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DEVELOPMENT OF GRAPHENE BASED NANOSTRUCTURES AND ITS APPLICATIONS IN FOOD QUALITY CONTROL AND HEALTH CARE

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ABSTRACT

Synthetic dyes are very often used as food colors to make the food stuff attractive. All the dyes however, are not good for health and therefore are not permitted to be used in food. There are total nine synthetic dyes approved by U.S Food and Drug Administration(FDA) which are commonly used in soft drinks, breakfast cereals, candies snacks, baked goods, frozen desserts etc. Recently certain dyes contain azo-groups are also found to be deleterious to the consumers causing cancers, hyperactivity and other disorders. So the determination of trace amount of these toxic food dyes in food stuff has become an analytical challenge. Graphene is a single layer of carbon atoms(sp^2 hybridized), that are packed in a hexagonal honeycomb crystalline structure. It has extremely high electron mobility at room temperature high surface to volume ratio and high electrical conductivity. Synthesis of graphene can be done either by chemical vapour deposition technique or by chemical exfoliation of graphite into Graphene Oxide(GO).The reduced GO(RGO) sheets are usually considered as one kind of chemically derived graphite whose properties can be altered depending on the reduction process. There is different domain of application in this graphene based nanostructures of which food quality control and healthcare is an important sector. In the present work our aim is to synthesize and characterize several graphene oxide based nanostructures and to study their applicability in the detection of trace amount of some carcinogenic food dyes.

KEYWORDS: Carcinogenic**,** Food and Drug Administration(FDA),Graphine, Graphene Oxide(GO), Nanostructures, Reduced Graphene Oxide(RGO).

I. INTRODUCTION

In recent times there are several media coverage reporting about the occurrence of toxic additives in processed foods, street foods, and restaurant cuisines. Centre for Science in the Public Interest (CSPI) has recently released a report claiming that certain dyes containing azo groups are found to be deleterious to the consumers causing cancer, hyperactivity, and other disorders. So the determination of these toxic food dyes in trace amount is becoming an analytical challenge as referred to Figure 1.

Recently several International groups are working in this field of developing systems for sensitive and selective detection of food colours in food and beverage samples. The photo catalytic activities of the synthesized pure ZnO nanoparticles(NPs) and ZnO-GO nanocomposite were investigated by photo degredation of azo-dyes.[1] A surface enhanced Graphine and Ag nanocomposite. In another work[3] an electrochemical sensor has been developed using chitosan and carbon nanotubes modified glassy carbon electrode to detect Sudan-1classified as category 3 carcinogen and which is forbidden in most countries. Currently, Gan et al[4] have developed an electrochemical sensor incorporating graphene and mesoporous TiO2 to detect sunset yellow and Tartrazine in food samples. In India Sudan dyes in red chilli powder has been detected by Masoodi et al following thin layer chromatography.[5] Binary mixture of synthetic dyes was detected via HPLC methods.[6] The optimal methods were applied to identify and determine the synthetic dyes in food stuffs and pharmaceutical samples.[7] So there is ample scope of research in the field of selective and sensitive detection of these toxic food colors in view of literature surveyed.

As we already know, Meat is an integral part of our diet which contains protein as major constituent which keeps our body fit. Preserving its quality from factors like atmospheric oxygen level, light intensity of storage, endogenous reactions and microorganisms, which influences its color, odor, flavor and texture and causes detrimental changes to the meat.[8] According to many research, the quality of meat gradually decreases till we consume them. This loss is attributed to chemical, physical microbiological and enzymatic changes occurring over period of time.[9]

With Ref. to an event before four decades, in many countries for safety evaluation of food special agencies and departments were responsible for controlling and supporting scientific facilities. In many countries there were lack of machinery, equipment and technology to handle such problems. The problems vary from one country to another depending upon climatic conditions, life style of the people. It was further made a guideline that FAO and WHO must carry out detailed survey for evaluation which consists of biological, chemical properties of the most used food additives.[10] Parvin Dehghan depicts that PS contains antimicrobial properties and are widely used in food and Pharma-Industries. In human digestive system, PS can be metabolized and oxidized into water and carbon-dioxide. Specified that it may enter into the biological chain through the consumption of food & drugs, which eventually results in deficiency of nutrition and leads forwards various diseases. The author projected that the usage of PS additive should be reduced and legalized.[11]

