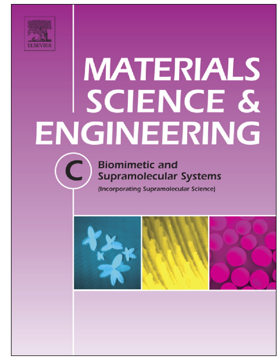

Current state of green reduction strategies: Solution-processed reduced graphene oxide for healthcare biodetection

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Current State of Green Reduction Strategies: Solution-processed Reduced Graphene Oxide for Healthcare Biodetection

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Abstract

Reduction of graphene oxide becomes an alternative way to produce a scalable graphene and the resulting nanomaterial namely reduced graphene oxide (rGO) has been utilized in a wide range of potential applications. In this article, the level of green reduction strategies, especially the solution-based reduction methods are overviewed based on recent progression, to get insights towards biomedical applications. The degrees of gaining tips with the solution-based green reduction methods, conditions, complexity and the resulting rGO characteristics have been elucidated comparatively. Moreover, the application of greenly produced rGO in electrochemical biosensors has been elucidated as well as their electrical performance in term of linear range and limit of detections for various healthcare biological analytes. In addition, the characterization scheme for graphene-based materials and the analyses on the reduction especially for the solution-based green reduction methods are outlined for the future endeavours.

Keywords: Solution-based reduction; Green methods; Graphene; Electrochemical; Photocatalytic; Biosensor.

Table of Contents

1. Introduction

Graphene is a single layer material (2-Dimensional) with an atomic thickness composed of carbon atoms arranged in honeycomb lattice structure. This material exhibits extraordinary properties in term of physical, chemical, mechanical, optical and thermal that offers enormous potential applications in various sectors [1]. The aforementioned properties are such as having high surface area (2630 m^2g^{-1}), very high electron mobility at room temperature (250,000 $cm^2 V^{-1} s^{-1}$), excellent thermal conductivity (5000 W $m^{-1} K^{-1}$), high mechanical strength (1 TPa) as well as high in optical transparency [2],[3]. Moreover, graphene-based materials such as graphene oxide (GO) and reduced graphene oxide (rGO) have specific advantages in biomedical and bioelectronics fields due to their oxygen-containing moieties (Eg; hydroxyl, carbonyl, epoxy and carboxyl groups) that makes the derivatives compatible with bio-environment [4],[5]. The biosensor is one of the great examples where the GO and rGO have been intensively utilized as a nanomaterial to develop myriad of nanodevices for medical diagnosis. For example, the detection of heart diseases [6],[7], cancer [8],[9] and diabetes [10],[11] which have been developed recently based on graphene-based materials.

The synthesisation of graphene or graphene-based materials becomes an active area of research since the discovery of graphene. This is because of the search for the large size of a graphene sheet with desired lateral dimension is still remaining as one of the big challenges. Graphene can be synthesised via various routes such as mechanical exfoliation, chemical vapour deposition, epitaxial growth, unzipping carbon nanotube or via reduction of GO [12],[13]. However, among these routes the reduction of GO is the widely accepted route because this strategic have the ability to produce graphene in a large scale besides having the possibilities to achieve a pristine graphene [14]. Moreover, the other aforementioned synthesisation routes have limitations such as smaller size turn out, low yields, requires expensive equipment, need high temperature and gaseous environment and the fact that needs to follow a complex protocol when transferring the produced nanolayers [12]. Furthermore, the solution-based green reductions of GO are considered as one of the best choices amongst various reduction strategies, see Figure 1. This is because the solution-based reductions have ruled out the use of any hazardous or toxic chemicals that can be harmful to human and nature [1],[15]. Moreover, it provides facile one-step process reduction with higher opportunity to get a large number of rGO nanosheets.

Hence, in this article, the level of green reduction strategies are outlined and arranged systematically. Moreover, comparative studies, especially for the solution-based reduction methods are done based on recent progression. Their related reduction methods, conditions, complexity and the resulting rGO characteristics are elucidated comparatively. In addition, the characterization scheme for graphene-based materials and the analyses techniques of reduction especially via solution-based green reduction methods are discussed in general perspectives. In this study, more than 80% of the references are within 3 years of range in order to get an insight on the recent development on green reductions as well as their rGO application in biosensing engineering.

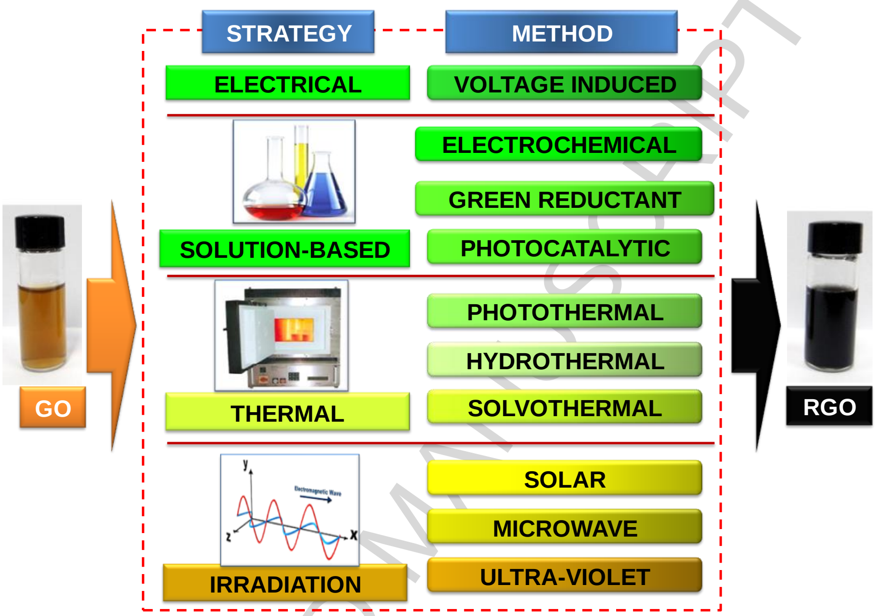

Figure 1: Level of green reduction strategies and their related methods.

