Decoration of Graphene with Metal Nanoparticles and Its Application for Bioanalysis

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Decoration of Graphene with Metal Nanoparticles and Its Application for Bioanalysis

by

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Abstract

Graphene is a two-dimensional monolayer of tightly packed $sp^2$ hybridized Carbon atoms. Formed from graphite, the theoretical surface area of the monolayer is about 2630 m$^2$/g, and the initial formation of graphene oxide from graphite oxide introduces a variety of functional groups, such as $-COOH$, $-C=O$, and $-OH$, on its surface. The high surface area and the rich presence of functional groups, along with the potential formation of $sp^2$ bonding networks within graphene oxide, all serve as critical factors allowing for additional chemical modifications. There are extensive research attempts being performed to explore and manipulate its shape, composition, and physical properties, rendering graphene as one of the most prominent and studied nanomaterial to date. It has been widely regarded as an incredibly strong and durable material, and as a good thermal and electrical conductor, can potentially replace silicon in its wide variety of applications. In addition to applying the conductive properties, graphene is also being considered as biodevices, such as biosensors, DNA sequencing components, and anti-bacterial hygiene product. The purpose of this research was to modify graphene oxide utilizing electroless gold plating techniques, and determine its viability in various applications. Previous research has shown that graphene oxide can provide reactive sites for nucleation and propagation of metal nanoparticles, due to the oxygen functionalization at the surface of graphene sheets. The electroless gold plating technique involved treating graphene oxide with a series of metal solutions, initially with $Sn^{2+}$, then with $Ag^+$, and finally with $Au^{3+}$, for a serial reduction of metals on its surfaces. The resulting graphene oxide product was then inspected with SEM and TEM instruments. Further investigation of its conductivity with electrochemistry techniques explored its variety of potentials, such as an electrode material for sensors, batteries, fuel cells and supercapacitors, as well as a supporting catalyst in chemical catalysis reactions, and a building block for new nanocomposites. In addition, the graphene oxide was considered as a potential biosensor, with its capability of detecting $H_2O_2$ as a silver nanoparticle deposited composite.
Keywords: Graphene Oxide, Electroless Metal Deposition, \( H_2O_2 \) Sensor
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A Thesis of Master Degree

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1. Introduction

1.1 Graphene

Graphene is a carbon nanomaterial receiving immense interest due to its unique properties and potential applications. It is a two dimensional sheet of tightly packed sp²-hybridized carbons arranged in a honeycomb lattice. Graphene is the fewest layer limit of graphite, in turn serving as building blocks for graphitic materials in other dimensionalities. In its 0 D form, graphene can be wrapped into forming fullerene, while also capable of forming its 1D nanotube structure when rolled, and can even be stacked to form 3D graphite. While graphite as a mineral has been known for about 500 years, investigation into graphene has been ongoing for only around 40 years, since it was mostly presumed to be only a theoretical model, as the formation of a 2 D material were thought to be thermodynamically unstable and thus inconceivable to exist in its free state [1]. However, with the discovery of isolated single-layer graphene sheet from graphite, by Giem and co-workers at Manchester University in 2004 [2], the investigation of graphene has rapidly become one of the most prominent areas of research in the fields of chemistry, physics, and material science. The extensive studies into graphene have produced many characteristic values of its properties, such as the large theoretical surface area at 2630 m²/g, high intrinsic carrier mobility of 200,000 cm²/ν-s, high Young’s modulus of around 1.0 TPa, thermal conductivity of around 5000 W/m·Km, and optical transmittance readings of around 97.7 % [3]. The qualitative analysis of graphene simply warrants further exploration into its boundless potential uses as a novel material in the realm of scientific research and technological advancements.
1.2 Graphene Oxide and Reduced Graphene Oxide

A simple method of graphene preparation involves the oxidation of graphite into graphite oxide. The process can be achieved either by the Brodie, Staudenmaier, or Hummers methods. Brodie and Staudenmaier methods encompass the usage of potassium chlorate (KClO₃) with nitric acid (HNO₃), while the Hummers method utilizes
potassium permanganate (KMnO₄) and sulfuric acid (H₂SO₄). Variations of such techniques have also been reported to achieve the desired results [3]. The oxidation of graphite introduces various oxygen containing functional groups onto its surface, such as hydroxyl, epoxide, carbonyl and carboxyl groups, rendering the resulting graphite oxide to be more polar and hydrophilic. Graphite oxide then can be easily exfoliated in polar solutions such as water, forming colloidal suspensions of graphene oxide (GO) sheets [4].