In this 21st century, besides all other technologies nanomaterial-engineering has become a boon which offers substantial growth towards health, production and processing farms. The intake of nanomaterial in our diet is substantially increasing. While problems occurs due to particle inhalation. Exposure to submicron-sized TiO2 particles for long time may prove fatal to the children age group. The committee of WHO and FAO jointly considered ADI-a daily intake, for TiO2 additives in food.[30] The consideration was taken upon its low solubility and less absorption into organs like liver. Many research still depicts that TiO2 bi-products upon mass consumption causes health hazards to fauna.[30,31] Within the European Union, TiO2 has been used as a food additive for 50 years for its intestinal absorption and less toxicity rate.[12] In the beginning it was used as a whitening agent-Opacifier in sweets and pasteries.[14,15] Marie Dorier evaluated the toxicity of TiO2 in vitro,upon a mucus-secreting intestinal epithelium. The ROS level increased in both of the TiO2 nanoparticle and E171 food additive. As a result they did not induce any deformation in cell viability. Hence, E171 food additive and TiO2 nanoparticles produces minor effects in vitro cell model.[12,13]

In most of the countries, chickens are raised in congested farms where a range of feed additives are used upon daily basis. Most of the trace metals are excreted via urine and feces and small proportion is absorbed in the body.[16] In his paper, Yuanan Hu conducted a study to differentiate the proportions of trace metal in fresh chicken tissues. To assess public health risk he conducted an experiment and found that with the exception of Pb, Cr and Ni the trace metals were found higher in interior lobe of the body apart from muscles.[17] Arsenic, being a toxic metal when leads exposure to mankind causes health issues. Upon collection of data from CNNHS, Gang Li., developed a model of monitoring average iAs consumption in different regions of China. It was found that Rice, being the largest contributor-60% of entire iAs intake. Which results more risk in Cancer in the Southern part of China rather than the North.[18] The total consumption of iAs, was estimated lower than what Gang Li., presented as all kinds of cereals were mixed which lead towards overestimation, as Rice consists of 10-fold more iAs than the other cereals.[19,20]

Among other food additives, enzymatic browing is also common in food industries causes negative effects upon nutrition level of the veges and fruits. Xanthone is well used for its biological activities but doubtful for enzymatic browning solutions. Kyoung Mi Moon, screened natural compound-Xanthone for its PPO activity and selected Maclurin for its strong nature. When maclurin was experimented with potato, maclurin quenched its enzymatic browning and thereby maintaining freshness for long term.[21] In Plants xanthones are secondary metabolities which are known for its biological activities-antioxidant, anti-cancer and antithrombotic possessions.[22]

Paulo E.S. Munekata conducted a detailed survey upon natural bioactive compounds-Hydroponics and its by-products,for the generation of nutraceuticals and food additives Hydroponics and its bi-products. By improving protein and mineral level the nutritional level can be maintained. Moreover modifications in the color of food products are found particularly in green Algae.[23] After the incident of cancer took place in 2018,supports efforts towards strategies and management. It was accounted that due to lungs cancer the mortality rate seems the maximum. Moreover breast, prostate and colorectal cancer were the leading sources of cancer as mentioned by Bray.[24]

Unconstrained break-up multiplication of abnormal cells apart from there conventional domain, probably the main factor towards Cancer.[25] Normal cells undergoes through series of modification and turns into cancer cells when carcinogenic factors subdue internal cellular mechanisms, which further causes mutation in genetic material. As affected cancer cells expands rapidly, the normal activity of tissue cells gets disorganized and leads towards cancer signs.[26,27,28]

In his review paper, Chandrakala Ravichandran depicts that R-(+)-limonene-an additive which is extensively used in food-cosmetics industries. Limonene is spread primarily through food(major) and ambient air(minor).He also suggested that the oral intake of limonene can be increased upon consumption of lemon juice. If consumed for a prolonged period of time at high concentrations would result fatal.[29]

Thus, concludes the introduction section. Objective of the proposed work is presented in the upcoming section. Detailed Methodology is present in Section III of the manuscript. The Results are projected in section IV., followed by Conclusion & Future works. The final Section consists of Acknowledgement and References.

