

Electrochemical sensing of biomolecules using nanomaterials-based electrodes

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Summary

This project aims to investigate the ability of graphene, which is a single sheet of carbon atoms, to detect glucose effectively in solutions with and without ascorbic acid (Vitamin C). This could prove to be a valuable and interesting alternative to the glucose biosensors available currently, which use enzymes to break glucose down and convert the energy released into an electrical signal. This relatively-new material is more stable and can function under a larger range of conditions compared to the highly unstable enzymes. The success of this project also opens up a range of other potential applications for graphene electrodes.

Abstract

Electrochemical biosensors based on graphene layers are described in this paper. Characterization of graphene electrodes is performed using cyclic voltammetry (CV) and compared with conventional glassy carbon electrodes, graphite paste as well as carbon nanotube paste electrodes with respect to its sensitivity and signal-to-noise (S/N) ratio among other factors. The results show that graphene electrodes have a much higher sensitivity attributed to the significantly lower background noise, which in turn improve the S/N ratio of detection. Further experimentation with the graphene electrode revealed that it was capable of non-enzymatic glucose detection, and interference such as the presence of ascorbic acid did not affect its ability to detect glucose concentrations in solution.

Introduction

Biosensors in lab-on-chip (LOC) devices often rely on the use of micro-electrodes or nanowires developed from nanomaterials, and they have had applications in many fields ranging from detection of river water contaminants in environmental science to monitoring blood glucose levels in diabetes patients.

They can directly measure the analyte in the sample, and are capable of responding continuously and reversibly without perturbing the sample. By combining the sample handling and measurement steps, the need for sample collection and preparation is eliminated. Furthermore, biosensors can be used in coloured or turbid media without interference from the many absorbing and fluorescing compounds in biological samples.

In the past decade, carbon nanotubes (CNTs) have been explored and tested for electrochemical sensing of electro-active biomolecules, including DNA with the use of oligonucleotide probes [1] or without [2], uric acid in the presence of ascorbic acid [3], and glucose in the presence of uric and ascorbic acid [4].

Recently, graphenes, flat monolayers of carbon atoms in a two-dimensional honeycomb lattice, are new nanomaterials that may have desirable properties for LOC device applications, including but not limited to non-enzymatic glucose detection in biological samples. Graphene exhibits exceptionally high crystal and electronic quality, in which charge carriers (massless Dirac fermions) can travel thousands of interatomic distances without scattering, but was only discovered recently in 2004 [5], leaving lots of room for exploration of its distinct properties.

An ideal biosensor should have a high effective electrode area, a small peak difference (which indicates higher electrode efficiency; ideal value is 59mV), and most importantly, a high S/N ratio for effective determination of substrate presence and concentration, and these three criteria will be used to evaluate the performance of the various electrodes.

Hypothesis

Based on the reported properties of graphene, it is hypothesized that graphene would have a significantly better performance as compared to conventional glassy carbon electrodes based on the above criteria.

Objectives

The aim of this project would be to assess the performance of graphene electrode for detection of a range of bio-molecules in comparison to those of different types of conventional electrodes.

Materials & Methods

Six different types of electrodes were obtained for this project. The conventional glassy carbon (GC) electrode (CH Instruments) was obtained commercially, the graphite/GC and MWNT/GC electrodes obtained by pipetting a solution of graphite and multi-walled carbon nanotubes (MWNTs) onto the surface of the GC electrodes respectively. The graphite and MWNTs were mixed with mineral oil in a w/w ratio of 4:6 to create the graphite paste and MWNT paste electrodes. The graphene electrode was prepared by electrically connecting the graphene sensing element (i.e. graphene sheets grown on a silicon carbide substrate) onto an electrode holder using silver paint.

CV was carried out with an electrochemical workstation (CHI 660a) to characterize the performance of the various electrodes, using 0.5 mM $K_3Fe(CN)_6$ dissolved in 1.0 M KCl solution as a basis for comparison. The redox behaviour of $[Fe(CN)_6]^{3-/4-}$ is well-studied and therefore commonly used as the redox probe for many electrochemical experiments. The setup is a 3-electrode cell, with the six different electrodes used as the working electrodes, a Ag/AgCl reference electrode and a platinum counter electrode. The GC electrodes were prepared via polishing with alumina powder (CHI Gamma Alumina Powder 0.3 and 0.05 micron), and sonicated (Bransonic Ultrasonic Cleaner 2510E-MTH) for one minute in deionised water (from Millipore system), and then for one minute in 70 % ethanol. The rest of the electrodes had their surfaces regenerated by running a series of CV scans (20 to 60 scans) in 0.5 M H_2SO_4 .