The oxidation of graphite and its subsequent GO formation is necessary as Reduced Graphene Oxide (RGO) tends to aggregate irreversibly in polar solutions, due to π-π stacking interaction of atomic orbitals and strong van der Waals interactions [5-6]. While the introduction of many oxygen functional group electrically insulates GO, rendering it nonconductive, the negative surface charges can be used to construct multilayer thin films of GO based on electrostatic interactions, and also allows its incorporation into polymers, ceramics, and metals for diverse applications. The restoration of conjugated networks and conductivity of GO can be established with the reduction of GO into RGO, utilizing chemical [6], thermal [7], electrochemical [8], photocatalytic [9], biomolecular [10], or bacterial agents [11]. This process reinstitutes viability of graphene to be incorporated into myriad of electrical devices.
Figure 2

(a) Discerning views of Graphite and Graphene. (b) Graphene Oxide (Go) is derived from the oxidation of graphite, and can subsequently be reduced back to graphene as Reduced Graphene Oxide (rGo) in restoring its conductivity.

1.3 Graphene Research

The current research into graphene can be categorized into two broad areas. First is the large-scale production of single layered graphene sheets, and the other is examinations into properties of graphene for targeted potential applications. As graphene is prepared from graphite, production research into graphene involves the optimal method for obtaining individual sheets of graphene from graphite. Currently, there are only a limited number of methods proposed in achieving this objective. The best method to date is the mechanical exfoliation as exemplified by the "Scotch Tape method" from Giem and coworkers [2] involving the ‘Scotch-tape’ method (Figure 3), but alternatives also include chemical, electrical, or thermal exfoliation of graphene in various solvents as previously discussed, and the bottom-up approach of either directly growing graphene from organic precursors or chemical vapor deposition on inorganic surfaces [3,5-6]. Each of these techniques entails its own drawbacks, as while the method of solvent exfoliation generates the best approach to obtaining large scale quantities of graphene, it does not completely uphold the stability of its crystal lattice structure. The mechanical exfoliation of ‘Scotch-tape’ method still produces the best qualities of single layer graphene, yet it is a non sized controlled method on a small scale [5-6].
Figure 3

Various properties of graphene offer exciting course of application in numerous settings. The large surface area, capacity for high thermal and electrical conductivity, high carrier mobility, and its tensile strength all offers the potential of graphene as capable supercapacitors [12], batteries [13], fuel cells [14], and solar cells [15]. The doping of graphene [16] and decorations with metal nanoparticles increases the surface area and conductivity of the nanomaterial [17], rendering graphene as excellent chemical or electrical sensors [18-19], catalysts for chemical reactions [20], and building blocks for nanocomposites [21]. Functionalization with biological molecules can transform graphene into biological sensors [22], biomechanical devices [23], and bioreactors [24]. Modifications of graphene, such as covalent attachment of polyethylene glycol polymers,
or pegylation, increases the solubility of graphene in buffers and serums without agglomeration, introducing the possibility of graphene as targeted drug delivery system for water insoluble drugs, while also allowing cellular imaging due to graphene’s intrinsic photoluminescence [25]. Graphene has also been presented with antibiotic activity, due to the sharpness of the edges on graphene nanosheets and the charge transfer between the bacterial and the nanomaterial, damaging the bacterial cell membrane [26]. The adsorptive properties of graphene may also be utilized in environmental applications, in isolation and transport of organic contaminants [27].

Figure 4

Figure 4. Various modifications of graphene. (a) Doping of graphene with Nitrogen increases its surface area and conductivity. Graphene can also be functionalized (b, c, and d) through attachment of various molecules to serve as chemical or biological sensors, catalysts for chemical reactions, building blocks for nanocomposites, bioreactors, and drug delivery systems.
1.4 Metal Deposited Graphene

Metal-graphene hybrid materials have received much attention due to their potential applications in fuel cells, batteries, and chemical or biosensors [13-14,18-19]. The decoration of Go with metal nanoparticles have shown to increase its electrical, thermal, mechanical, optical, and catalytic properties [17]. Gold graphene oxide nanocomposites (Au-Go) have been of particular interest, as being an inert metal, it is suitable in many biological and electrochemical applications [28-29]. Previous attempts for synthesis of metal nanocomposites utilized various electrochemical, chemical vapor, thermal, and polymer incorporated approaches [30]. Yet, current methods apply a solution based electroless deposition of metal nanoparticles, such as gold, to obtain the metal-graphene hybrid. While many of the previous models used in the production of the hybrid employed a perpetual reducing agent, the electroless deposition technique is performed in the absence of an external reducing agent, as the reducing electrons are provided by the lattice of Go. The controllable synthesis process permits the homogeneous size distribution of metal nanoparticles, resulting in the clean deposition of the metals with large facial areas [31].
Figure 5

Decoration of graphene with metals such as gold nanoparticles offer potential applications of graphene nanocomposites in various biological and electrochemical settings.