2. Solution-based Green Reduction Methods

There are various green reduction strategies devised over the years in an attempt to produce a less defect and a high quality of reduced GO nanosheets. These reductions strategies can be arranged from the greener methods to less greener in the order of electrically, solution-based, thermally and irradiation, respectively (Figure 1). Moreover, under each strategy there are various sub-methods have been developed up to date that can be considered for reducing the GO. However, among all the strategies and methods, GO reduction via solution-based is widely adopted approach because it is well matured and had gone thru several improvements since the discovery of graphene. For example, the shift from hazardous reductants to green reductants is a very noticeable and appreciable improvement. Firstly, it also removes the needs for high temperature (>100 ˚C), gaseous, vacuum environment and radiation energy thus simultaneously rule out the need for high-end equipments or costly apparatus for the synthetization. Secondly, the preparation of GO in a solution-based medium becomes the precursor process in order to execute the other green reduction methods like in thermal or irradiation strategies. For example, Niu et al. [16] had recently synthesized the rGO by using hydrothermal reduction by preparing the GO powder in DI water and reduced it at various temperatures ranging from 100 - 200 ℃. On the other hand, Shaikh and co-workers [17] had prepared the TiO_2-rGO nanocomposites by solvothermal reduction where they prepared the GO and TiO_2 in solvent medium followed by reduction process at a temperature of 180 ˚C. Furthermore, Mohandoss et al. [18] reported the reduction of GO by using sunlight (irradiation strategy) where they prepared the GO dispersion in DI water. Similarly, Ding et al. [19] had reduced the GO using irradiation route by preparing the GO dispersion in polyvinylpyrrolidone solution before exposed it to the UV-light. Based on above retrospect, the solution-based technique has a significant role in the reduction process. Moreover, the technique is extremely required as a precursor process in other reduction strategies too. Thus, the momentous in producing rGO nanosheets from the solution-based methods and their classifications without incorporation of any additional process is vital and need to be reviewed.

There are three main methods to reduce GO via solution-based namely; 1) by using green reductants, 2) electrochemically and 3) by photocatalytically. Figure 2 shows a schematic illustration of the process technology for the three main methods. A comparative summary of these methods are engulfing from reduction conditions, reduction complexity and the rGO sheet morphology as well as their related applications are shown in Table 1. Clearly, the solution-based green reduction methods are facile because it only involves one-step process (Table 1) without the need for high-end equipments. Further elucidations based on recent progression on each reduction methods are comparatively discussed in the subsequent sub-topics.

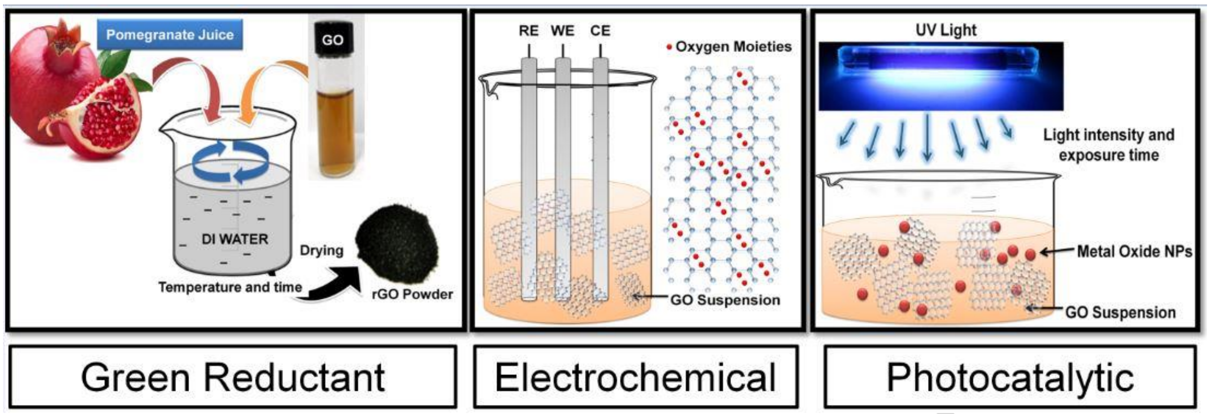

Figure 2: Schematic illustration of solution-based green reduction methods.

2.1 Green reductants

Reduction by using green reductants is facile and quick where the GO reduction is accomplished in an aqueous medium mediated by a mild heating treatment. This method eliminates the use of toxic chemical such as hydrazine and the need for any special laboratories equipment. The oxidation of graphite becomes the precursor step for the GO reduction. However, the oxidation process developed by Brodie, Staudenmaier and Hummer are literally hazardous because these methods evolve of toxic gaseous such as chlorine dioxide and nitrogen dioxide during the process. However, the Tour method is considered as improved green oxidation method because it is relatively safe to human and environment. The primary indication for the reduction took place is when the color of GO suspension gradually change from yellowish brown to black color, forming rGO [12]. To date, researches have tried to reduce GO by using various biological agents such as lemon juice [20], alanine [21], guava dried leaf [22], and barberry fruit extract [23] to name a few. However, there are more than 60 green reducing agents that have been utilised in the past and the list of green reductants can be found in this literature [24]. Among them, ascorbic acid (Vitamin C) is the most widely used agent for the reduction [25],[26],[27]. Besides Vitamin C as a reducing agent, there are other emerging green reductants which have been used recently. For example, recently, Wang et al. reported a one-step green reduction of graphene oxide using a biomolecule (alanine). They have presented that the operating conditions such as temperature, time and reagent concentration contribute to the reduction. Moreover, they found the optimal condition for the reduction using alanine was at a concentration of 10 g/L at 85˚C with a treatment time of 24 hours without any alkaline medium or additional chemical [21]. Similarly, Nasrollahzadeh et al. synthesized a nanocomposite consists of palladium nanoparticles supported on rGO through one step green reduction using barberry fruit extract. The reduction has been carried out at 80˚C for 12 hours which is lower in treatment time compared to the study by Wang et al. [21]. Moreover, Bozkurt et al. [28] reported a new approach to reduce GO by using sodium citrate as a green reducing agent via sonochemical technique. They found the combination of such reducing agent and the technique have leads to the formation of nanocomposite and economical advantageous.

2.2 Electrochemical reduction

Electrochemically rGO (ErGO) has been widely used in biosensor applications for detecting various analytes such as horseradish peroxidase [29], chlorpromazine [30] and glucose [31] because the end-product shows high in electrocatalytic activity. This method can be accomplished by using a standard three-electrode cell dipped in electrolyte medium and the reduction process took place when applying a constant potential for a period of time at room temperature. This method can reduces foreign contamination compared to the reduction by using a reducing reagent [14]. In addition, the major processes such as oxidation, reduction and deposition can also be done simultaneously via electrochemical reduction method. Thus, this method is considered straight-forward in preparation, requires less chemical and inexpensive as well as having the potentiality for a large-scale production [32]. Very recently, [33] Narayan et al. had reduced the GO sheets via electrochemically directly on the glassy carbon electrode. They reported the reduction is taking place about 10% in removing oxygen moieties compared to pure GO that shows a successful reduction. They observe large wrinkles and rougher rGO surfaces where such topologies can enhance the exposure and the diffusion of the electrolyte ions for the electrochemical-based application. Moreover, the ErGO has shown an enhancement in peak current of electro-oxidation for mycophenolate mofetil detection from bulk samples. They suggested the electrochemical reduction method is simple, cheap and suitable to be used for clinical applications.