The electrodes were then tested for the non-enzymatic detection of glucose and ascorbic acid. 30 mM glucose solution was prepared in a 0.1 M NaOH supporting electrolyte from a 2.0 M glucose stock solution. CV measurement was subsequently conducted on glucose detection by the six types of electrodes. The CV measurement was conducted on a similar concentration of ascorbic acid by the graphene electrode, and its ability to measure varying glucose concentrations was then investigated in a solution containing a mixture of ascorbic acid and glucose in NaOH.

Results & Discussion

Characterization in $K_3Fe(CN)_6$

From a typical CV curve of a GC electrode in $K_3Fe(CN)_6$ solution (Appendix I), the calculation of electrode area can be obtained by measuring the value of the anodic peak current (Appendix II) and substituting it into the Randles-Sevcik electrode equation for reversible reactions (Appendix III). However, in this experiment, the effective area of the electrodes was obtained by measuring the current response over a range of scan rates of different voltages, manipulating the equation by plotting I_{ap} against $v^{0.5}$, and calculating the resulting gradient, as shown in Fig. 1. There was only one reading for graphene due to the limited number of graphene samples.

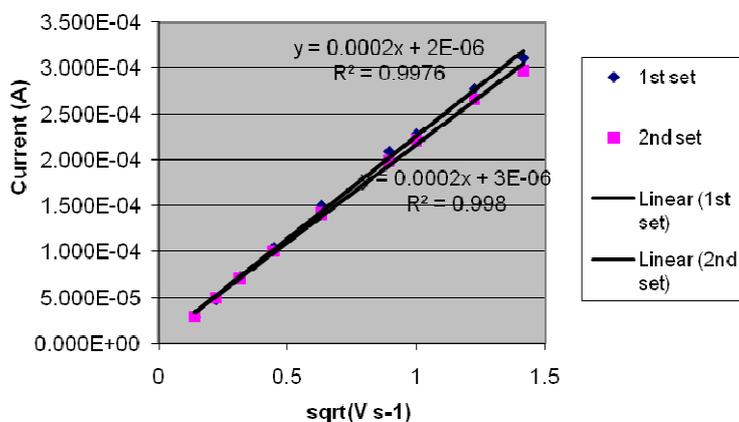


Figure 1: I_{ap} against $v^{0.5}$ graph of a GC electrode

Table 1: Summary of the effective surface area of the different types of electrodes

Type of electrodes	Average Area \pm standard deviation ($\times 10^{-2} \text{ cm}^2$)	Replicates
GC	5.79 ± 0.00	6
graphite/GC	5.79 ± 0.00	3
MWNT/GC	21.22 ± 0.02	3
graphite paste	1.64 ± 0.01	6
MWNT paste	2.08 ± 0.01	5
Graphene	6.23	1

Table 1 shows that graphene has an effective detection area second only to that of the MWNT/GC electrode. MWNT and graphite paste electrodes have a significantly lower effective surface area as compared to the adsorbed form. This may be because they are aligned randomly as they are mixed with mineral oil and compacted into paste form, hence drastically reducing the surface area to volume ratio. For comparison among electrodes with different surface area, the current responses of each electrode were normalized by the respective electrode surface area. Figure 2 shows the resulting current density plots, where the current density graphs of the paste electrodes are omitted due to their much lower current density. From Fig. 2, we can see that MWNT/GC and graphene have around the same current density, indicating that the performance of graphene would be comparable to the highly-conductive carbon nanotubes.