The electroless deposition of gold process exploited in this experiment was designed by Ahn and coworkers [30]. Although the method was designed for Au plating on island and thin films [32], the experiment carried out in this research will use Go as a substrate for the subsequent production of Au-Go hybrids. The technique employs a series reduction of metals on the Go surface, initially with the treatment of Sn$^{2+}$ solution, then with a solution of Ag$^+$, and finally a solution of Au$^+$, all utilizing the electrons from the Go substrate to reduce the metals onto its surface. The resulting process produces three different metal deposited samples: Sn-Go with deposits of Tin(II) nanoparticles on Go surface, Ag-Go with Ag nanoparticles on Go, and Au-Go with Au nanoparticles.
Electroless Gold Deposition Technique

Figure 6. (a) A pictorial representation of the electroless gold deposition technique. (b) A detailed view of the electroless gold deposition process. With the initial formation of Sn-Go through the attachment of Sn\(^{2+}\) atoms onto Go surface during the treatment of aqueous Go with 0.026 M SnCl\(_2\) solution, the addition of 0.029 M ammonical AgNO\(_3\) allows the reduction of Ag\(^{+}\) atoms onto Sn\(^{2+}\) particles, which in turn oxidizes the tin atoms into its Sn\(^{4+}\) state. Finally, the Ag-Go treated with Gold Sulfite solution allows the replacement of Ag atoms with reduced Au nanoparticles, and the formation of Au-Go product.
1.5 H₂O₂ Sensor

Due to its exceptional surface-enhanced Raman Scattering activity, while also displaying high electrical and thermal conductivity, silver nanoparticles have been extensively examined for its catalytic properties [33-34]. One of these roles explored in this manner has been for its biosensing applications, in particular determining the presence of hydrogen peroxide (H₂O₂) in various mediums, as it provides valuable information in food, industry, biology, pharmaceutical, and environmental research. While there are many expensive and time consuming methods that serve this role, electrochemical techniques offer a relatively simple, low cost, and efficient approach in achieving the desired outcome, as studies have shown the catalytic activity of Ag nanoparticles in reducing H₂O₂ [35]. Accordingly, in this research, Ag-Go was explored as a potential H₂O₂ sensor, utilizing electrochemical techniques.

2. Materials and Methods

2.1 Materials
Sodium Hydroxide, Sodium Chloride, Ba(OH)₂·8 H₂O, H₂O₂ (30-32 wt. %), Hydrochloric acid (37 %), and Trifluoroacetic acid (99%) were all purchased from Sigma-Aldrich and used as received. Tin (II) Chloride dihydrate, K₄Fe(CN)₆·3 H₂O, and KAuCl₄·xH₂O (Au 49 % min.) was purchased from Alfa Aesar and used as is. Potassium Nitrate, Sodium Sulfite, and Nitric Acid (50-70 %) were purchased from Fisher Scientific and used as received. Silver Nitrate was purchased from Electron Microscopy Sciences
and used as is. All compounds used were reagent grades and used without further purification. With the exception of the synthesis of Sodium Gold Sulfite solution, all solutions were prepared with DD H₂O.

2.2 Graphene Oxide Preparation

Graphite purchased from Sigma-Aldrich was used for the preparation of graphene oxide by a modified Hummers’ method. Graphite powders were first oxidized by sulfuric acid. Then 5 g of the graphite powder was first treated with a solution by mixing 25 ml of concentrated H₂SO₄ with 5 g K₂S₂O₈ and 5 g P₂O₅. The mixture was kept at 80 °C for 6 hours using a hotplate. Subsequently, the mixture was cooled to room temperature and diluted with 1.0 L of de-ionized (DI) water and left overnight. The mixture was then filtered and washed with de-ionized (DI) water to remove the residual acid. The product was dried under ambient conditions overnight. The pre-oxidized graphite was then subjected to oxidation by Hummers’ method. The pretreated graphite powder was put into cold (0 °C) concentrated H₂SO₄ (250 ml). Then KMnO₄ (30 g) was added gradually under stirring, and the temperature of the mixture was kept below 20 °C by cooling. The mixture was then stirred at 35 °C for 4 hours and then diluted with DI water (500 ml). Because adding water to concentrated sulfuric acid medium releases a large amount of heat, the dilution was carried out in an ice bath to keep the temperature below 50 °C.