Clearly the electrochemical reduction method shows the ability to produce rGO with cost-effectiveness procedure besides reduces the number of complex steps in transferring the nanosheets onto a substrate which is advantageous compared to the others solution-processed rGO.

2.3 Photocatalytic reduction

Another reductant free method for GO reduction is via photocatalytic reaction where the process is carried out by using photocatalyst nanomaterials like semiconductor oxide (eg: TiO_2 or ZnO) assisted by UV or visible light illumination [34]. This method will eventually leave the nanomaterials attached to the rGO basal sheets and forming a nanocomposite which is desirable for some applications such as optoelectronic or supercapacitor [35],[36]. The reduction principle is based on how many electrons can be accepted by the GO from the excited photocatalyst nanomaterial. Moreover, this strategic does not require the use of high temperature which paved the path for much safer reduction. Recently, Chakraborty et al. have utilized semiconductor metal sulfide Zinc Sulfide (ZnS) for the first time as a photocatalyst material to reduce the GO. They reported that the reduction and the formation of the rGO-ZnS hybrid are attainable and both processes take place simultaneously. Moreover, the ZnS nanorods were firmly anchored onto rGO [37]. Similarly, Chih et al. have demonstrated the growth of cuprous oxide (Cu_2O) and the reduction of GO occurs simultaneously to form nanoheterostructures. They found the density of Cu_2O nanocubes growth on the rGO sheets can be controlled by adjusting the GO concentration. However, their procedure is rather utilized nitrogen gas and involve of multi-steps procedures [38] Hence, the photocatalytic reduction method is recommended and very suitable to produce a hybrid nanocomposite effortlessly.

Table 1: A comparative summary of green reduction strategies in term of methods, conditions, complexity, sheet morphology and the potential applications of rGO

Reduction Strategic	Reduction Method	Reduction Conditions	Reduction Complexity /*Remark	TEM-Sheet Morphology	rGO Sheet Thickness/ *Remark	Application of rGO / *Remark	Ref.
Electrical	Voltage-Induced	Bias Voltage: 5 V Relative humidity at 70% Reduction time: Depending on electrodes gap size	*No additional chemicals were used	N/A	N/A/ *rGO thickness was pre-defined during GO deposition	Resistive memory Optoelectronic/ *Removes a large portion of oxygen groups.	[39]
Solution-based	Green Reductant	Green Reductant: Alanine Reductant concentration: 10 mg of GO with 200 mL of alanine solution. Temperature:85˚C Treatment Time:2-24h	Process: One-step	Few-layer of aggregated and wrinkled rGO	N/A	Biomaterial/ *Demonstrated a safer oxidation method.	[21]
		Green Reductant: Dry leaves of *Psidium guajava* Reductant concentration: GO (0.5 mg/ml) in 8 ml of *P. guajava* phytoextract Temperature: 90˚C and 94˚C Treatment Time: 6 h	Process: One-pot reflux	Restacking of multiple graphene layers	Graphene interlayer spacing of 0.37nm	Textile industries and aquatic environment.	[22]
		Green Reductant: Barberry fruit extract/ Reductant concentration: 30 mL of the aqueous extract of barberry fruit into 50 mL of 0.003 M Temperature: 80˚C	Process: One-step	Large number of Palladium NPs with spherical morphology were immobilized	N/A	Organic amines production.	[23]

		Treatment Time : 12 h		onto rGO sheets			
	Electrochemical	Using three electrode cells. Reduction under constant Amperometric i–t curve (with -1.2 V or -3.0 V). Electrolytes: potassium chloride (KCl). Room Temperature.	Process: One-step	N/A	rGO film interlayer spacing of 0.37 nm.	Potential for various kind of application because having a good electrical conductivity.	[14]
		Reduction by applying 25 cyclic potential sweeps (with 0.6 and- 1.6V) Electrolytes: phosphate buffer with pH 6.0 Room Temperature.	Process: One-step	SEM images show large wrinkles and rougher surface of rGO	Average GO film thickness of 7.2 nm	Bioelectronics/* Reduction of oxygen moieties by 10% compared to GO.	[33]
	Photocatalytical	GO powder (1 mg/mL) and ZnS nanorod (20 mg/mL) dispersion in double distilled water and sonicated for 30 min. Reduction under high-pressure mercury vapor lamp for 12 h at room temperature under constant stirring.	Process: One-step	ZnS nanorods firmly attached to a single layer of rGO sheet	GO film with d-spacing of 0.85 nm	Optoelectronic devices.	[37]
		The final mixtures purged with N_2 in the dark condition for 30 min followed by reduction under UV irradiation for 12 h.	Process: Multi-steps	Cu_2O nanocubic structure formation on rGO sheets	GO sheets with d-spacing of 0.71 nm	Potential for harvesting solar energy and organic pollutants degradation.	[38]
Thermal	Photothermal	10 mL of GO (2 mg/mL) solutions drop-casted on the surface of DVD disc and dried overnight. Scribing with	Process: One Step	Porous and ripples structure	* The thickness of rGO was expanded to	Electronic, optoelectronic and electroacoustic devices/ *Allows for transfer-free graphene.	[40]

		788 nm laser pulses with 5 mW of maximum power			10 times after laser scribed		
	Hydrothermal	GO suspension (1 mg/mL) in DI water. Pot: Teflon-lined autoclave. Reduction temperature between 100-200 ˚C.	Process: One pot	RGO sheets reduced at 180 ˚C was larger than that at 200℃	N/A	*GO sheets could be simply reduced at a temperature of 180-200℃.	[16]
	Solvothermal	Preparation of GO and TiO_2dispersion in solvent. Solvent: DMF. Pot: Teflon-lined stainless steel autoclave. Reduction at 180 °C for 8 h.	Process: One pot	Confirms the formation of TiO_2 and rGO nancomposites	Calculated rGO sheets with d-spacing of 0.34 nm	Environmental pollution/*As a catalyst material for photoreduction process of heavy metal like hexavalent chromium Cr (VI).	[17]
Irradiation	Solar	GO with 0.01% dispersion in DI water. Sunlight irradiation from 11 am to 4 pm, at a range of 42 W m^{-2} to 373 W m^{-2}	Process: One-step	Layer sheet, folding and wrinkled	0.7–1.4 nm (~2-4 layers)	Biosensing and Energy storage/* Energy saving method.	[18]
	Microwave	Reduction using a conventional microwave oven operated at 1000 W for 1- to 2-s pulses.	Process: Rapid	A highly ordered structure	Monolayer	Electronic devices/* This technique is claimed to yield a high quality of graphene features like in CVD graphene.	[41]
	Ultra-Violet	GO dispersions in Polyvinyl Pyrrolidone. UV irradiation (365 nm, 125 W, height ~5 cm) for 0 - 48 h.	Process: One-step	N/A	0.9 nm	*After the reduction, the rGO solution was very stable even for several months.	[19]

