxygenation reaction.[25] Generally, the capacitance of an oxide is higher than the corresponding pure metal due to its increased value of dielectric constant.[26] In addition, almost a similar broadening was reported previously, by Shan et al in the CV of a GC electrode layered by oxidized graphite.[27] This observation indicates the generation of oxygenated carbon sites at the electrode surfaces. We also considered the contribution of surface roughness to the increased capacitance. The surface roughness results from the electrochemical corrosion reaction, which is accelerated by long time anodization, higher operating voltage and higher conductivity of solution, especially in presence of any supporting electrolyte. However, we maintained lower electrode potential (10 Volts), an optimized treatment time (10 minutes) and ultrapure water to minimize the corrosion. The existence of oxygenated sites was further justified by raman spectroscopy. Figure 1b represents the raman spectra of electrodes VG and MG. For both, two characteristic peaks of carbon materials termed as 'D band' (~1350 cm^{-1}) and 'G band' (~1580 cm^{-1}) are noticeable. These two peaks rise from the breathing mode (A_{1g}) and the in plane bond stretching motion of pairs of C sp^2 atoms (E_{2g}), respectively.[28] The G band position shifts from 1577 cm^{-1} to 1580 cm^{-1} after treatment. This hardening results from the change in electronic structure during oxidation and complies with the softening explained in some previous reports.[29] The ratio of peak height for D and G-band (I_D/I_G) was calculated as 0.656 and 0.776 for VG and MG, respectively. The difference corresponds to an 18 % increase from VG to MG conversion. For carbon materials, the I_D/I_G value is inversely proportional to the extent of sp^2 domain. Therefore, the increase in this value signifies destruction of sp^2 network and generation of some sp^3 sites.[30] In graphitic stake the carbon atoms are in sp^2 hybridized state. During oxidation some of the carbon atom has been converted to epoxy sites (-C-O-C-) and sp^3 hybridized form. Therefore, the displayed variation in raman spectra is as per the expectation. However, the question still arises about the functional nature of the oxidized sites. To investigate this, we further studied the electrode surface by XPS spectroscopy. Figure 1(c) represents the C1s XPS spectra for VG and MG. Oxygen containing carbonaceous groups are observed in the form of epoxide and hydroxyl (-C-O-C- and -OH) at 286.8-287.0 eV, carbonyl (-C=O) and carboxyl (-COOH) groups at 287.8-288.0 and 289.0-289.3 eV, respectively.[12,31] The carbon to oxygen ratio converted from 91: 5.3 in VG to 75:21 in MG. Compared with VG, a decrease in peak height for C=C bond and increases in peak heights for C-OH, -C-O-C and O=C-OH functional groups are observed in MG. Statistics reveals that the quantitative amount of hydroxyl, epoxy and carboxylic groups changes from 5.1, 1.4 and 2.5 %, respectively in VG to 17, 5.2 and 10 % in MG.

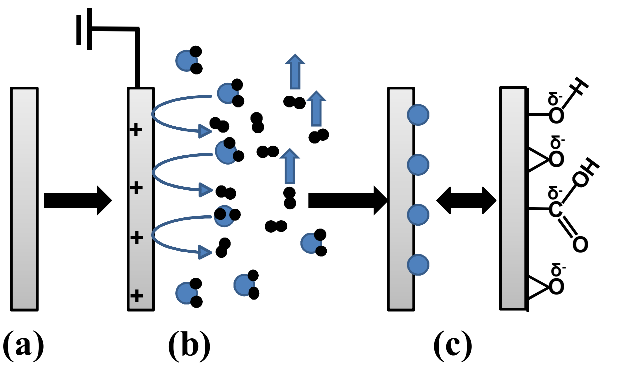

Figure 2. Schematic of Electrochemical oxidation process: (a) The virgin rod. (b) Polar water molecules results oxidation, when the rod is connected to positive terminal of DC voltage source. The associated chemical reaction is suggested as: $H_2O + 2C = H_2\uparrow$ + -C-O-C-.(c) The modified surface gains partial negative charge.