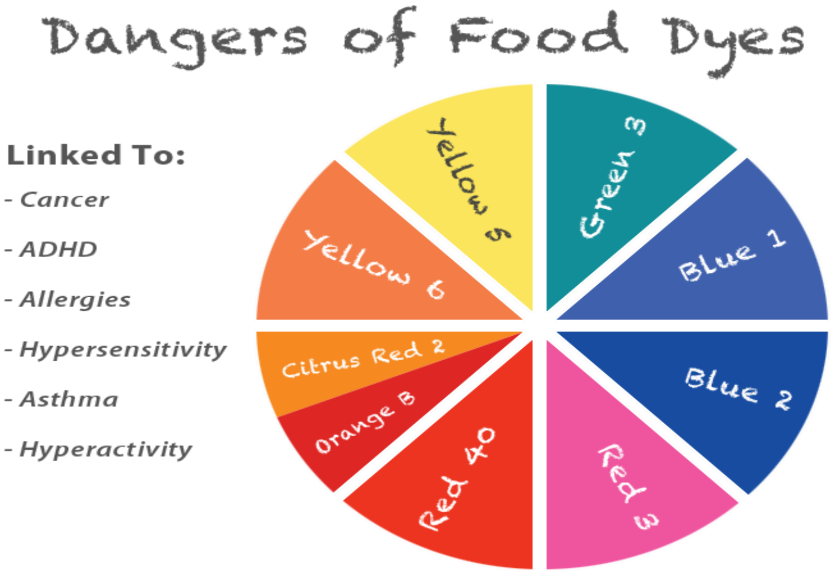


Figure 1: Cons for the over usage of Food Dyes.

II. Objectives of the current research topic

In his previous analysis the authors drew a review upon various applications of grapheme and graphene oxide based nanostructures in the field of electronics, food and biological sciences which would help to develop graphene oxide based nanostructures for detection of synthetic food dyes.[32] There are some selected colors, which can be allowed as food additives, food dyes, candies. But some chemicals used are carcinogenic and causes health hazards to body and skin.

Researches shows that many people are affected by throat and stomach cancer. Hence our study is related to the data analysis and detection of prohibited food colorants even in minute scale like Tartrazine, Sunset yellow, Allura red etc. We also exhibited some field trials of the samples through electrochemical sensing device, based on suitable chemical and biological receptors as shown in Figure 2.

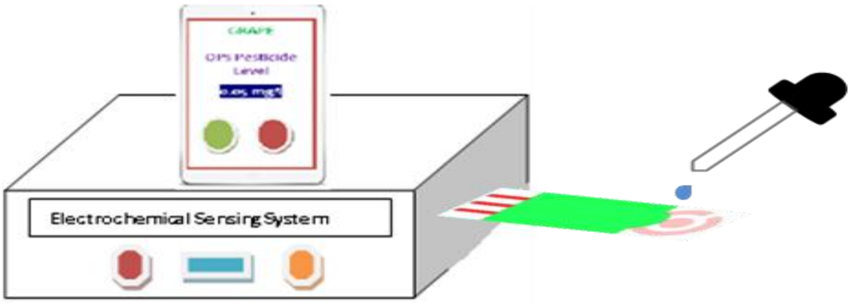


Figure 2: Development of electrochemical sensing device.

Advantages of using this device

Minimum sample volume and low cost reagent ease of automation.

The poor signal can be amplified by using advanced nanomaterials or nanoparticles like Graphene.

Key points for the usage of Graphene

High Electron Mobility at room temperature. High surface to volume ratio and high electrical conductivity.

Graphene is a single layer of carbon atoms (sp^2 hybridized) that are packed in a hexagonal honeycomb crystalline structure.

III. METHODOLOGY

III.A Process flow chart of Synthesis of Graphene

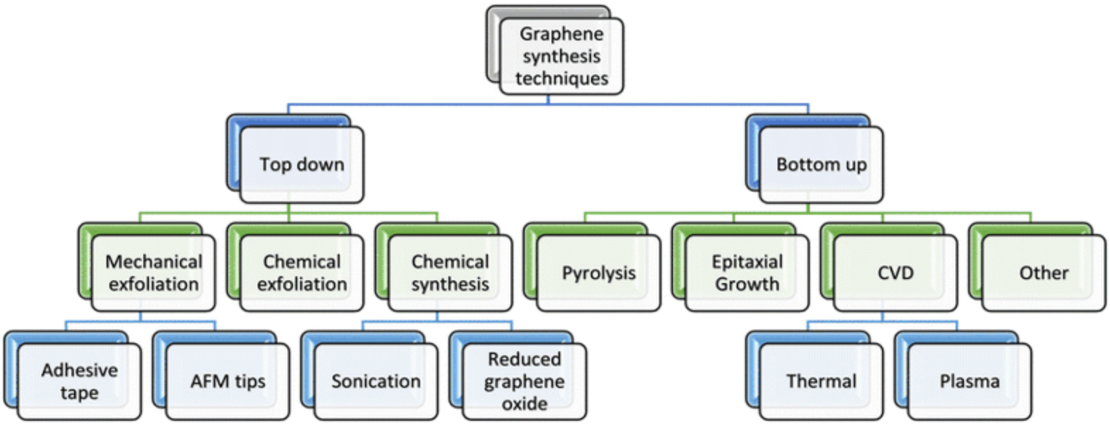


Figure 3: Flow Chart showing - Synthesis of Graphene.