*Note: *Remark represents the important findings from the literature.*

3. Characterization Techniques for Graphene-Based Materials

Several characterization techniques can be used to analyse the unique properties graphene-based materials (graphene, GO and rGO.) These techniques are classified as the morphological study, spectroscopy analysis and structural study. Special equipments are required to conduct the characterization and to analyse the graphene-based materials (Figure 3). In general, the morphological study is carried out to visually inspect and evaluate the surface texture, shape, lateral size and the thickness using high-resolution microscopes such as atomic force microscopic (AFM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), high resolution-TEM [42]. Normally, the scale of magnification for the best morphological images for the graphene-based materials are seen in the range of 0 - 25 μm by using AFM [15], 1 - 400 μm by using SEM [43],[44] 10 - 500 nm by using TEM [45],[46],[47] and 2 - 5 nm by using HR- TEM [48],[17]. These scales of magnifications are suggested as primary indication or setup to search and evaluate the nanomaterial morphology. Meanwhile, the spectroscopy techniques are used to identify the material authenticity and defects [22], [49], the presence/absence of functional groups [12], the nanomaterial's transparency [21] and their elemental composition analysis [33],[50] via Raman spectrometer, Fourier transform infrared spectrometer (FTIR), Ultraviolet-visible (UV-Vis) spectrophotometer and X-ray Photoelectron Spectroscopy (XPS) or Energy Dispersive X-ray spectroscopy (EDX), respectively. The combination of these spectroscopy techniques are the commonly used technique to evaluate the GO reduction effectiveness or in other words the conversion from GO to rGO. Moreover, the crystal structure and their interlayer spacing of graphene-based materials can be analysed via X-ray diffraction (XRD) [21],[51]. From our observations, these techniques are indeed requires high-end equipments that bear a lot of costs but the outcome of the research are worth and beneficial to human and for the sustainable development.

The morphological, structural and spectroscopy analyses of rGO that have been reduced via various solution-based green reduction methods are illustrated in Figure 4. Figure 4a shows the AFM image of rGO nanosheets that have been reduced by using green reductant (Vitamin-C). The image clearly reveals the rGO nanosheets exhibit a flat-like shape with a thickness of 1 nm. Meanwhile, in Figure

4b, the TEM image reveals the layered and the wrinkled rGO as well as the distribution of TiO_2 nanoparticles on the rGO nanosheets. Evidently, both morphological results give insightful information on evaluating the atomic thickness and the shape of the rGO. Furthermore, the structural analysis of rGO is shown in Figure 4c, where the GO has been reduced via electrochemically. Noticeably, the diffraction peak of GO (before reduction) occurs at a lower angle (2Theta = ~10.8˚) with a higher value of interlayer spacing (~0.82 nm) [52]. However, upon the reduction process the diffraction peak shifts to a higher angle (2Theta = 24.3˚ which corresponds to the lattice plane of (002) that shows an ordered structure) with a lower value of interlayer spacing (0.37 nm), as shown in Figure 4c. The Raman spectroscopy for the rGO (red-profile) which has been synthesized by using green reductant (extract of dry leaves of Psidium guajava) is shown in Figure 4d. Notably, the two distinct peaks of D- and G-bands from Raman spectra reveal the defects and the graphitic nature of the materials, respectively. Thus, it serves as a fingerprint to identify the graphene and considered as the most important characterization technique. A further confirmation on the GO reduction can be analyzed by using FTIR and UV-Vis spectroscopy analyses as shown in Figure 4e and 4f, respectively. The GO has been reduced by using ascorbic acid. The FTIR result revealed the presence of all oxygen functional groups of GO and the intensity peaks of the specific functional groups decreases or removed completely upon the reduction process. Similarly, the UV-Vis spectroscopy shows the maximum absorption peak of GO shifts to a higher wavelength upon the reduction process.

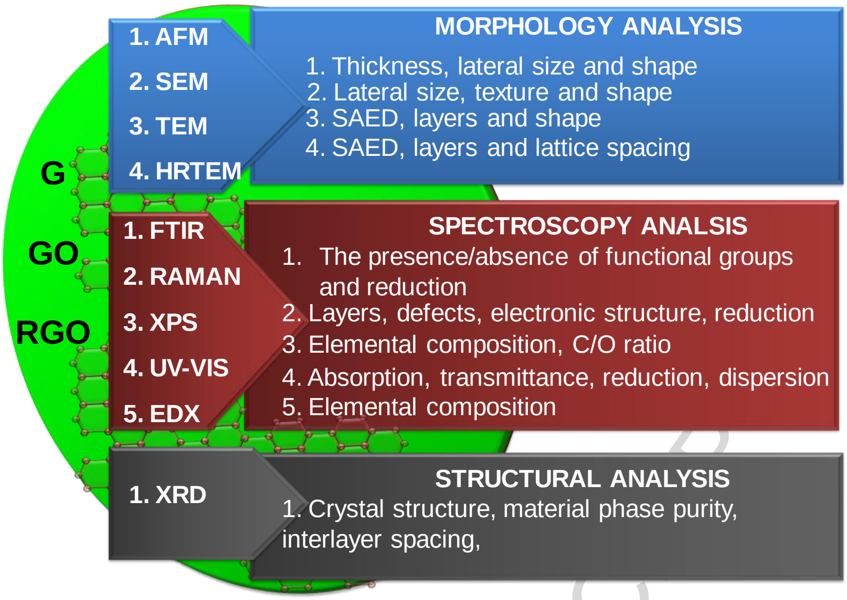

Figure 3: Schematic illustration of characterization techniques for graphene-based materials and their related equipment.

