A Short Review on the Impact of Graphene-Based Nanomaterials (Graphene oxide and Reduced Graphene Oxide) in the Biomedical Industry

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***Abstract*— Graphene is a highly densely packed sp2 hybridized single layer carbon atom two-dimensional honeycomb structure. Due to its chemical, physical, physicochemical, and biocompatible properties day by day use of graphene oxide (GO) and reduced graphene oxide (RGO) has been increased in biomedical, biotechnology, and pharmaceutical research fields. Graphene oxide is used as an energy storage device (battery and supercapacitors), biosensors, photocatalysis and biomedicine purposes, etc. Not only GO but its modified nanomaterials like RGO have applications in medical biotechnology. RGO nanocomposites are broadly used in drug delivery systems to deliver insoluble drugs, nucleic acids, and other molecules into cells for bioimaging. Due to its antimicrobial and cytotoxic properties, its uses have been increased as a therapeutic agent. For therapeutic effect, it should be noticed its size range and dosage. Hybrid materials like gold-based GO and RGO sheets have excellent properties as catalytic biosensing agents. Though graphene-based nanomaterials like GO and RGO has several novel applications in biomedical, biotechnology as well as a pharmaceutical research field, these have several drawbacks for the environment and human health also. But proper synthesis procedures could reduce its adverse effect on the environment. In this review, we studied the different methods of synthesis of RGO from GO like green synthesis, hydrothermal method, solvent assistant thermal reduction, etc., and their applications in the biomedical field as well as health care also.**

***Keywords:* Graphene, Graphene oxide(GO), Reduced Graphene oxide(RGO), Nanomaterial, Biomedical, Biosensing, Green synthesis.**

I. INTRODUCTION

Carbon is one of the most abundant elements of earth. It takes 4th position as an abundant element after hydrogen, helium and oxygen. In this present era, the uses of carbon based nonmaterial have taken a great role in the research field of biomedical, pharmaceutical as well as medical due to biocompatibility and others properties. Graphite and its different derivatives already used in several segments of our daily life due to their non toxic and biocompatibility nature up to a certain range [1].

Graphene is synthesized from graphite. Graphene is hexagonal densely packed sp^2 hybridized single layer two dimensional hexagonal lattice arrangement allotrope of carbon atom [2]. It has honeycomb like three dimensional frame block structure. Due to pi electron bond, graphene has good electronic property. Not only this, it is one of the lightest element but mechanically strongest of the earth. It is around 40 times stronger than diamond [3]. Mostly useful methods to synthesize graphene are top-down strategy and bottom-up strategy [4].

Graphene oxide (GO) is synthesized from graphene. Due to its biocompatibility, physiochemical attributes, heamocompatibility, non-toxic in a certain limit it is used in biomedical as well as pharmaceutical research since last few years. In biomedicine GO is used as biosensors, novel drug delivery system, medical image processing, chemotherapy etc. [5].

GO is oxygen group contained 6 carbon atomic honeycombs structured which formed a ligand so it is used as medicine. Due to its sp^2 hybridization it has large surface area; for this property it is used in utilization of single stand DNA (ssDNA), detection and selection of pi-pi electron bond stacking molecule [2]. GO has also antibacterial, anti-fungal, cytotoxic etc. properties.

Reduced graphene oxide (RGO) is synthesized by removal of functional groups of GO by different reduction method such as green synthesis, thermal treatment, solvent assisted thermal reduction, electrochemical reduction, thermal reduction and hydrothermal method etc. [6, 7, 8, 9, 10].

RGO nano composites have been used for biosensors, medical image processing. Due to its anti- microbial as well as cytotoxic activity, it has been used in biomedicine and cancer treatment also.

In this article, different methods of synthesis of GO and RGO have been discussed. Application of GO and RGO in biomedical industry have reviewed.

II. METHODS TO SYNTHESIZE GRAPHENE OXIDE

Tour Method: GO is prepared in tour method. This method has few steps:

Oxidation or Intercalation of Graphite powder: Graphite powder reacts with mixture of potassium permanganate ($KMnO_4$) with sulfuric acid (H_2SO_4) and phosphoric acids (H3PO4) are mixed in 9:1 ratio for 12 hours at 50℃. Here, H_2SO_4 is used as one of the most common intercalating agent. [11]

Exfoliation: The oxidized dispersed in distilled water distilled water and heated with magnetic stirrer for next 12 hours which makes black precipitate of GO. Then centrifuged and dried at 60 ℃ and collect the GO.