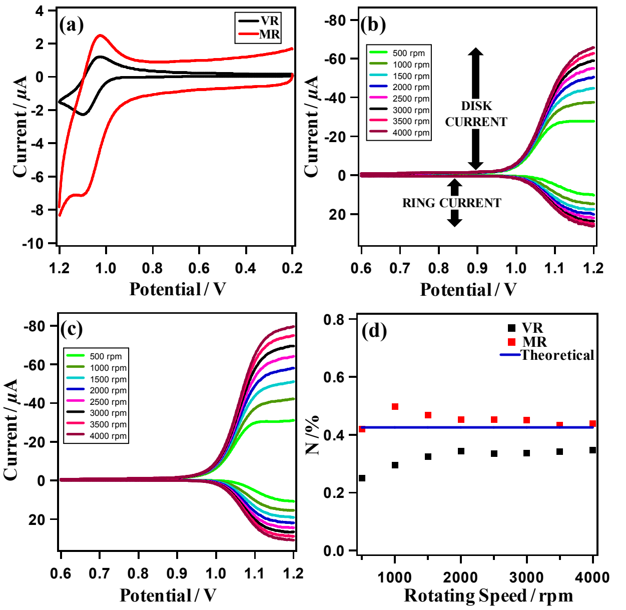

Figure 3. (a) CV of 1 mM $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl at scan rate 50 mv/sec. (b), (c) Voltammogram of 1 mM $Ru(bpy)^{2+}$ in 0.1 M KCl in variable rotational speed of electrode VR and MR, respectively. (d) The collection efficiencies (N%) with respect to rotation rate.

Therefore, almost a 3-4 fold increment of hydroxyl, epoxy and carboxylic functional sites during the electrochemical treatment confirm the successful oxygenation reaction at the graphite electrode surface. As the oxidation occurs at surface only, electrode MG can be considered as a graphite core coated by a very thin oxidized layer. We further compared this thin oxidized layer with GO and rGO, as GO is the oxidized form of exfoliated graphite. Figure S1 and S2 represents the XPS spectra of GO and rGO that we synthesized through reported method.[23] GO is abound with oxygenated groups, but in chemically reduced rGO these are almost ceased except some residual hydroxyl and carboxylic sites.[32] However, the intensity of functional sites for electrode MG lies between those for GO and rGO. Interestingly, this intermediate amount of oxidation is significant and can be considered as the key point of this experiment. The oxidized sites are necessary for the enhanced attraction of electrodes toward charged species or rGO mediator, whereas excessive amount of those is undesirable, as the oxide formation results increased capacitance and reduced conductivity.[12] We measured the surface conductivity for further justification of this issue. The I-V curve in Figure 1(d) shows that the surface conductivity has slightly decreased in modified electrode. In graphite surface, a conductive molecular orbital results from the linear combination of unhybridized p_z orbitals. After electrochemical treatment, some epoxy sites with sp^3 hybridized carbon atoms are generated at the surface. These sp^3 sites function for some break through within the conductive network, which is reflected by the lower conductivity of the modified electrode than the virgin sample. Considering the origin of enhanced dielectric value and reduced conductance of the oxidized surface, we were most careful to control the treatment time of the electrodes. As explained in the experimental section, an optimization was essential to decorate the electrode with significant amount of oxygenated sites (confirmed XPS measurement), but minimum capacitance (confirmed by CV). For bulk sampling of MG, after several times trial, we successfully scratched out the oxidized powders to measure the IR and PXRD spectra. In IR spectra (figure S3) the broad absorption peak around 3400 cm^{-1} shows the presence of oxygenated carbonaceous functional groups in electrode MG. In PXRD pattern (figure S4) the presence of broad peak around 11.34^O confirms the presence of intercalated oxygenated functional groups at the modified electrode. Finally, we propose Figure 2 as the mechanism of electrochemical oxidation.