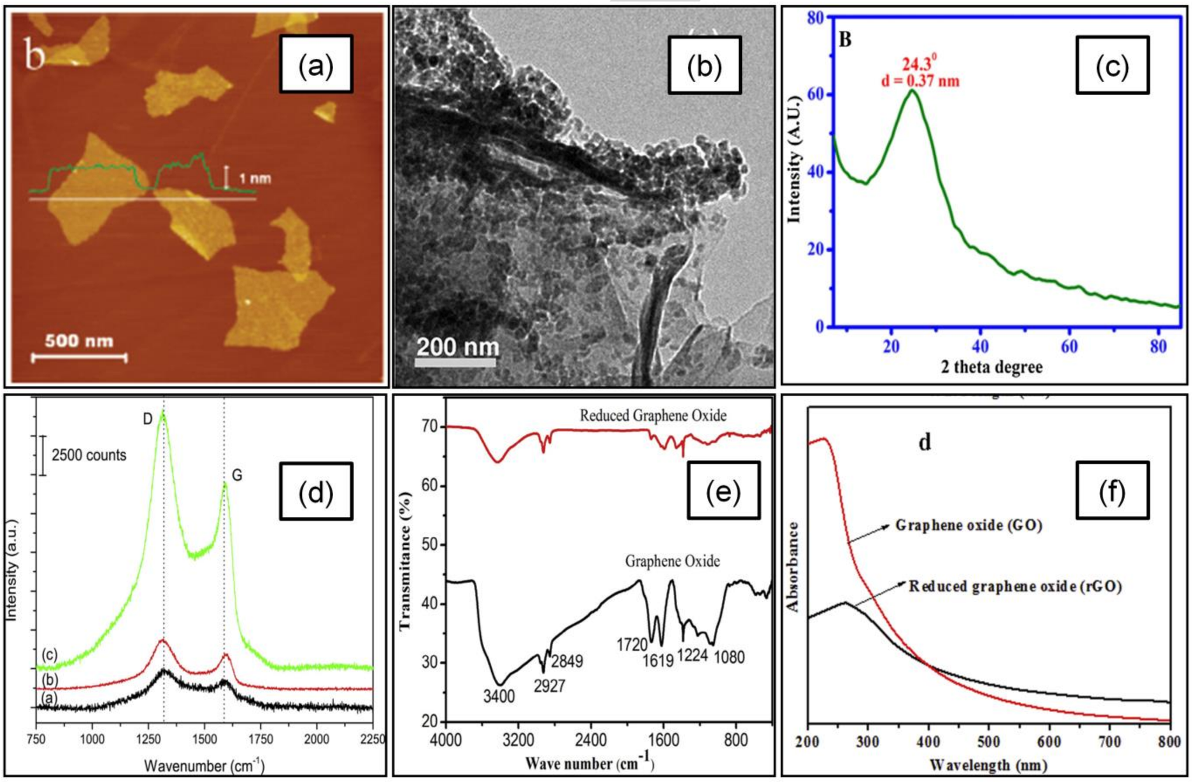

Figure 4: (a) AFM image of rGO reduced by using green reductant Vitamin-C, (b) TEM image of TiO_2-rGO reduced via photocatalytically, (c) XRD pattern of rGO reduced via electrochemically, (d) Raman spectra of rGO (red) reduced by using aqueous extract of dry leaves of Psidium guajava, (e) FTIR spectra of (rGO) powder reduced by using ascorbic acid, (f) UV–Visible spectra of rGO (blue) by using

ascorbic acid. Figures reproduced with permission from (a) Ref. [53], Copyright 2010 American Chemical Society., (b) Ref. [35], Copyright 2012 American Chemical Society., (c) Ref., [54] Copyright 2017 Elsevier B.V., (d) Ref. [22] , Copyright 2017 Elsevier B.V., (e) and (f) Ref. [12] Copyright 2017 Elsevier B.V.

4. Application of Green-rGO in Electrochemical Biosensors

Recently, the research and the application of rGO in biomedical fields can be seen from diverse aspects such as drug delivery [55],[56], tissue engineering [57],[58], biosensor [59],[60] and bioimaging [61],[62]. However, the biosensor field-related research in the past two decades has seen tremendous growth because it serves a major role in early detection and enabled for prompt treatment. Moreover, the biosensor approach offers a low-cost diagnostic method and the product can be used directly by any individual for regular check-up to monitor health condition. There are various types of biosensors have been developed up to date. One way to classify the variety of biosensors is thru the types of transducer used such as optical-based [63], physical-based [64] or electrochemical-based transducers [65]. Among these types of biosensors, the electrochemical biosensor becomes a mainstream research because it offers a facile method in fabrication and further brings down the cost. Thus, engineering in material science is continuously carried out to improve the performance of the biosensor in term of sensitivity and selectivity. Therefore, various transducers architectures have been used to develop an electrochemical biosensor. For example, paper-based electrode, field effect transistor (FET), interdigitated electrode (IDE), screen-printed electrode (SPE) and glassy carbon electrode (GCE) are the commonly used transducers. Furthermore, the incorporation of nanomaterials especially rGO in transducer have enhanced the biosensor performance. This is because the rGO exhibit a high surface area and contains a large number of oxygen moieties that ease for surface functionalization. Moreover, the greenly synthesized rGO has also paved for cleaner and green environment. Table 2 shows the various types of electrochemical biosensors developed in the past three years for the detection of biological samples using greenly produced rGO. Clearly, the greenly produced rGOs shows the ability to detect the various types of analytes and also showing an excellent detection performance where the limit of detection of target had reached to micromolar. Further elucidation on application of greenly produced rGO in biosensing engineering can be found below.