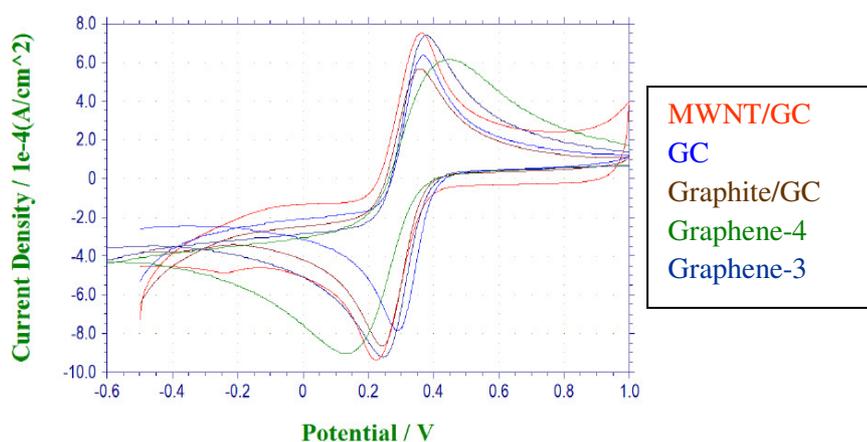


Figure 2: Cyclic voltammograms of the electrodes in 5ml of 5mM $\text{K}_3\text{Fe}(\text{CN})_6$

The difference in the anodic and cathodic peaks' potential (ΔE_p) were also measured and compared to the Nernstian value expected for an ideal one-electron reversible redox reaction for an ideal surface electrode, which is 59 mV. Table 2 shows that graphite paste is the most efficient at conducting a response in the electrolyte to the sensor, possibly due to its superior contact with the surface of the electrode that the graphite was adsorbed on. This also indicated that improvements could be made on optimizing the protocol for the attachment of the graphene layer to the transducer surface so that the transfer of the signal to the electrode is more efficient.

Table 2: Summary of the ΔE_p values of the different types of electrodes

ΔE_p (mV)	Mean \pm standard deviation	Replicates
GC	93.74 ± 0.01	19
graphite/GC	110.13 ± 0.02	8
MWNT/GC	149.17 ± 0.02	6
graphite paste	82.56 ± 0.01	9
MWNT paste	201.50 ± 0.21	10
graphene	171.83 ± 0.08	6

The S/N ratio of the electrodes were obtained by running a CV scan in $\text{K}_3\text{Fe}(\text{CN})_6$ solution (Fig. 3), where the signal at the peak is expressed as a ratio to the baseline signal. Successful detection should return a S/N ratio of more than 3; the sensitivity of a biosensor is proportional to the S/N ratio of detection.

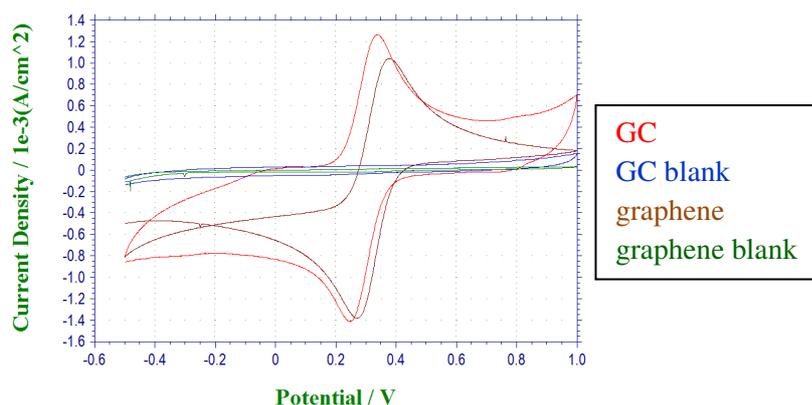


Figure 3: Cyclic voltammetry curve of graphene and GC electrodes in 5 ml of 5 mM $K_3Fe(CN)_6$ overlaid with CV curve in 1.0 M KCl blank

Table 3: Summary of the S/N ratios of the different types of electrodes

S/N	Mean \pm standard deviation	Replicates
GC	66.5 \pm 41.5	6
graphite/GC	7.18 \pm 1.44	7
MWNT/GC	5.20 \pm 0.22	3
graphite paste	45.7 \pm 4.50	3
MWNT paste	8.37 \pm 0.71	3
graphene	59.3 \pm 17.6	5