3.2 Enhanced activity in RRDE experiment.

The surface analysis confirms the presence of oxygen containing functional groups on modified graphitic surface. If so, in comparison with virgin electrode the modified electrode is expected to show stronger electrostatic attraction towards positively charged species. We justified this possibility by monitoring the collection experiment using RRDE in $Ru^{2+/3+}$ couple. At first, the GC ring electrodes (VR and MR) were characterized by cyclic voltammetry as per the phenomenon we already explained for VG and MG. Figure 3 (a) represents the CV of $Fe(CN)_6^{3-/4-}$ recorded by using VR and MR as working electrodes. Similar to the case of MG, here also the MR electrode generates a CV with increased capacitance. Besides, this pattern matches with the previously reported graphehe/Au nanoparticles grafted on GC electrode.[33] These observations suggest the formation of oxygenated sites on GC ring surface. Figure 3b and 3c represents the voltammograms of collection experiments for $Ru(bpy)^{2+/3+}$ couple in KCl solution using VR and MR, respectively. Both the disk and ring current increase with respect to rotation speed and reflects the hydrodynamic migration of oxidized species from the disk to the ring. Besides, at all rotational speeds the disk and ring current generated by electrode MR is higher than those for VR. In Figure 3d, N% for MR is

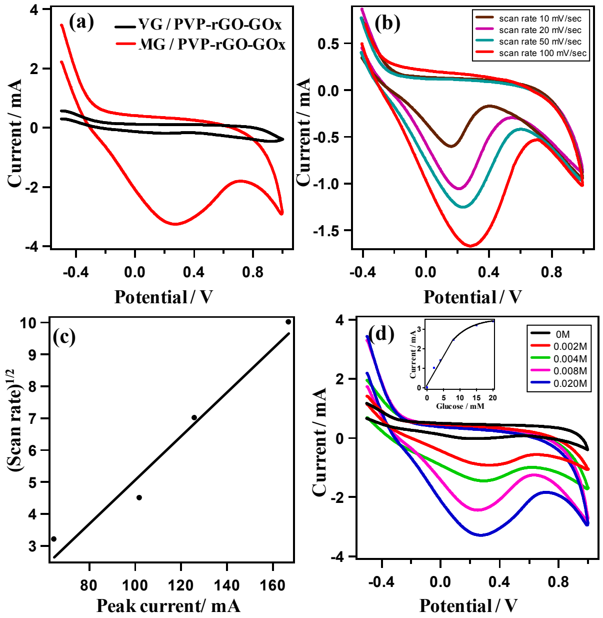

Figure 4. (a) CV of 0.02 M glucose solution at scan rate 100 mV/sec. (b) Cyclic voltammogram for 0.05 M glucose solution at various scan rates. (c) Peak current Vs (scan rate)$^{1/2}$ for 0.005 M glucose solution. (d) Concentration dependent cyclic voltammograms at scan rate 100mV/sec.

is interpreted to have higher accuracy than that for VR. The RRDE experiment involves sequential electron transfer phenomenon, and is usually applied to monitor redox reaction kinetics and efficiency of electron transfer process. In a typical RRDE experiment, the oxidized species generation rate at the disk oxidizer and the collection rate of those at ring reducer is not necessarily the same, and this variation depends on the structure of the electrode. The theoretical N% can be derived according to the mathematical model proposed by Levich.[34] However, in a kinetic study, the real collection efficiency is calculated from the ratio of ring current and the disk current.[18,19,35] The N% is usually constant with respect to rotation rate, and for a simple reversible redox reaction it depends on the geometry of the electrodes only.

In our experiment, initially the Ru(bpy)$^{2+}$ ions exist in reduced form. At the disk electrode it is converted to the oxidized form as Ru(bpy)$^{3+}$, and the disk current is generated. The hydrodynamic movement resulting from rotation of RRDE, sweeps Ru(bpy)$^{3+}$ ions from the disk to the ring, and the ring current is resulted from the reduction of Ru(bpy)$^{3+}$ back to the Ru(bpy)$^{2+}$ ions. Using the calculation method described by Albery et al the theoretical N% of VR and MR was calculated as 0.424.[36] Figure 3(d) reveals that for electrode MR, N% lies closer to the theoretical line. Besides, MR represents values of N% with higher precision, and express better agreement with the rotation independent collection efficiency. In comparison to virgin GC ring electrode, the oxygenous functionalized GC ring exhibits stronger electrostatic attraction toward positive ions. The migration rate of oxidized specie is therefore accelerated, and an increased value for N% with better accuracy and precision is resulted.