Graphene is not directly obtained in the market, It is coming from graphite flex. In laboratory, graphene was made from graphite flex undergoing different processes like exfoliation. Then graphite oxide was converted into powered form and obtained.

III.B

From Graphite to GO and RGO

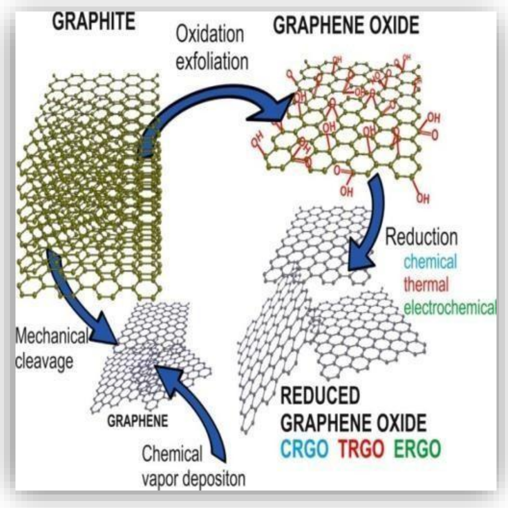


Figure 4: Graphene Oxide(GO)-precursor material of Graphene.

The grapheme-oxide film would be prepared by modified Hummer's method. This may be synthesized by taking a selected amount of graphite flake/powder in a glass container followed by the addition of different oxidizing agent with variable proportion under hot condition. Then, the resulting mixture may be sonicated for the proper exfoliation of the graphite layers in an aqueous medium. After that, the obtained GO will be isolated by centrifugation and sonicated in anaqueous medium to use as a precursor as mentioned in Figure 4.

III.C Key points

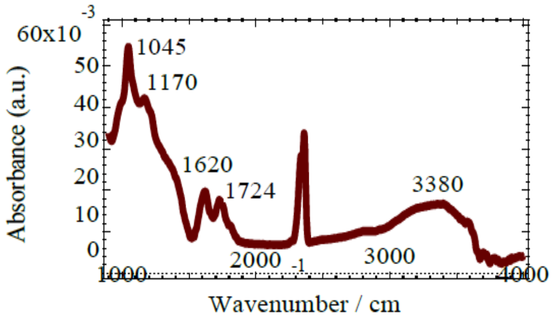
Non- Stoichiometric Material of C, H & O in variable ratios; C:O Ratio in between 2.1 – 2.9 Layered Structure but Larger and Irregular Spacing.

Phenolic –OH, Epoxy, Carboxylic Acid Groups in Basal Plane & Sheet Edge Disruption of sp^2 bonding network of Graphene.

III.D Spectrophotometric Characterization of GO and rGO

The synthesized GO was characterized by Raman spectrum, FTIR spectrum, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Interlayer spacing of GO was determined by X-ray diffraction (XRD).[33]

GO



FTIR

Figure 5.1: Variation between Absorbance and Wavenumber.

RGO

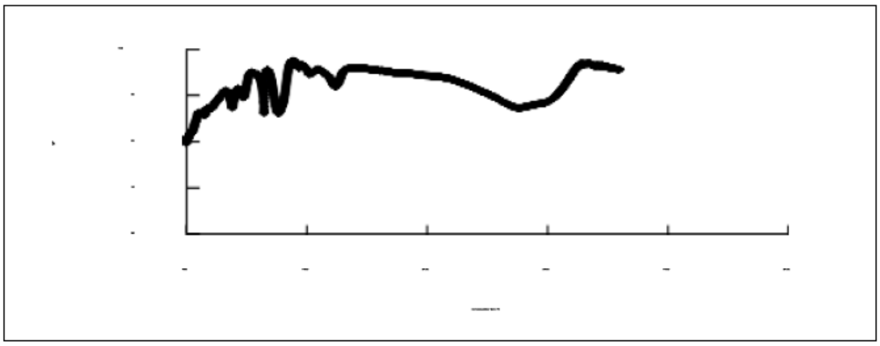
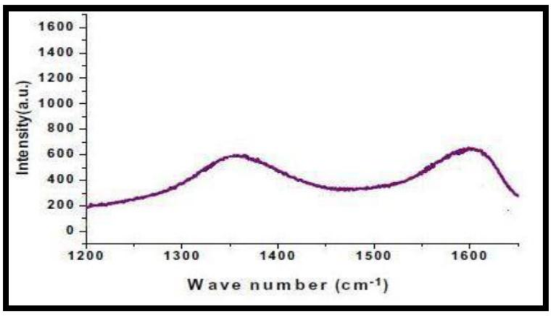


Figure 5.2: Variation between Time period(%) Vs Wavenumber(cm-1).