Hummer's Method: To synthesis GO from graphite powder, Hummer's method is one the most used method [12]. Graphite powder is mixed with $NaNO_3$ and H_2SO_4 and kept in ice bath. Then under the stirring condition $KMnO_4$ solution is added with the mixture in ice bath. Then this solution is transferred into water bath where the temperature is more than water bath.

Then the solution is sonicated and after that H_2O_2 and Deionized water is added and color is changed. Now the mixture is centrifuged and washed until see the clear decant. Dry the sample and collect the powder sample [13, 14, 15].

In Hummer's method, for pH adjusting, dilute H_2SO_4 is used as acid solution and dilute KOH is used as alkaline solution.

There are different methods which are used to synthesize GO from graphite powder except above mentioned Tour and Hummer's method. Different methods are responsible for different concentration of oxygen of functional groups which are present in GO molecule [14]. This oxygen concentration variation impacts on their specification like size and shape of the powder nano particles, mechanical strength as well as some chemical properties.

III. SYNTHESIS OF REDUCED GRAPHENE OXIDE (RGO)

Electrochemical Reaction: RGO is synthesized from the GO which is produced in Hummer's method. Here, the carbon nanotube is prepared by deposition of carbon vapor. After that, it is refluxed with HNO_3 and washed with deionized water. In this method, graphite powder used as cathode electrode and platinum plate as anionic electrode. GO powder is dispersed in electrochemical cell where potassium nitrate (KNO_3) is used as electrolyte solution. The reduction is done by applying different voltage [16].

RGO is also prepared by this electrochemical method by using oxygen electrode. Here, carbon nano-tubes are oxidized with heptavalent manganese. Hypophosphite and sulfite are used as reducing agent [17].

One pot synthesis method: Here, RGO/metal nano composite is prepared by few following steps; at first GO and metallic compounds mixed and heated on magnetic stirrer. Then after certain time reducing agent mixed into the heated solution and again continue the stirring when the color is changed the RGO is formed GO. To remove excess metal then it is centrifuged and washed several times with deionized water and then dried in lyophilizer. Finally collect the dried sample [18, 19].

Tour Method: GO powder dispersed into the deionized water and ascorbic acid is mixed as reducing agent and heated for 30 minutes at 60 ℃. Then the reducing mixture is centrifuged and washed with ethanol and deionized water for thrice followed by centrifugation. After this, collected sample is dried in oven at 60 ℃ for 24 hours to collect the dried powder sample [11].

Synthesis of reduced graphene oxide by green method*:* Here, any plant based material or homeopathic mother tincture is used as reducing agent. The use of this method has been increased due less adverse effect and less toxicity than synthesized chemicals. Here, plant extract solution is added with the mixture of GO powder and Deionized water and heated with magnetic stirrer for one hour at 70 ℃ [20]. At this temperature reducing agent and capping agent is removed by evaporation. To remove the excess material except RGO, H_2O_2 is added to separate the solvent and the black precipitate is RGO which is collected by Whatman number 1 filter paper. Then collect the filtered sample and dried in oven at 50 ℃ for 5 hours and collect the cake formed dried sample which ground by using glass mortar and pestle [21].

Among these above mentioned method, Green synthesis method is more used to manufacture graphene derivatives in industrial level due to more ecofriendly, economically cheaper than others way, availability of reducing agent, less adverse effects than chemicals etc.

RGO Synthesis by Hydrothermal Method: Here, GO powder in dispersed in DI water and add reducing agent; then 30 min in an ultrasound bath. After this, this GO solution transferred into Teflon autoclave at 140 ℃ for 6 hrs. The internal pressure 140 KPa. After autoclave when it comes in to room temperature, then collect the sample and washed several times with ethanol or DI water to remove the excess metals from RGO. Then the sample is dried in air dryer or oven and collects the sample [23].