After adding all of the 500 mL of DI water, the mixture was stirred for 2 hours, and then an additional 1.5 L of DI water was added. Shortly thereafter, 40 ml of 30% H₂O₂ was added to the mixture, and the color of the mixture changed into brilliant yellow and began bubbling. The mixture was filtered and washed with 0.1 M HCl to remove
metal ions, followed by 1.0 L of DI water to remove the acid. The resulting Go solid was dried in air.

2.3 Electroless Metal Deposition onto Graphene Oxide

Solid Graphene Oxide was initially dissolved in DDH$_2$O for a 1 mg/mL Go solution. The Go solution was then treated according to the Electroless Au Plating technique employed by Ahn and coworkers [30], adapted for metal nanoparticle deposition onto Go surface. The initial deposition step required the gradual addition of 0.026 M SnCl$_2$ solution containing 0.07 M trifluoroacetic acid in drops to the stirring Go solution. During this step, Sn$^{2+}$ nanoparticles are nucleated onto the graphene surface, thereby “sensitizing” the surface for further activation. The SnCl$_2$ solution was added in drops to avoid aggregation of the Go. The resulting mixture was left stirring overnight and then washed repeatedly with DD H$_2$O. The Sn deposited Go (Sn-Go) was then treated with a 0.029 M ammonical AgNO$_3$ solution, where the Sn(II) is oxidized to Sn (IV), at which point the Ag$^+$ is reduced to Ag and deposited onto the Sn$^{4+}$ molecules. The solution again was added in a drop by drop fashion and left stirring overnight with sufficient washing of the product with DD H$_2$O afterwards. The final step entailed the treatment of Ag deposited Go (Ag-Go) with a Na$_3$[Au(SO$_3$)$_2$] solution in the same gradual fashion, at which instance the Ag nanoparticles are galvanically replaced by the more noble metal, Au. The reaction mixture was once again left overnight and the abundant washing of the subsequent Au-Go product in DD H$_2$O.
2.4 SEM Microscopic Analysis

Each resulting Go solutions during the electroless deposition steps (Sn-Go, Ag-Go, Au-Go), were observed with the Hitachi S-3400N SEM. Each of the metal decorated Go solutions were heavily diluted with DD H$_2$O, sonicated for ~1 hour, and a drop of the prepared sample solution was loaded unto gold etched Hi-Grade Mica plates. The sample plates were then placed in a vacuum oven overnight to ensure complete evaporation of liquid before examined with the SEM.

2.5 Electrochemical Analysis

The electrochemistry of the metal decorated Go was carried out utilizing the Electrochemical Workstation with Picoamp Booster & Faraday Cage from CH Instruments, Inc., along with the CHI660C software. The method incorporated a standard three electrode system, including a Ag/AgCl reference electrode containing saturated KCl and a gold counter electrode. The working electrodes utilized were glass carbon electrodes coated with 2.5 µL or around ~ 10 µg of the various Go solutions prepared and then layered with a small amount of 1 % naphthalene solution. The cyclic voltammetry of the Go modified electrodes were observed within ranges of -0.4 to 0.8 V at a scan rate of 0.06 V/S or 60 mV. The reduction and oxidation of the modified Go was observed with 10.0 mM Fe(CN)$_6^{4-}$ in 0.2 M KCl solution.

2.6 Ag-Go Reduction Potential of H$_2$O$_2$

The ability of the Ag-Go to reduce H$_2$O$_2$ was observed with cyclic voltammetry and chronoamperometry techniques. The Ag-Go solution was mixed with 1 %
Polyethylenimine (PEI) solution to maintain and stabilize the integrity of Ag nanoparticles on Go surface [36-37]. The cyclic voltammetry was carried out in 0.5 M KNO₃ solutions, with the reduction of H₂O₂ observed in solutions of 0.5 M KNO₃ containing either 1.0 mM or 100 mM of H₂O₂ at 60 mV. The chronoamperometry technique was performed in 0.5 M KNO₃ solution with periodic 50 μL additions of 30–32 wt. % H₂O₂ solution at every 50 second intervals. The parameters of chronoamperometry included application of a constant voltage at -0.4 V and pulse width of 600 sec.