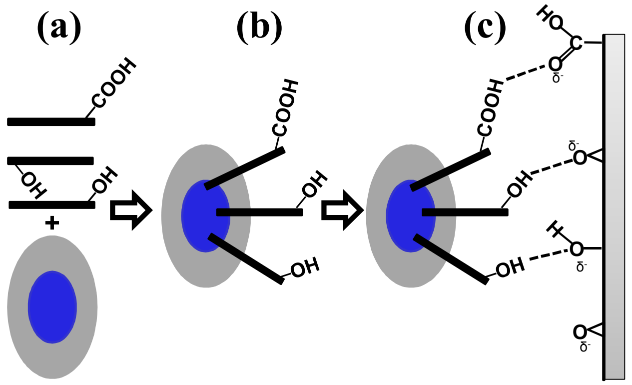

Figure 5. Perspective mediator-electrode interaction. (a) Mixing up GOx with PVP-rGO having some residual functional sites. (b) The tinny rGO fragments establish electrical communication between the active centre (FAD) of GOx and the electrode. (c) Electrostatic interaction between oxygenated graphite rod and PVP-rGO mediated to GOx.

3.3 Enhanced electrode-mediator interaction.

So far, due to the partial negative charge attributed by oxygenated functional sites, the modified GC ring electrode shows increased affinity for charged species ensuring better application for electrokinetic study. In this phase, we have justified the activity of the electrode for capturing mediator. For a model representation, we choose PVP protected rGO mediated to the enzyme GOx. The mediator rGO was produced by reduction of GO in a milder condition than conventional methods.[32] Prior to the immobilization of PVP-rGO-GOx, MG was found to be reversible in $K_3Fe(CN)_6$ solution with KNO_3 as supporting electrolyte (Figure S5). Figure 4(a) represents the cyclic voltammogram of 0.020 M glucose solution in PBS. Electrode VG is irresponsive, and fails to produce signals corresponding to the oxidation of glucose. This observation confirms the absence of electrical communication between the mediator and virgin electrode surface due to unsuccessful immobilization of PVP-rGO-GOx layer on the inert surface of VG. In reverse, electrode MG responds significantly. The oxidation peak near 0.28 V implies that the MG-PVP-rGO–GOx electrode assembly is working for glucose sensing. In Figure 4(b), the 1.69 mA peak height corresponding to glucose (0.005 M) oxidation at 100 mV/sec scan rate decreases gradually at lower scan rates. The linear line for current verses root scan rates (Figure 4c) confirms that the electrode assembly is reversible and utilizable for glucose detection. Figure 4(d) represents the potential verses peak current for electrode MG in glucose solutions of different concentration at ambient condition under oxygen bubbling. A typical full scan CV in a wide potential window is presented in supporting information (Figure S6).

GO and rGO has been reported to function as mediators in biosensor.[20,21] In place of rGO we used PVP-rGO as it is dispersible in aqueous media and can be processed easily. In addition, PVP doesn't interfere in electrical conductivity of rGO. The tiny sized PVP-rGO fragments establish electrical communication between the active centre (FAD) of GOx and the electrode surface. To study the electrochemistry of biological materials, electrodes are usually connected to the active centre of