4.1 Organically-reduced graphene oxide in biosensing

Recently, the rGO synthesized via green reductants are applied to develop electrochemical biosensors for various analytes detection. Herein, to ease our discussion we label the rGO synthesized via green reductants as organically reduced graphene oxide (OrGO). The OrGO show wrinkled nanosheet morphology besides showing the ability to attach nanoparticles [Figure 5(a-d)]. Generally, varieties of nanoparticles have been anchored onto OrGO nanosheets to develop electrochemical biosensors. Such OrGO and the nanoparticles composite have showed a further growth in surface area and enhance the biosensors sensitivities as well as selectivity. For example, Amanulla et al. [66] fabricated a non-enzymatic biosensor for hydrogen peroxide detection. They used ipomoea pestigridis leaf extract as reducing agents to reduce GO. They further decorated the rGO sheets with iron nanoparticles (FeNPs) to form a nanocomposite material. Then, the as-prepared nanocomposite was used to construct the biosensor on the GCE. They found the TEM image confirmed the decoration of FeNPs into rGO nanosheets and the FeNPs are in irregular shape (Figure 5a). Moreover, the as-prepared nanocomposite (FeNPs/rGO) shows an enhancement in reduction of current response to 9 folds in detecting the hydrogen peroxide than rGO alone electrodes. Their observation shows the as-fabricated biosensor having high selectivity towards the hydrogen peroxide detection. Recently, Akkaya et al. [67] developed a novel biosensor based on direct electrochemistry approach that can decently detect glucose oxidation. The biosensor was constructed using GCE. They used a natural reductant of tannic acid to reduce GO and anchored platinum nanoparticles (PtNPs), simultaneously (Figure 5b). The as-fabricated biosensor shows the detection limit of glucose of 1.21 μM. Moreover, they also demonstrated that the tannic acid with stimuli-responsive polymer such as poly(N-isopropylacrylamide) (PNIPAAm) can develop pH and temperature controlled switchable biosensor. In general, they have demonstrated the versatility attributes of the natural tannic acid in developing the biosensor with less-complex procedures. On the other hand, Burss et al. [68] had fabricated electrochemical biosensors to detect glucose (enzymatic-biosensor) and *Escherichia coil* O157:H7 (RNA aptasensor) using rGO that had been prepared via thermal and ascorbic acid reductions. They used paper-based electrode to make the biosensors. They demonstrated the rGO with platinum nano-cauliflower shows a high electroactive surface area (≈ 0.29 cm^2) for the detection of glucose and *Escherichia coil* O157:H7 (Figure 5c). Moreover, the biosensors show a competitive sensitivity with commercial electrodes with the limit of detection to 0.087 μM and ≈4 CFU m/L for both glucose and *Escherichia coil* O157:H7, respectively. In addition, their electrochemical analysis shows the thermally rGO shows poor in charge transfer compared to rGO synthesized via both treatments (thermally and ascorbic acids). Very recently, Zheng et al. [69] have reported the simultaneous detection of uric acid and tyrosine using a nanocomposite composed of molecularly imprinted polymer (MIP)/rGO deposited on the GCE. They reduced the GO using ascorbic acid. They found the incorporation of MIP and rGO increases the surface area of the sensing interface (Figure 5d). The as-fabricated biosensor shows the limits of detection for uric acid ~0.0032 μM and tyrosine ~0.046 μM with wider linear ranges of 0.01–100 μM and 0.1–400 μM, respectively. Moreover, they reported that the utilization of MIP/rGO as a nanocomposite resulted in the enhancement of electron transfer rate, provides large sensing sites, having good selectivity, exhibit long-term stability, good reproducibility and the achievement of simultaneous determination of both targets.

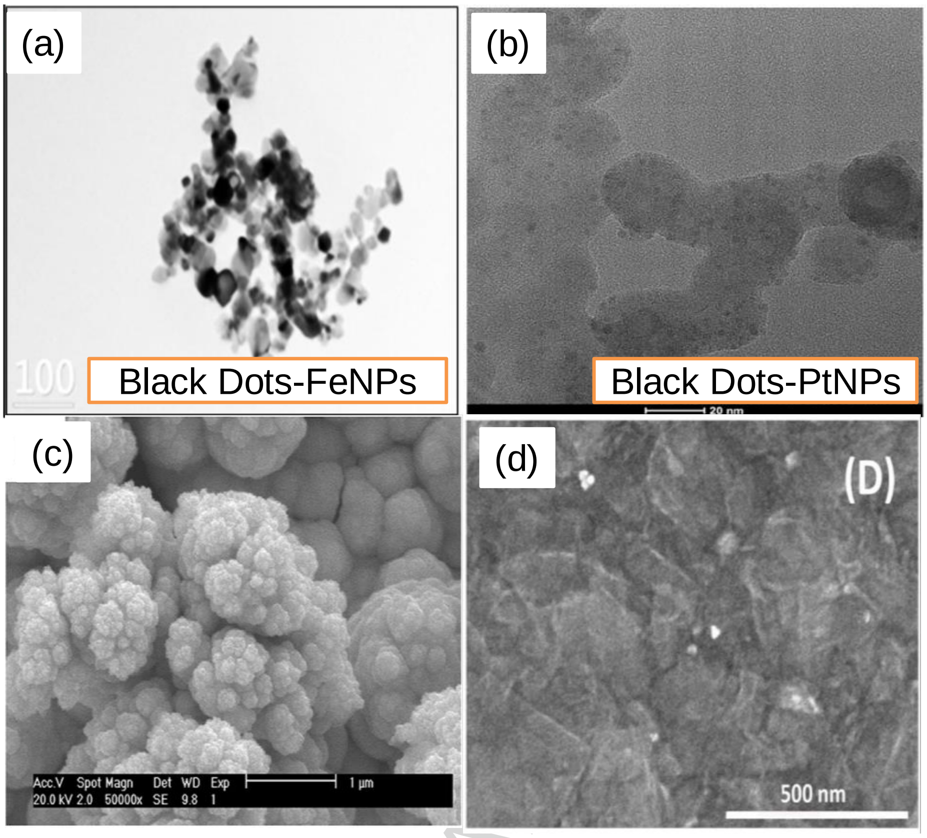

Figure 5: (a) TEM image of rGO/FeNPs nanocomposite, (b) TEM image of rGO-PtNPs with platinum particles size distribution in the range of 2.0 nm to 4.5 nm, (c) SEM image of pulSED nano-cauliflowers on rGO, (d) SEM image of MIP/rGO modified on the GCE. Figures reproduced with permission from (a) Ref. [66], Copyright 2017 Elsevier B.V., (b) Ref. [67], Copyright 2018 American Chemical Society., (c) Ref. [68], Copyright 2016 Elsevier B.V., (d) Ref. [69], Copyright 2018 Elsevier B.V.

4.2 Electrochemically-reduced graphene oxide in biosensing

Apart from using green reductants, GO reduction via electrochemically (ErGO) have also shown a good electrocatalytic activity, improved in sensitivity, exhibit a good selectivity, having a wider linear range of detection, showing reproducibility as well as having long-term stability. The ErGO have been used to develop both enzymatic and non-enzymatic biosensors. For example, Yajiv et al. [70] fabricated a non-enzymatic biosensor using a composite of ErGO-nafion and gold nanoparticles (AuNPs) to detect hydrogen peroxide on the GCE. SEM images reveal that the ErGO-nafion looks tiled-thick sheets with the appearance of wrinkles [Figure

6 a(i)] and the as-electrodeposited AuNPs shows uniform distribution on ErGO nanosheets [Figure 6 a(ii)]. They found the nanocomposite shows a good electrocatalytic activity for the detection of hydrogen peroxide [Figure 6 a(iii)] with a detection limit of 2 μM, good sensitivity (574.8 μA mM^{-1} cm^{-2}), good selectivity, long-term stability and reproducibility. Moreover, very recently, Li et al. [71] fabricated a novel non-enzymatic biosensor for glucose detection using gold nanoparticles (AuNPs) and ErGO. They assembled the nanocomposite via π-π stacking assembly of phenoxyl-dextran on the screen-printed electrode. They used Concanavalin A as biorecognition element to covalently link AuNPs and make a specific biorecognition probe for glucose detection. They reported a sensitive transduction signal was observed due to the reduction of GO via the electrochemical method. SEM images reveal the morphology of ErGO with 3D porous net with a plentiful of wrinkles and fluctuated nanosheets [Figure 6 b(i)] and a large amount of AuNPs decorated on the ErGO surface [Figure 6 b(ii)]. They found the biosensor exhibits a detection limit of 0.02 mM for a wider linear range of 0.05–100 mM evaluated by using differential pulse stripping voltammetry (DPSV) [Figure 6 b(iii)].