GO



Raman

Figure 5.3: Variation between Intensity(a.u) Vs Wavenumber(cm-1).

RGO

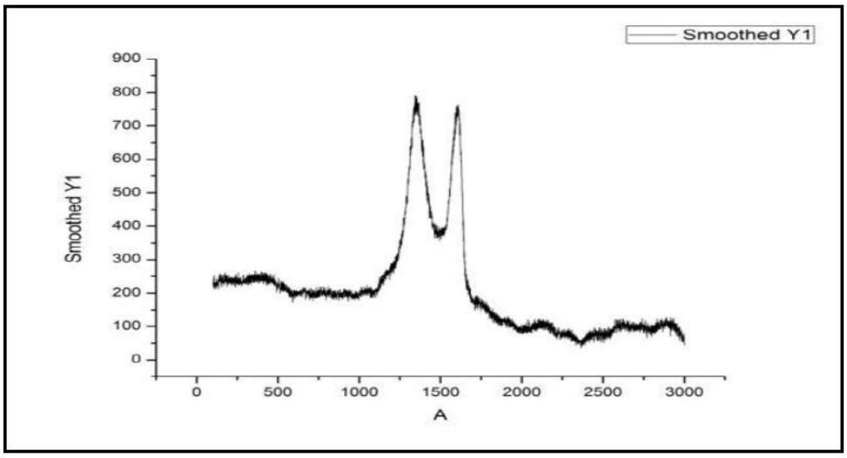
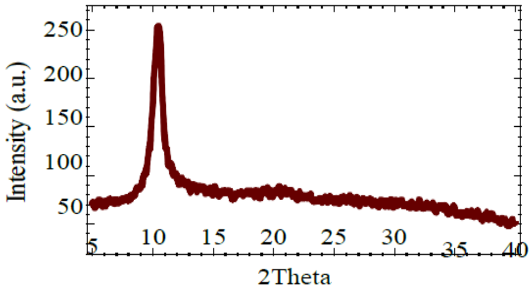


Figure 5.4: Variation between Intensity(a.u) Vs Wavenumber(cm-1).

GO



XRD

Figure 5.5: Variation between Intensity(a.u) Vs Angular Degree(cm-1).

RGO

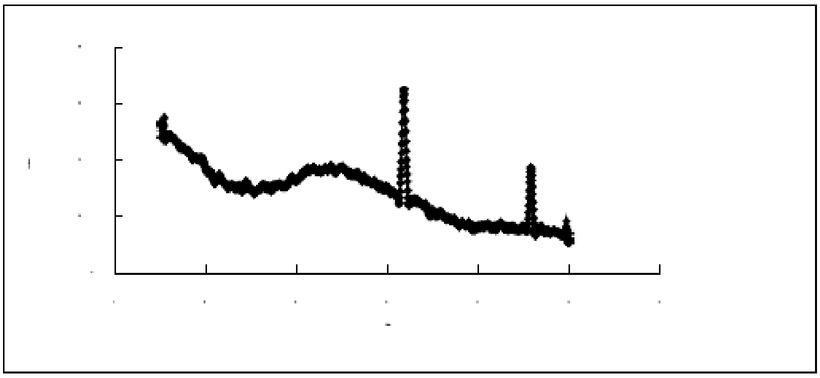


Figure 5.6: Variation between Intensity(a.u) Vs Angular Degree(cm-1).

With Ref.to Figure 5, the FTIR spectra of GO (graphene oxide) and rGO (reduced graphene oxide/graphene). The GO (graphene oxide) spectrum as shows the peak at 1066 cm-1 is attributed as C-O streching. The peak at 1288 cm-1 is confirmed as C-O-C bending and C-OH bending is observed at 1587 cm-1. The carbonyl groups are also shown at 1724 cm-1 as C=O stretching and a broad peak at 3448 cm-1 is attributed as O-H stretching vibration of the C-OH groups and water content in the material.