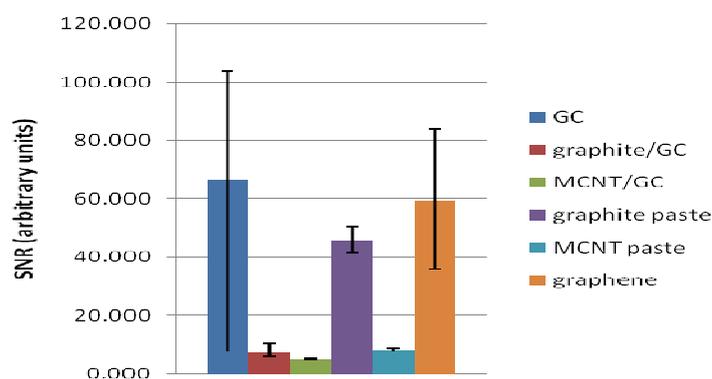


Figure 4: Chart of the S/N ratios with maximum-minimum error bars

From the results in Table 3 and Fig. 4, we can see that the commercially available GC electrodes as having the highest S/N ratio, followed by graphene. However, the GC electrodes vary a lot in their S/N ratios, as can be seen in the large error bar and standard deviation value. This indicates that it produces inconsistent results, and hence may be unreliable as a biosensor in large scale practical applications such as screening. Graphene also has a high S/N ratio, but it also has a large error bar despite being smaller than the GC electrode. This maybe be attributed to the fact that detection conditions and protocol for assembling the graphene electrode have not been optimized, accounting for the relatively inconsistent readings.

Efficiency in glucose detection

Cyclic voltammetry testing was conducted subsequently to determine the ability of the graphene electrode to detect glucose without the action of enzymes. (Appendix IV)

Table 4: SNR of 4 electrodes in 1.5 mM glucose with 0.5mM NaOH supporting electrolyte

Type of electrode	S/N \pm standard deviation	Replicates
MCNT/GC	1.02	1
graphite paste	1.24 \pm 0.20	3
MCNT paste	1.23	1
Graphene	9.97 \pm 2.8	3

Table 4 shows the SNR of detection in glucose for the different electrodes. For the S/N testing, graphene outperformed 3 other electrodes by a significant margin, being the only electrode that could register a SNR larger than 3. All the other electrodes exhibited high absolute responses, but also high baseline noise, resulting in poor sensitivity to the addition of glucose.

A further experiment was conducted to investigate the specificity of glucose detection by the graphene electrode in solutions containing ascorbic acid, which is commonly found in human urine, hence it would be a good preliminary test for the potential application of the graphene electrode as a biosensor for the detection of diabetes. Ascorbic acid and glucose are oxidised at different voltages, 0.2 V for the former and 1.0 V for the latter, although ascorbic acid does increase the glucose peak to a certain extent (Appendix V).

After determination of the graphene electrode's ability to differentiate between glucose and ascorbic acid in solution, the experiment was repeated by using a blank with ascorbic acid to investigate the effect of cumulative glucose addition on the magnitude of the response displayed. From Fig. 5, it can be seen that the glucose peak (or the "tail-end" of the CV graph) increases progressively with increasing glucose concentration in the solution.

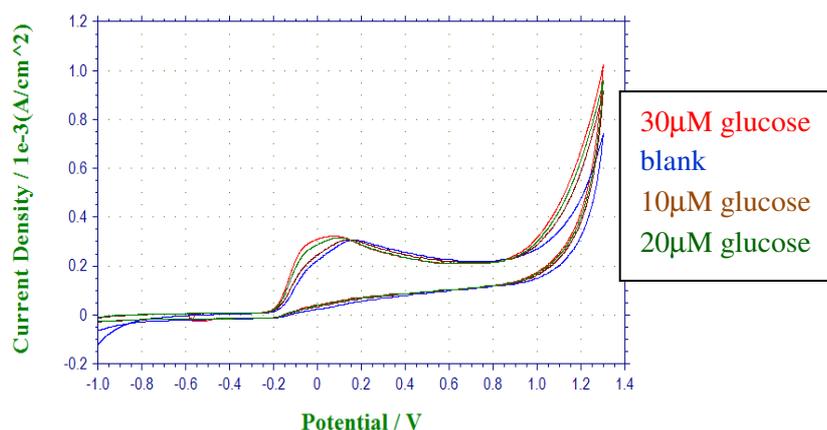


Figure 5: CV in 0.5mM NaOH + 10 μ M ascorbic acid solution blank with 3 cumulative additions of 10 μ M glucose solution

Subsequently, an amperometric curve (Appendix VI) was obtained by measuring the current response of graphene sensor towards glucose at a constant potential of +1.0V. The amperometric curve facilitated the plotting of the calibration curve (Fig. 6), where the current density can be read off more easily in a linear graph as compared to a stepwise progression of the amperometric curve.

From the calibration curve, we can see that the sensor can respond linearly to the amount of glucose, and with the calibration plot, it can be used to detect and determine the concentration of glucose in unknown samples.