Besides that, Sethuraman et al. [72] fabricated an enzymatic biosensor using conduction polymer poly (3,4-ethylenedioxythiophene) PEDOT, ERGO, Fe_2O_3 nanoparticles, and polyphenol oxidase as a nanocomposite matrix on the GCE to detect catechol [Figure 6 c(i) and (ii)]. The as-fabricated biosensor shows a wider linear range of 0.04-62 μM with a limit of detection of 0.007 μM towards catechol [Figure 6 c(iii)]. Moreover, they reported that biosensor have no interference influence from various compounds (L-glutamic acid, D-glucose, o-nitrophenol and ascorbic acid) and having stability at least for 75 days by storing in a buffer medium at 4°C. However, the fabrication of this type of biosensor involves complex and multistep procedures. Another example of a novel biosensor using ErGO was developed by Wen et al. [73]. They developed an amperometric biosensor using a nanocomposite of ErGO and biopolymer (chitosan) on the GCE. They immobilized hemoglobin on a GCE to detect an organic nitro compound of nitromethane. They found the as-fabricated biosensor shows an excellent catalytic activity and faster in electron-transfer rate towards the organic nitro compound detection. Moreover, the biosensor displays a faster electrochemical response within 5 seconds to detect the nitromethane with the detection limits of 1.5 μM and exhibit a wider linear range of detection of 5 μM-1.46 mM, under optimal condition. They also reported that the developed biosensor shows high selectivity, stability and reproducibility as well as the possibility for monitoring in real samples.

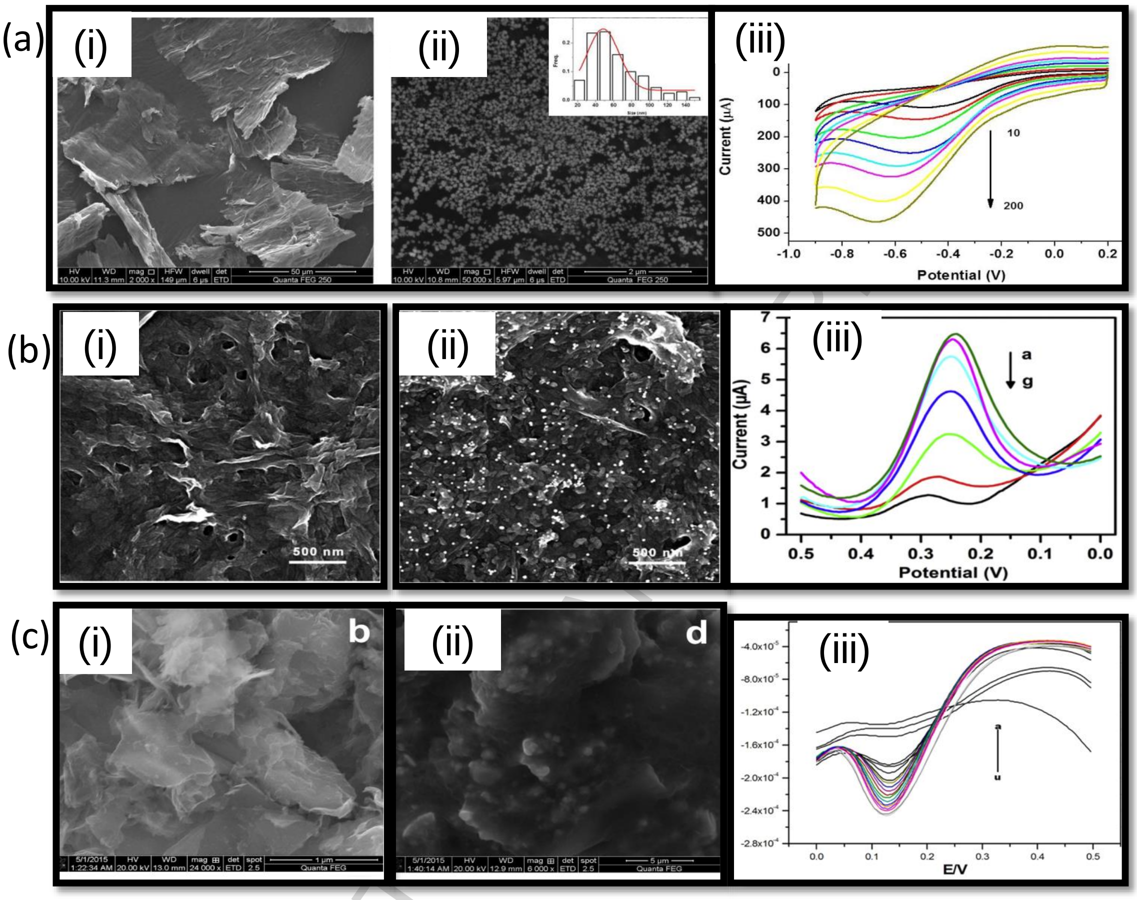

Figure 6: (a) SEM images of (i) ERGO-Nafion on the surface of GCE, (ii) AuNPs on ERGO-Nafion. (iii) CV curves of the GCE/ERGO-Nafion/AuNPs electrode in detecting hydrogen peroxide at various scan rate of 10 -200 mV/s. (b) SEM images of (i) ERGO-modified electrode, (ii) AuNP/Con A decoration (iii) DPSV responses toward glucose detection at various concentrations of 0.01 to 500 mM (a→g). (c) Fe-SEM images of (i) PEDOT-rGO, (ii) PEDOT-rGO-Fe2O3-PPO modified electrodes (iii) DPV responses towards catechol detection at various concentrations of 0.025 - 100 mM (a→u) . Figures reproduced with the permission from (a) Ref. [70] Creative Commons Attribution License 3.0 2016, (b) Ref. [71], Copyright 2018 Elsevier B.V, (c) Ref. [72], Copyright 2016 Elsevier B.V.