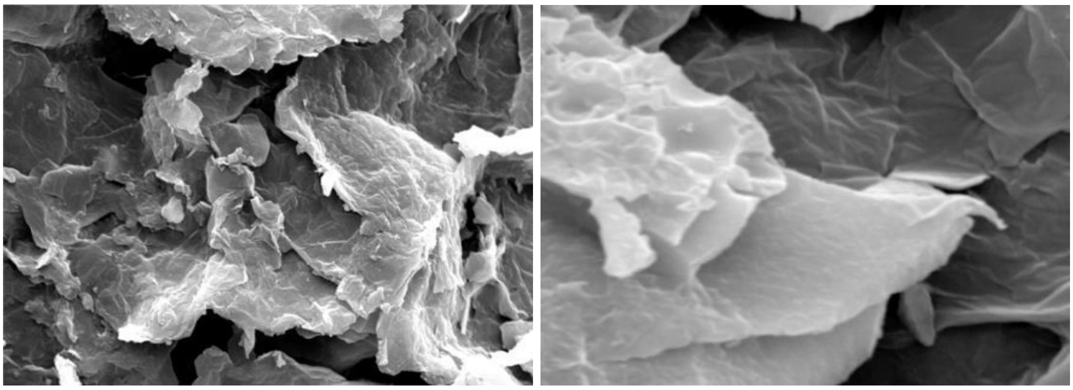
In case of RGO, The peaks in 1066, 1288 and 1724, 3448 cm-1 disapeared and decreased dramatically, which is an indication of the removal of oxygen-containing functional groups in GO. These rGO spectra confirmed that most oxygen containing functional groups in the GO were removed although some residual oxygen-functionalities on GO still present on the rGO surface with weaker intensity.

XRD of GO shows single peak at 2theta = 11.5 whereas in XRD of RGO we are getting two peaks These XRD results are showing the exfoliation and reduction processes of GO and the processes of removing intercalated water molecules and the oxide groups.
Whereas, Raman spectra do not show any remarkable changes.

III.E Morphological Characterization of GO and rGO

SEM image of GO sheets are smooth with small wrinkles and folded at the edges (Figure 6), and reduced GO material consists of stacked and aggregated, thin, crumpled sheets (Figure 8).Similarly, GO exhibited a closely packed lamellar and plate structure and rGO showed typical sheet-like silky waves, wrinkled appearance, and clumped structure in a plain surface (Figure 7,9).

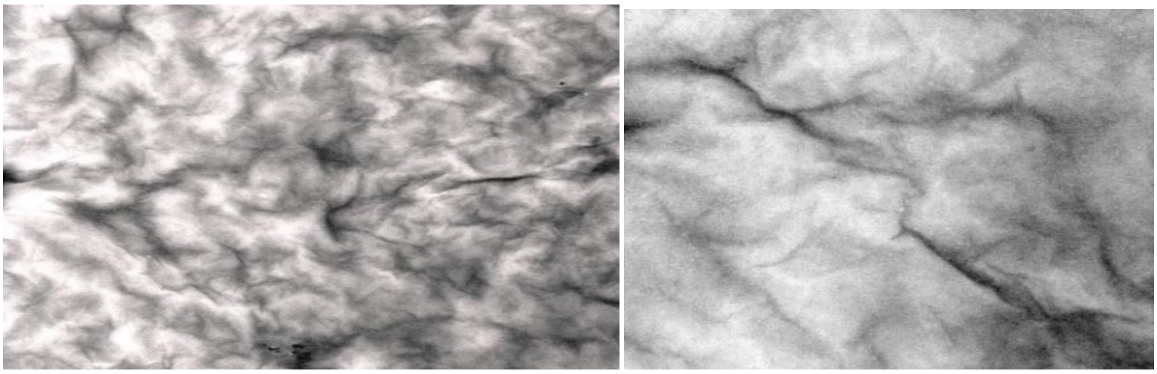
SEM image of GO



1µm 200 nm

Figure 6: This figure depicts SEM image of Graphene Oxide(GO).