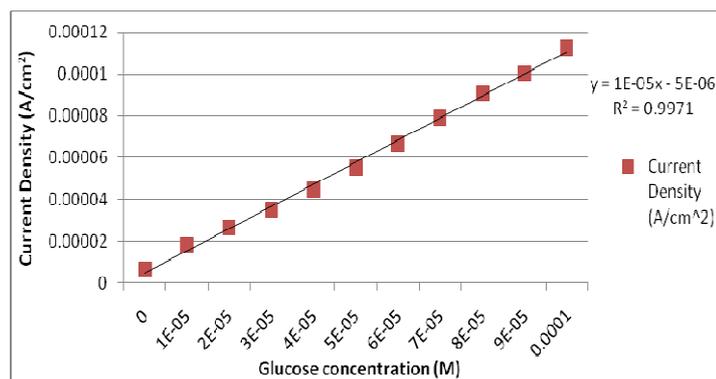


Figure 6: Linear calibration curve of graphene electrode in 0.5mM NaOH blank with cumulative addition of 10µM glucose solution at +1.0V

6 CONCLUSIONS

In conclusion, the test results show that the graphene electrode, as predicted in the hypothesis, does indeed have potential applications as a biosensor for diabetes detection due to its specificity and good S/N ratio. The non-enzymatic approach as compared to conventional glucose biosensors with glucose oxidase enzymes also offers it an enhanced range of applications.

Further tests could be conducted to test its ability to differentiate glucose in a solution also containing more biomolecules such as uric acid and other mono- and di-saccharides, as well as to possibly optimize the detection conditions. Extensions in other areas include detection of DNA without adsorbed or conjugated DNA probes for the detection of free ribonucleotides, which may also yield interesting and exciting results for new applications of this new nanomaterial.

ACKNOWLEDGEMENTS

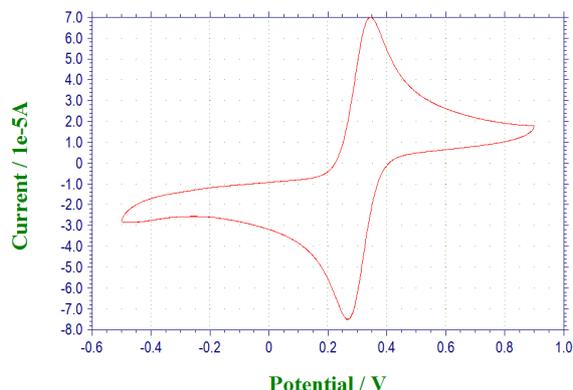
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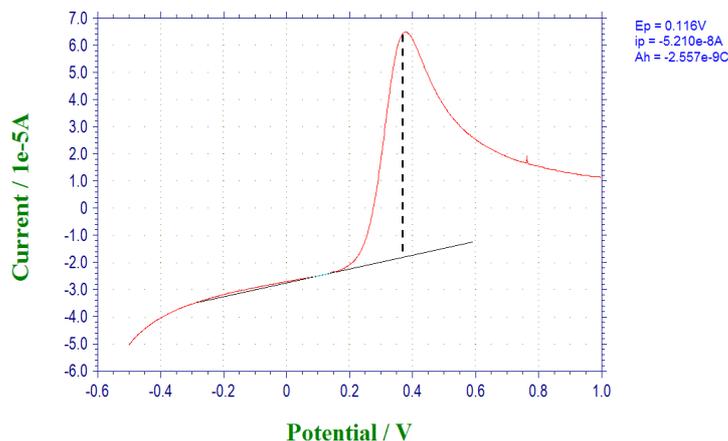
APPENDICES

Appendix I: Typical cyclic voltammetry curve in potassium ferricyanide



Appendix I shows a typical CV curve in potassium ferricyanide, where there are two segments, with the top segment being the anodic peak for oxidation, and the bottom segment the cathodic peak for reduction.

Appendix II: Determination of anodic peak current



The program was used to calculate the anodic peak current both for the determination of the effective electrode area as well as calculating the S/N ratio.

Appendix III: Randles – Sevcik equation

$$I_{ap} = 0.4463nFAc(nF/RT)^{0.5}D^{0.5}v^{0.5}$$

Where I_{ap} = anodic peak current, n = electrons per molecule reaction, F = Faraday constant, A = electrode area, c = concentration of ions, R = gas constant, T = temperature (in K), D = diffusion constant, v = scan rate.