3. Results and Discussions

3.1 Microscopic Analysis of Metal Decorated Go

The SEM analysis of the Go provided images of single layer graphene oxide sheets, confirming the feasibility of method utilized for the production of single layer Go from graphene. Figure 7 also shows the presence of Ag nanoparticles embedded onto the Go surface. The size of Ag nanoparticles appears to vary, as the electroless metal deposition step was not designed for size controlled purposes. Yet, as the efforts of the experiment focused on the deposition of metal nanoparticles on the surface of Go as a whole, the clear presence of dispersed Ag nanoparticles reinforces the viability of the metal-Go composites, produced by the electroless metal deposition method utilized.
Figure 7. The analysis of Go and Ag-Go with SEM. The SEM clearly shows the presence of Ag nanoparticles on Go surface with the Ag-Go sample (Right).

3.2 Cyclic Voltammetry Analysis

The cyclic voltammetry of metal deposited graphene samples were analyzed in two different solutions, 10.0 mM Fe(CN)$_6^{4+}$ in 0.2 M KCl and 0.5 M KNO$_3$, as shown in Figure 8. Using the electroanalytical technique of cyclic voltammetry, an effective representation of the electric potential capacities regarding the metal deposited graphene samples can be observed. When a voltage is applied to the Fe(CN)$_6^{4+}$ solution, Fe(CN)$_6^{4-}$ ions are reduced to Fe(CN)$_6^{3-}$ on the electrode surface. Thus, within this solution, the conductance of various metal deposited Go samples can be analyzed, based on the rate of electron transport on its surface. The expected response can be observed in Figure 9, displaying the behaviors of various metal deposited Go in standard 10.0 mM Fe(CN)$_6^{4+}$ in
0.2 M KCl solution. It was evident that all the metal deposited Go samples possessed a much higher electrocatalytic activity than Go by itself. The size of current signals seen in the metal deposited electrodes correlated with the conductivity of the metal nanoparticles deposited unto the Go surface. Accordingly, being the most conductive of the metals being examined, Ag-Go was able to produce the largest flow of current among all the metal deposited graphene samples, indicating the higher presence of Fe(CN)$_6^{4-}$ on the electrode surface. Au-Go also displayed significant rate of electron transport properties, yet at a slower rate than the Ag-Go sample containing the more conductive Ag nanoparticles, evidenced by the decreased current signal. The Sn-Go modified electrode displayed the lowest signal of all the metal deposited Go samples, as the least conductive of all the metal samples.
Figure 8. Cyclic voltammetry comparison of GC electrode in both 10 mM Fe(CN)$_6^{4-}$ with 0.2 M KCl and 0.5 M KNO$_3$. The analysis shows that the bare GC electrode is highly conductive in the 10 mM Fe(CN)$_6^{4-}$ with 0.2 M KCl solution, actively participating in the reduction and oxidation of Fe(CN)$_6^{4-}$ to Fe(CN)$_6^{3-}$. As KNO$_3$ is an inert solution, no signal is observed in this solution.
Figure 9. Cyclic voltammetry analysis of metal deposited Go in 10.0 mM FeCN₆⁴⁻ in 0.2 M KCl displaying the difference in current flow for the samples examined. The size of the current flow observed correlates to the conductivity of the metal nanoparticles deposited onto the Go surface. The Ag-Go displayed the largest current flow among the metal deposited Go samples, as it is the most conductive of the metals examined, with the second largest current signal displayed by Au-Go, being the second most conductive metal of the three. Having a lower conductivity compared to Ag and Au, the Sn-Go gave the smallest current flow of the three metals examined. However, as these samples are all composed of Go, which is hindering the contact surface of the electrode with the analyte solution, all the metal deposited Go samples display a lower signal than the bare GC electrode.

The metal deposited Go samples did not match the current signal displayed by the bare GC electrode. As Go is a conductive material, likely repelling the negative Fe(CN)₆⁴⁻ ions with the already present negatively charged oxygen atoms on its surface disallowing their passage to the electrode surface, the reduction of Fe(CN)₆⁴⁺ ions to Fe(CN)₆³⁻ is understandably hindered by the coating of the electrode surface with the Go.
This is evidenced by the lack of signal given by the Go modified electrode while analyzed in the Fe(CN)$_6^{4-}$ solution. Thus, as the metal nanoparticles are present on the Go surface, the conductivities of these samples are less than the bare GC electrode. However, as there are only about $\sim$10 $\mu$g of the Go sample on each electrode, the conductivity of the metal deposited electrode can match or even surpass the fixed conductivity of the GC electrode, with the increased concentrations of the Go samples placed unto the electrode surface. Modifying the electrodes in this manner, allows greater control of the electrodes, not only in the levels of conductivity, but also the size of the surface area, and specificity of the electrodes for analytes concerned, all in a simple preparatory procedure.