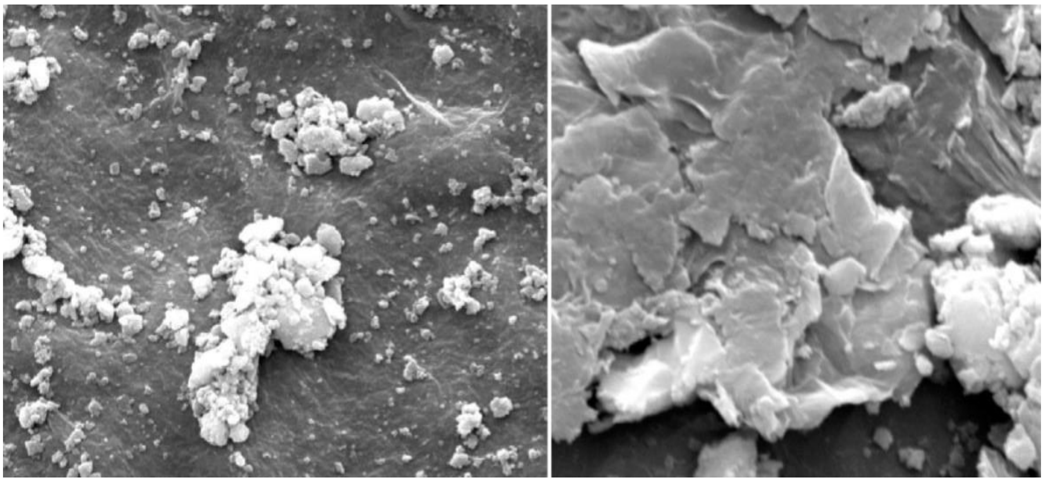
TEM image of GO



0.5 µm **100nm**

Figure 7:This figure depicts TEM image of Graphene Oxide(GO).

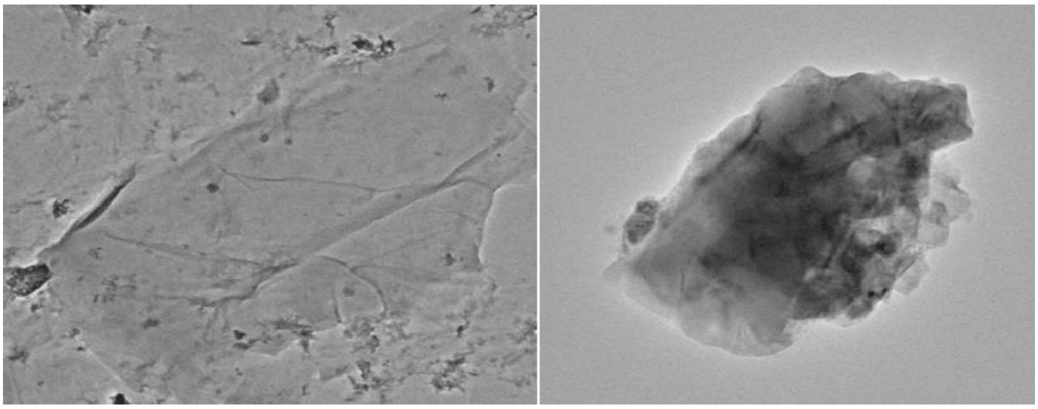
SEM image of RGO



1µm **200nm**

Figure 8: This figure depicts SEM image of Reduced-Graphene Oxide(RGO).

TEM image of RGO



0.5µm **100nm**

Figure 9: This figure depicts TEM image of Reduced-Graphene Oxide(RGO).