Table 1: Different Methods to Synthesis RGO:

Methods	Reducing Agents	References
Tour Method	Ascorbic acid	[11]
Green Synthesis Method	Plant extract	[20]
One Pot Synthesis Method	Ammonium Hydroxide (NH4OH)	[19]
Electrochemical reduction	Hypophosphite and sulfite	[16]
Thermal treatment	Hydrazine monohydrate	[22]
Hydrothermal Method	Effect of pH (Using HCl or NaOH)	[23]
Solvent-assisted thermal reduction	Dimethylformamide, Ethylene glycol	[24]

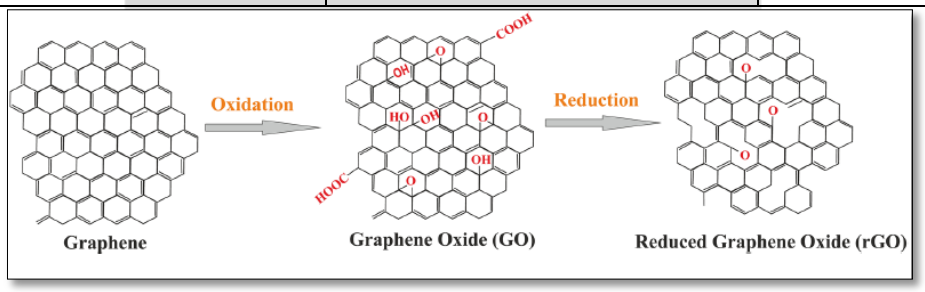

Figure 1: Covertion to Graphene to GO and GO to RGO [25].

IV. CHARACTERIZATION OF GO and RGO

GO and RGO is characterized by UV-visible spectrometer in 200-800 nm wavelength range to evaluate the photo catalytic activity. Fourier transform infrared spectroscopy (FTIR) is used to detect the presence of functional groups in synthesized GO and RGO. The X-ray diffraction (XRD) is used to check the interlayer spacing [26, 27].

V. APPLICATION OF GO IN BIOMEDICAL INDUSTRY

Gene Delivery: Polyethyleneimine (PEI) is mostly used as surface enhancer of GO sheet to deliver the gene to the targeted cells in complex formation by electrostatic interaction and this coagulates with plasmid DNA (Pdna) by covalent bond [28]. GO forms covalent bond with branched [29] and linear [30] Polyethyleneimine which showed high gene e transfection efficiency with low cytotoxicity in comparison with PEI/pDNA complexes [31].

GO's role in Biosensor: Due to presence of pristine structure and oxygen containing functional groups, GO is used as a advance leveled biosensor material to detect active biological molecule which is one of the most important part of biomedical as well health industry [32, 33]. It has also good biocompatibility, high water dispersibility, specific detection power of biological molecule so it is used in various novel applications of biomedical field, like electrochemical detection and laser desorption/ionization mass spectrometry (LDI-MS) [34, 35], surface-enhanced Raman spectroscopy (SERS), fluorescence resonance energy transfer (FRET) based biosensors etc. [36].

Fluorescent sensors for DNA/RNA detection:

DNA Detection: The surface interaction between DNA (phosphate backbone which contained negative charge) and the functional groups of GO (-OH, -COOH, C=O etc.) help to design and optimization of different biomedical devices to detect biological molecules with high sensitivity capacity which takes role in diagnosis and treatment of several diseases [37]. GO has behavior of surface adsorption of DNA molecule of quenching of the adsorbed fluorophores increased its uses as biosensors in several novel applications. The interaction of GO and DNA depends on several parameters like temperature, ionic concentration, hydrophobic interaction, pH of the buffer solution, electrostatic repulsion etc.

Fluorescent based micro RNA detection: Micro RNA (miRNA) is a very small non coding RNA molecule which regulates the gene expression as well as dysregulated in various types of human diabetes and cancers [38, 39, 40] This miRNA acts as biomarker of various diseases. It helps to diagnosis of several genetic disporder by dysregulation and abnormal expression of miRNA. So, GO is mostly used in biosensors or as biomarker in modern devices [41, 42].

Glucose Biosensors: Diabetes mellitus is one of the most common chronic diseases in the world. Since last few years, in advance biomedical research scientists have been used GO as glucose biosensor in several biomedical devices [43-46]. Due to insufficient production of insulin from beta cells of pancreas is responsible for diabetes mellitus [47]. This novel innovation helps to detect blood glucose level easily which reduce the leading causes of death rate due to diabetes [48].

Cancer Therapy and Drug Delivery System: Cancer is caused due to the uncontrolled cell growth which is affects the large percentage of people in every year and leads up to death. In every nine women, one is effected in breast cancer and died one in twenty nine women due to breast cancer. In this present era, this breast cancer is oneof the most common invasive malignancy through all over the world [49, 50].