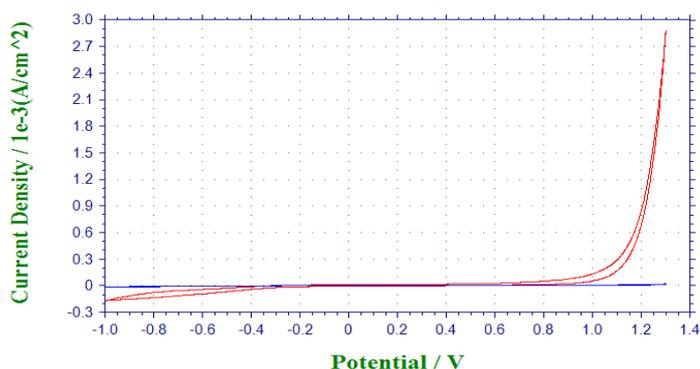
Which can be simplified into:

$$I_{ap} = 2.69 \times 10^5 \times n^{1.5} \times A \times c \times D^{0.5} \times v^{0.5}$$

Using the following values:

$n = 1$, $F = 9.65 \times 10^4 \text{ C mol}^{-1}$, $c = 5.00 \times 10^{-6} \text{ mol cm}^{-3}$, $R = 8.31 \text{ J K}^{-1} \text{ mol}^{-1}$, $T = 298\text{K}$, and $D = 6.1 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$

Appendix IV: CV curves of graphene in glucose and NaOH

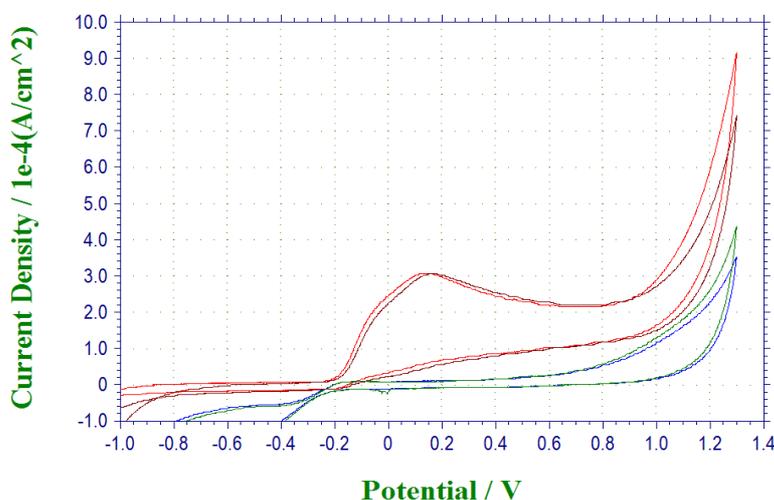


graphene in
1.5mM glucose +
0.5mM NaOH

graphene in
0.5mM NaOH
blank

Appendix IV illustrates the anodic oxidation peak of glucose at +1.0V as compared to the blank.

Appendix V: Illustration of different oxidation peaks



10µM ascorbic acid
+ 10µM glucose

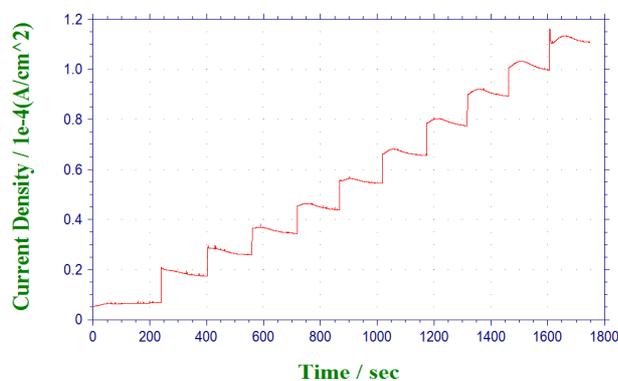
0.5mM NaOH blank

10µM ascorbic acid

10µM glucose

Appendix III shows the cyclic voltammetry curves of 0.5mM NaOH blank and individual reagents (either ascorbic acid, glucose or both), where it is evident that the ascorbic acid and glucose peaks start at +0.2V and +1.0V respectively. This is important because the appearance of the oxidation peak at different voltages indicates the possibility of differentiation in a solution containing these two reagents by fixing the applied voltage.

Appendix VI: Amperometric curve of graphene electrode in glucose



The figure shows the amperometric curve of the graphene electrode with cumulative additions of glucose represented by the vertical rise in current density at various points such as 250s.