Concerning the behaviors of metal deposited Go samples in the 0.5 M KNO$_3$ solution, Figure 10 shows that the only species with any pronounced redox activity were Ag-Go and Au-Go. As the KNO$_3$ solution is an inert solution, the behaviors observed in this solution would be the ability of Go to either reduce or oxidized the metal nanoparticles deposited unto its surface. With the Ag-Go and Au-Go being the only samples able to produce a signal in this solution, this indicates that the already reduced Ag and Au nanoparticles on Go are able to be oxidized. Within the linear sweep ranges of -0.4 and 0.8 V, Ag-Go was oxidized with the anodic peak of around $\pm$ 0.42 V, similar to the peak seen during the oxidation of Au-Go. The redox reaction of Sn-Go was not observed in the 0.5 M KNO$_3$ solution, as studies have shown that any of the transitional metals with standard reduction potentials of less than $+0.38$ V cannot be reduced by Go, as it is the reduction potential of Go. Thus the Sn$^{2+}$ nanoparticles on the Go surface cannot be reduced back to its Sn form, or oxidized to Sn$^{4+}$, rendering the Sn-Go sample
inactive in the KNO₃ solution. As expected, Go did not display any redox potential in the 0.5 M KNO₃ solution, similar to the behavior observed in the Fe(CN)₆⁴⁻ solution, being a nonconductive material that cannot be reduced back to its graphene form in this manner. As stated previously, since KNO₃ is an inert solution, there are no analytes present to be reduced or oxidized, thus the bare GC electrode was observed to be inactive.

Figure 10

**Electrode Comparisons in 0.5 M KNO₃**

![Figure 10: Cyclic voltammetry analysis of metal deposited Go in 0.5 M KNO₃. The Ag-Go and Au-Go are the only Go samples displaying any peaks. Both the Ag-Go and the Au-Go displayed their respective oxidation peaks at around +0.45 V. This indicates that the two different metal nanoparticles deposited onto the Go surface are behaving in a similar manner, as they are both reduced to their ground states from a single electron transfer during the preparation step, and they are both attached to the Sn⁴⁺, which appears to be predominantly dictating the similar behaviors of the two metal nanoparticles. The difference in current between Ag-Go and Au-Go can be explained by the fact that Ag is more conductive than Au. Sn-Go cannot be reduced or oxidized in the single electron transfer of electrodes within the inert 0.5 M KNO₃ solution. The Go is essentially nonconductive, and also cannot be reduced in these experimental parameters.**
3.3 Ag-Go Biosensor

*Ag Deposited Go Electrodes.* Ag-Go exhibited similar electrocatalytic behavior towards reduction of \( \text{H}_2\text{O}_2 \) as compared to other studies incorporating silver nanoparticles onto carbon based platforms [33-35]. In this study, two types of Ag deposited graphene oxides were examined. Ag-Go was obtained after initial activation and nucleation of \( \text{Sn}^{2+} \) nanoparticles as devised by Ahn and coworkers. Go+Ag was synthesized through direct addition of 0.029 M ammonical \( \text{AgNO}_3 \) to a Go solution without the \( \text{Sn}^{2+} \) nucleation step. The initial scans through cyclic voltammetry revealed the different states of the Ag nanoparticles in each of the samples, as seen in Figures 12 and 13. The Ag nanoparticles in Ag-Go are already in a reduced Ag state, as the initial nucleation by \( \text{Sn}^{2+} \) nanoparticles onto the graphene surfaces reduces the \( \text{Ag}^+ \) during the subsequent treatment with ammonical \( \text{AgNO}_3 \), which in turn oxidizes the \( \text{Sn}^{2+} \) to the \( \text{Sn}^{4+} \) state [38]. The Ag nanoparticles in Go+Ag however maintains the metal in its already oxidized state at \( \text{Ag}^+ \), during the addition of ammonical \( \text{AgNO}_3 \) to the Go. This is supported by the cyclic voltammetry of GC electrodes modified by each samples, as Figure 11 displays only a single peak relevant to the anodic peak of the Ag-Go electrodes, at around + 0.42 V, while Figure 12 displays both the cathodic and anodic peaks, at around + 0.38 V and + 0.14 V respectively, for the Go+Ag electrodes. In addition, it is worth to note that the varying of the peaks displayed by the two modified electrodes may be due to the Go platform in the case of Ag-Go, as the higher potential it displayed in comparison to the Go+Ag modified electrode, may be due to its association of \( \text{Sn}^{4+} \) nanoparticles already on Go surface stemming from the nucleation step, which is likely participating as a \( \text{Sn}^{4+} \)-Ag nanoparticle complex during experimentation.
Proposed $\text{H}_2\text{O}_2$ reduction mechanism