GOx through leaking the non conductive protein polypeptide chain by conductive nanomaterials. Functional carbon nanotubes are the most common one dimensional materials for such purpose.[37,38] Some two dimensional materials have also been used successfully.[39,40] Recently, the graphene materials have also been reported to be used as conductive mediator.[20,21,27] The rGO is reported to contain some residual functional groups (-OH and -COOH), which we also have noticed in our sample (Figure S2). Moreover, to ensure the presence of residual polar groups we reduced GO in milder condition (used relatively small amount of reducing agent with lower reduction time).[32] Therefore, the PVP-rGO-GOx could be attached to electrode MG by electrostatic bonding between the residual functional sites of rGO, and the polar oxygenated sites of the electrode surface. A perspective mechanism for such interaction is presented in Figure 5. The intermolecular interactions are highly dependent upon the solution pH. The residual polar sites of rGO might tend to form bond with the excessive concentration of hydroxyl ions. To avoid this possibility we buffered the solution at pH 7.0. In this condition the modified electrode stands as a model substrate to anchor rGO mediator with increased strength.

GOx immobilization on solid electrode is usually suffered by poor electrical communication between the active site of the enzyme and the electrode.[41,42] But using electrode MG, we successfully developed stronger mediator-electrode attachment with better electrical connectivity. The peak current is almost linear (inset of Figure 4d) up to 0.008 M. For higher concentration the linearity reduces. On the other side, the lowest concentration that we could measure with distinguishable peak is 0.0005M. At extremely dilute solution the electrode could produce distinguishable signals, but the detection is rendered as the cyclic voltammogram becomes diffused along with the shifting of peak position to higher voltage. As a result, at present time the detection efficiency of electrode MG is comparatively lower than some recently reported glucose sensor.[25,43] Nevertheless, the most important feature of this report is, the increased electrode-mediator interaction. The current observation suggests the possibility of functionalized graphite surface as a new platform to construct charged carbon based substrate for detection of biological analyte.

4. Conclusions

In this work, we investigated the increased activity of electrochemically oxidized graphite rod and glassy carbon RRDE. In a controlled manner the surfaces of these carbon based materials were functionalized by oxygenated functional groups through facile, quick and reproducible method. The increased capacitance measured from cyclic voltammetry implies the chemical change at the surface. Raman spectra imply the generation of sp^3 hybridized carbon atoms by breaking some of the graphitic sp^2 network. The XPS spectra confirm the presence of oxygenated functional sites on the modified electrode surface. In addition, the measurement of surface conductivity reveals that the chemically modified surfaces have some break through within the conductive sp^2 network of graphite, which is attributed by the formation of some sp^3 carbon sites. The IR and PXRD spectra also confirm the oxidation process. The effect of negatively charged oxygenated sites on ion collection was evaluated by electrodynamic study using RRDE. In comparison with an untreated RRDE, the modified RRDE exhibited higher N% for Ru^{3+} ion produced at the disk oxidizer. The accuracy and precision for the values of N% obtained by using modified RRDE was also higher. In case of modified RRDE, the enhanced negative charge at the oxidized surface of glassy carbon ring is responsible for faster movement of oxidized species, and the increased value of N%. Finally, the modified graphite rod was utilized for direct attachment of mediator impregnated GOx. Compared with the virgin graphite rod, the modified rod exhibited stronger affinity toward PVP-rGO mediator. It is supposed that the tiny PVP-rGO fragments mediates electrons through the protein polypeptide chain surrounding the core of GOx enzyme, and the residual functional sites of rGO could make good electrical communication with the modified electrode surface through strong electrostatic interaction. At present time, the sensitivity of the electrode is low, but the control experiment indicates that a much stronger analyte-electrode binding is attainable by using electrochemically modified graphite electrode. We suppose that in future this surface modification technique might be useful to recover the appeal of once elusive carbon based electrode materials for their better application in electrokinetic study or sensing biological analyte.

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† *Electronic Supplementary Information (ESI) available: The synthetic procedure for GO; XPS spectra of GO and rGO; IR and PXRD spectra of electrode VG and MG; CV of electrode MG in Fe$^{2+/3+}$ couple; and a full scale CV in wide potential window using electrode MG -PVP-rGO-GOx assembly as working electrode.*. See DOI: 10.1039/b000000x/

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Text for table of contents:

Carbon electrode's affinity for charged species and reduced graphene oxide mediator increased significantly after its oxygenous functionalization by controlled anodization.

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