Due to pi-pi electron stacking it takes role in anti-cancer drug delivery. Graphene nano composite produces reactive oxygen species (ROS) [51]. This ROS can destroy the tumor cells under radiation. Hydrophobic drug have the power to transmit the GO into the target cell. Due to large surface area and presence of active functional group in surface edges of GO, more amount of drug loaded is possible.

A novel drug delivery GO nanogel (polyaspartamide or hyaluronic acid based with double network GO) was able to deliver large quantity of drug (irinotecan) into the cancer cell [52-54].

Doxurubicin (DOX), anticancer drug delivery with GO gives a synergistic effect of cytototoxic activity on cancer cell (HepG2). We know that the chemotherapy is one of the most useful method to treat the cancer but due to use since last century, this has dearbck of drug resistance to treat cancer cell, so now the use of combinations of chemo-photothermal and chemo-chemo have tried to use to cancer treatment [55].

Antibacterial activity of Graphene oxide (GO): From antimicrobial assay of GO on gram negative bacteria, *E. coli* and *P. aeruginosa* strain which gives a standard result with marketed antibiotic drugs [56].

Go also has activities like water purification, super capacitors, Solar cells Coatings, hypothermia capabilities etc. [57-59].

Table 2: Application of Graphene Oxide (GO) in Biomedical Industry.

Applications	Reason of this Application	References
Cell Imaging	Strong mechanical strength	[60]
Cytotoxicity	Pi-pi electron stacking in the structure	[57]
Super capacitors	Great fluorescence quenching capacity	[59]
Biosensor	High dispersion rate in water	[58]
nti-bacterial effect	Complex formation	[57]

VI. APPLICATIONS OF REDUCED GRAPHENE OXIDE (RGO) IN BIOMEDICAL INDUSTRY

Cytotoxicity: Reduced graphene oxide has cytotoxicity activity in very small dose (it can be use up to (up to 50 µg/mL dosage range). It affects on mitochondrial activity and the cell membrane integrity. It also takes place in metabolic activity, decreasing cell adhesion. RGO takes place to prevent cell apoptosis [61]. Several factors are responsible for this cytotoxicity nature like, surface area, lateral size including dose of RGO particle. Large particles phagocytes where small particles can enter into the cell through clathrin-mediated endocytosis [62, 63].

RGO in tissue Engineering: Stem cells have capacity of auto-renewal and the ability to differentiate different cells according to their function. RGO have a strong impact on the differentiation of the stem cells [64, 65]. If we consider RGO in 3D scaffolds, it is closer than GO to mimic the natural microenvironment and it can be used as a good alternative for the translation of Graphene oxide application in biomedicine. This application is used to regenerate nerve, cardiac, bone etc. tissue regeneration [65-69].

Biosensors: Reduced graphene oxide is also used as biosensors to detect several biological molecules. RGO also used in biomedical devices like FRET. RGO based ion sensitive field-effect a transistor (ISFETs) is used to detect several biomolecules. This biosensing mechanism is a label free approach for analyzing and detection in contrast to the ELISA method [37-40].

VII. CONCLUSIONS

From this review it can be concluded that, graphene based materials GO and RGO are broadly used in biomedical industry for its physical, physiochemical, antibacterial, biocompatible properties. Due to GO's mechanical strength, presence of functional groups, electronic bond etc. properties it has different applications in biomedical industry like chemotherapy, biosensors to detect DNA, RNA and others biological molecule, biomedicine, gene delivery etc.[55, 56]. In different temperatures prepared GO has different significant properties due to presence of different functional groups. Due to presence of hydrophobic group it can be easily entered in to the cancer cell which helps the efficacy in cancer treatment as well as gene delivery also. RGO is also used as career of drug delivery, tissue engineering, bone regeneration, chemotherapy, biosensors, and antimicrobial agent, antibacterial agent also [67-69] RGO can easily regenerate the tissue like nerve tissue, cardiac tissue and bone also. For this above mentioned huge applications in different segments of health industry, it is needed to produce in large amount. Though there is several synthesis methods have been used since last few years but now scientists use green synthesis method due to less toxicity and side effects than synthetic product.

VIII. ACKNOWLEDGEMENT

The authors are thankful to School of Bioscience and Engineering, Jadavpur University to access all types of sources for this review.

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