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{H}^+ + e^- & \rightarrow \text{OH}_{\text{ads}} + \text{H}_2\text{O} \\
\text{H}^+ + \text{OH}_{\text{ads}} + e^- & \rightarrow \text{H}_2\text{O}
\end{align*}
\]

Possible reduction process with Ag-Go

1) Adsorption of OH

\[
\text{Go-Ag(0)} + \text{H}_2\text{O}_2 \rightarrow 2 \text{[Go-Ag(1)-OH]}
\]

2) Reduction of Ag

\[
2\text{[Go-Ag(1)-OH]} + 2e^- + 2\text{H}^+ \rightarrow \text{Go-Ag(0)} + \text{H}_2\text{O}
\]

Figure 11. The proposed mechanisms regarding the reduction of $\text{H}_2\text{O}_2$ and the role of Ag nanoparticles participating in the reduction process on the Go surface. The addition of an electron and a proton allows the degradation of $\text{H}_2\text{O}_2$ molecules into OH and $\text{H}_2\text{O}$. With the oxidation of Ag into Ag$^+$ this allows the adsorption of OH molecules onto Ag nanoparticles deposited on the Go surface. The adsorbed OH molecule then can also be released from the Ag nanoparticles, with the addition of another pair of proton and electron, releasing the OH as $\text{H}_2\text{O}$, and the subsequent reduction of Ag back into its ground state.
Figure 12. Cyclic voltammetry analysis of Ag-Go in 0.5 M KNO₃ displaying the shift in the oxidation peak due to the addition of PEI. While the oxidation peak of Ag-Go sample was observed around +0.45 V, when the Ag-Go sample is mixed with PEI to preserve the integrity and stability of the Ag nanoparticles, the oxidation peak is shifted to around +0.25 V. This indicates that the PEI is indeed protecting the Ag nanoparticles on the Go surface with possible disassociation from the Sn⁴⁺ nanoparticles, where they were initially attached.
Figure 13. Cyclic voltammetry analysis of Go+Ag in 0.5 M KNO₃ solution displaying the difference in peaks observed with the addition of PEI. The Go+Ag sample shows both the reduction peak at +0.38 V and the oxidation peak at around +0.25 V. This is consistent with the preparation of Go+Ag sample, as AgNO₃ is directly added to Go, producing Ag⁺ nanoparticle attachments to the Go surface. However, when this sample is mixed with PEI, Ag⁺ is reduced to Ag during the treatment, and the only peak seen in the mixed sample is the oxidation peak at around +0.25 V, similar to the results observed in the Ag-Go-PEI sample.

Both the Ag-Go and Go+Ag samples, nevertheless could not be used directly for the purposes of H₂O₂ sensoring system, since the current flow during numerous sweeps in cyclic voltammetry gave unstable readings, as the current began to decrease over each potential sweep. This process was hence corrected with the addition of 1 % PEI solution to the Ag deposited Go samples, which stabilized the rate of current flow in each potential sweeps [36-37]. The ratio of 1 % PEI added to the Ag deposited Go samples were relative to its concentration. In the case of Ag-Go, an aliquot of 4-5 mg/mL Go
sample was prepared, disregarding the weight of Ag nanoparticles. The Ag-Go and 1 % PEI mixture that gave the most stable current readings were obtained from 14:1 ratio of Ag-Go aliquot to 1 % PEI. For the Go+Ag sample, a 5-6 mg/mL aliquot was obtained, and was mixed to prepare 5:1 ratio of Go+Ag to 1 % PEI sample. The cyclic voltammetry readings gave the stable reduction peaks for both samples, at around + 0.25 V indicating that PEI was able to maintain the integrity of the Ag nanoparticles on the Go surface, while prohibiting the Ag nanoparticles to be oxidized to its Ag⁺ state, in the case of Go+Ag. With the Ag-Go, PEI was able to limit the association of the Sn⁴⁺ nanoparticles with the Ag, allowing the anodic peak to occur close to the literature values, at around + 0.28 V [33-35]. The difference in the current flow shown by each of the Ag deposited Go sample can be attributed to the difference in concentrations of the Go samples employed in each case to stabilize the signals, with varying ratios of 1 % PEI.