4.3 Photocatalytically-reduced graphene oxide in biosensing

Recent studies show the synthesisation of rGO via photocatalytically is still ongoing (Table 2) and the research is slowly shifting towards applications-based research in various sectors like oxygen production from water [74], organic pollutant degradations [75] and the degradation of methylene blue dye [76]. Moreover, we found not many works is dedicated to biosensing engineering using photo catalytically rGO (PrGO), recently. However, the PrGO has been suggested for specific application area like in optoelectronics devices [37],[77] degradation of organic contaminant [78] and lithium-ion batteries [35]. Nevertheless, until very recently Kong et al. [43] had developed an electrochemical biosensor based on the tetrapods-like titanium nitride (TiN) and PrGO nanocomposite to determine chloramphenicol. They reported that GO is effectively reduced under photo-reduction process assisted by ultraviolet illumination and finally obtained a nano-hybrid composite which was then used to modify the GCE. The as-fabricated biosensor demonstrates a good electrochemical response due to the synergic effect of both materials with detection limits of 0.02 μM and wider linear range of 0.05–100 μM towards chloramphenicol detection. Moreover, they reported the nanocomposite synthesisation method is simple, fast and efficient without need for any additional reducing agent to form such nano-hybrid composite.

Very recently Soni et al. [79] have demonstrated that the photo-reduction process can be used directly for patterning GO-rGO nanosheets in the flexible substrate by using a photomask. Their method could pave a way for facile electronic device fabrication because the method could possibly reduce the number of standard lithography processes in involves in fabricating the electronic device. Hence, the utilization of PrGO in biosensors development is still very limited and more work can be concentrated in the near future to explore the resulting nanocomposite performance and fitness in biosensors.

Table 2: Application of green rGO in biosensing and their electrochemical performance

Green Reduction	Device Architecture	Target/analytes	Transduction mode	Linear Range	LOD (μM)	Ref.
Organically	GCE	Hydrogen peroxide	Amperometric	0.1 μM - 2.15 mM.	0.056	[66]
Organically	PBE	Glucose	Amperometric	0 - 500 μM	0.08±0.02	[68]
Organically	GCE	Glucose	CV	2 - 10 mM	1.21	[67]
Organically	GCE	Uric acid and Tyrosine	DPV and CV	UA: 0.01 - 100 μM TY: 0.1- 400 μM	UA: 0.0032 TY : 0.046	[69]
Electrochemically	GCE	Hydrogen peroxide	Amperometric and CV	0.02-23 mM	2.0	[70]
Electrochemically	GCE	Nitromethane	Amperometric and CV	5 μM - 1.46 mM	1.5uM	[73]
Electrochemically	SPE	Glucose	DPSV	0.05 - 100 mM	20.0	[71]
Electrochemically	GCE	Catechol	DPV	0.04 - 62 μM	0.007	[72]
Photocatalytically	GCE	Chloramphenicol	CV	0.05 - 100 μM	0.02	[80]

GCE: Glassy carbon electrode, PBE: Paper-based electrode, SPE: Screen printed electrode, CV: Cyclic voltammetry, DPV: Differential pulse voltammetry, DPSV: Differential pulse stripping voltammetry, UA: Uric acid and TY: Tyrosine.

5. Conclusion and Perspectives

In this article, various green reduction strategies to synthesis rGO nanosheets are arranged and systematically outlined. A strategy particularly the solution-based green reduction methods have been comparatively elucidated based on the recent progression in term of their reduction methods, conditions, complexity, characteristics and the application of rGO in electrochemical biosensors. Clearly, the solution-based reduction methods (of green reductant, electrochemical or photocatalytic reductions) have shown a good ability in producing a hybrid nanocomposite with one-step process of synthesis. Moreover, the methods also offer numerous advantageous like eliminates the use of additional chemicals, high temperatures or the need for high-end equipments hence a low-cost process that is suitable for large scale-production and friendly-environmental. Besides that, the application of resulting nanocomposite in fabricating the electrochemical biosensors has shown a good ability in detecting the various biological analytes. Furthermore, the biosensors show an excellent detection performance in determining dopamine, uric acid, L-tyrosine, hydrogen peroxide and glucose when compared to the rGO nanocomposites which were synthesized by using the chemical reduction methods [81],[82],[83],[84]. Based on the recent progression, we noticed that the ErGO is more suitable to be applied in electrochemical biosensors because it offers a good electrocatalytic activity [70],[73]. Meanwhile, the PrGO is suggested for optoelectronic based applications because the hybrid nanocomposite have shown an enhancement in the photocatalytic activity [37],[77]. Furthermore, the OrGO have been applied for various applications ranging from drug delivery, textile industry, besides biosensors development because the reduction process itself is simple and easily observable through the changing of GO suspension color from orange to black. We also noticed that other green reduction strategies (voltage-induced, thermal and irradiation) have shown a good capacity in producing rGO. Among these strategies, the irradiation strategy especially reduction via microwave pulses have shown a superior ability to produce a pristine like-graphene. In addition, we noticed that the reduction of GO via electrically is the most green method, faster and safer approach than the other three matured strategies [35]. However, a lot of investigation is required as this method is still at infancy stage.

In the near future, more research is required in order to obtain a desired size of nanosheet with high quality or defect-free rGO nanosheets. Further, study on solution-based green synthesis can be focused towards the parameters optimization such as the effect of time, temperature and reductant concentrations in effort to produce a single layer of rGO nanosheet. Moreover, one should clearly define the characteristic of the produced rGO in term of their thickness, reduction degree and sheet morphology. This is because of the rGO thickness itself affects the physical, optical, mechanical and electrochemical properties where it directly controls the performance of electronic devices. Besides that, the application of greenly produced rGO could be extended to various fields and is not limited to the electrochemical biosensors. The other potential sectors which need more attention on utilizing the greenly produced rGO could be extended pristine graphene for the application in the transistor fabrication, micro-supercapacitor, transparent electrodes, in addition to rGO application itself, i.e. flexible electronic sensors, electroacoustic devices, heavy metal detection sensor, gas sensor, bio-imaging, food industry, environmental monitoring as well as a coating materials and of course not limited to this sector. Recent advancement clearly has shown an active research is going on because the demands to produce rGO nanosheets via greener methods are much preferable.

Furthermore, hybridization between greenly produced rGO with other 2D nanomaterials, for example, Molybdenum disulfide (MoS_2) nanosheets need to be explored apart from hybridizing with metal nanoparticles.

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Highlights

- Reduction of graphene oxide resulting nanomaterial and rGO are utilized widely.
- Green reduction, especially the solution-based reduction is overviewed.
- Degrees of gaining tips with all aspects are elucidated.
- The applications in electrochemical biosensors are elucidated.
- Analyses scheme for the graphene-based material are outlined.