$H_2O_2$ Detection. The evidence of $H_2O_2$ detection and reduction by the Ag deposited graphene samples were observed through cyclic voltammetric methods. Figures 14 and 15 clearly shows that when the Ag-Go and Go+Ag modified electrodes were analyzed in 0.5 M KNO₃ solutions containing either 0.1 M $H_2O_2$ or 1.0 mM $H_2O_2$, the reduction peak around -0.45 V, similar to literature values [33-35], was observed in both electrodes corresponding to the reduction of $H_2O_2$. This was in contrast to the bare GC electrode and the Go only modified electrodes which did not display any sign of $H_2O_2$ reduction. In addition, there seems to be a shift in the anodic peak for the Ag nanoparticles on the Go samples, as a slight increase in the peak of + 0.28 V to around + 0.30 V was observed, indicating the possibility of a Ag and $H_2O_2$ complexes, such as with radicals, while the $H_2O_2$ is being reduced. Further examination of the $H_2O_2$ reduction potential by the Ag
deposited Go was performed through amperometric response of the modified electrodes. As observed in Figure 16, with the successive additions of H$_2$O$_2$ to the 0.5 M KNO$_3$ solution, the Ag-Go-PEI modified electrodes displayed a fast response to the presence of H$_2$O$_2$, similar to observations made in other studies concerning carbon based Ag electrodes [33-35]. The linear relationship between the current and the concentrations of H$_2$O$_2$ present within the solution is also observed, derived from the signals displayed in the amperometry data. The H$_2$O$_2$ detection limit derived from the plot was around ~ 600 μM. Overall, both the Ag-Go and Go+Ag samples offer a potential as efficient biosensors in the detection of H$_2$O$_2$. 
Figure 14

Cyclic voltammetry comparisons of Ag-Go with Go+Ag modified electrodes, utilizing Go as a control, in 1.0 mM H₂O₂ in 0.5 M KNO₃ solution. Both samples appear to be a good sensor for H₂O₂ detection, indicating the legitimacy of both preparation methods, as the current signal is similar to each other, much higher than the Go sample. In addition, both Ag deposited Go samples display a clearly defined H₂O₂ reduction peaks at this concentration, whereas the Go sample does not.
Figure 15. Cyclic voltammetry comparisons of Ag-Go-PEI with Go+Ag-PEI modified electrodes, utilizing Go as a control, in 1.0 mM H₂O₂ in 0.5 M KNO₃ solution. Like the Ag-Go and Go+Ag counterparts, these samples also appear to be a good sensor H₂O₂ detection, indicating the effectiveness of PEI preserving the integrity of the Ag metal nanoparticles on the Go surface. The current signals are similar to each other, much higher than the Go sample, and the H₂O₂ reduction peaks can be clearly seen at this concentration, whereas the Go sample does not. The slight decrease in current for both sample, compared to the Ag-Go and Go+Ag counterparts, are due to the addition of PEI to the sample, decreasing the presence of Ag deposited Go molecules.
Figure 16

a) Amperometry analysis of Ag-Go mixed with PEI in 14:1 ratio of Ag-Go to PEI compared to control samples: Go-PEI, Go, and bare GC electrode. 50 uL of H$_2$O$_2$ was added at every 50 second interval in 0.5 M KNO$_3$ solution correlating with the increase in current with each addition, indicating the detection of H$_2$O$_2$ within the solution for each experiment condition. As expected Ag-Go with PEI sample was able to detect the highest level of H$_2$O$_2$, indicated by the size of current. The control samples were unable to detect H$_2$O$_2$ at significant levels compared to the Ag-Go-PEI. b) The calibration curve derived from the amperometry response of successive addition of H$_2$O$_2$ to 0.5 M KNO$_3$ solution as shown in a). The calibration curve shows the linear relationship between the concentrations of H$_2$O$_2$ with the currents observed and the approach to determining the detection limit for the Ag-Go nanocomposite.
4. Conclusion

Electroless gold deposition technique devised by Ahn and coworkers provides a convenient and efficient method of metal nanoparticle deposition onto Go surface. This process was successful in producing three different metal deposited Go samples: Sn-Go, Ag-Go, and Au-Go. The Ag-Go was of particular interest due to its capability of being utilized as a H$_2$O$_2$ sensor. While the detection limit of the Ag-Go modified electrode was around ~ 600 µM, this may be improved with better stabilization of Ag-Go with PEI, along with higher concentrations of the Ag-Go sample onto the electrode surface. Exploration into the other metal deposited Go samples as various sensors and devices can also be performed, as studies have been shown the potential of Sn-Go in heavy metal detection systems [39], along with Au-Go being utilized an efficient enzyme based sensors and fluorescent probe or imaging components [40]. Overall, this study provides an initiation point for further examination of the various metal deposited Go nanocomposites as excellent modules for potential sensor systems, produced in a fast, convenient, and cost effective manner.
References