IV. RESULTS OF OUR WORK: IV.A

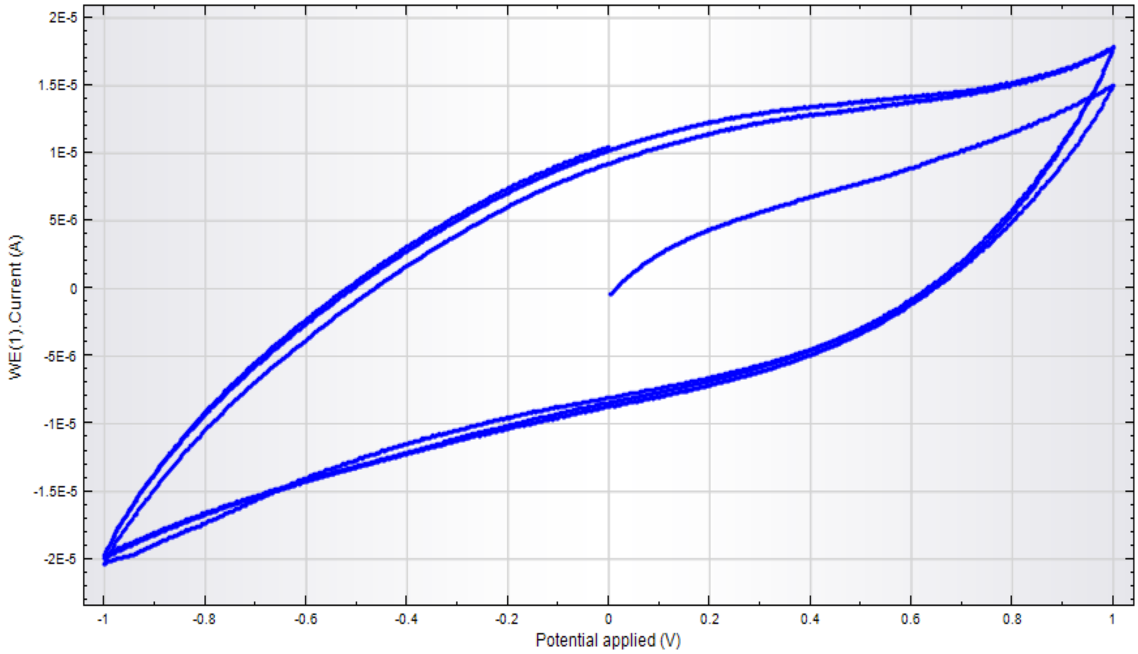


Figure-10.1: CV for new RGO on buffer added without RGO pasted Silk screen electrode (for 16 Stop Crossing) and at 0.1 V/s scan rate.

We have operated in the electrochemical workstation in C-DAC, Kolkata through the instrument Autolab PGSTAT 302N. Figure 10.1, is done using Nova 1.1 after we use the auto lab, connected by the electronic sensor and the computer.

Primarily, we prepared sunset yellow and tartrazine raw sample ranging from 0.5 mili Mole to 5 mili mole and also prepared in phosphate buffer solution of pH value 7.46.Initially,the silk screen electrode connected with Autolab was added with 1 milligram graphene oxide that is GO prepared in 1 ml of distilled water with 1 milligram of graphene oxide in powder form. This prepared graphene oxide solution is sonicated for 1 hour with a frequency of 12 minutes each and finally vortexed.

The CV operation is then started and 16 stop crossed means 8 complete cycles was completed. Therefore the graphene oxide was converted into reduced graphene oxide. Then with the use of Nova 1.1 software the graph is prepared by the computer connected with the autolab system. The graph obtained was regular and smooth graph which was not possible when we have done the same process without using buffer solution.

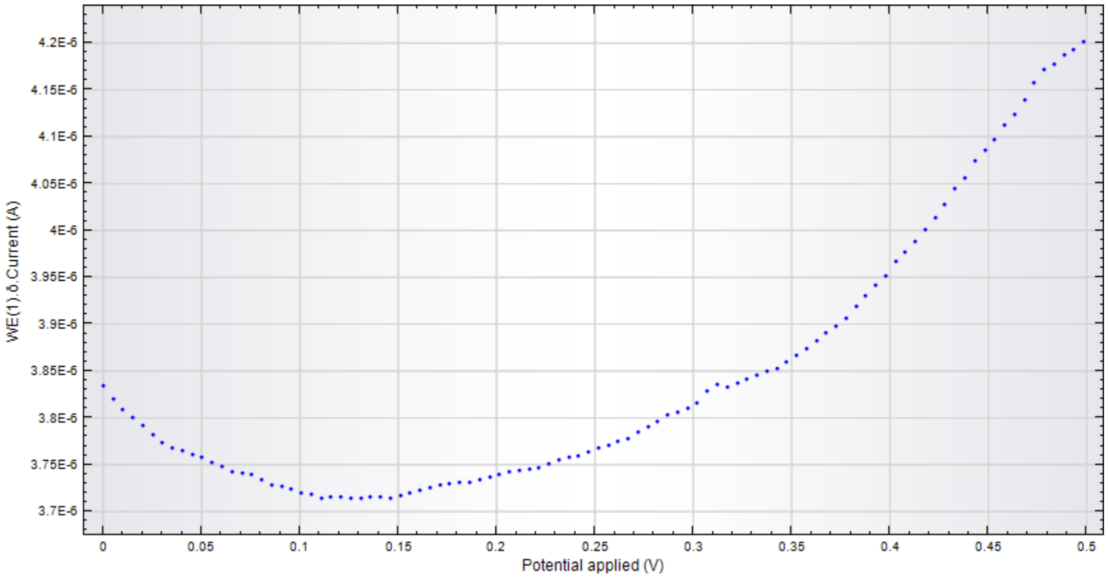


Figure-10.2: DPV for 1mM sunset yellow prepared in buffer used on silk screen electrode (without RGO pasted electrode).

Figure 10.2 graph is almost like the first one but the steps are slightly different and here the silkscreen electrode dopped by previously prepared 1 millimole Mole sunset yellow solution then differential pulse voltametry or simply DPV was done. Now using Nova 1.1 software connected in Computer and the computer connected Autolab and silk screen electrode the graph was generated. The graph was not very good quality because the buffer solution was not used earlier which maintains standard constant PH value.

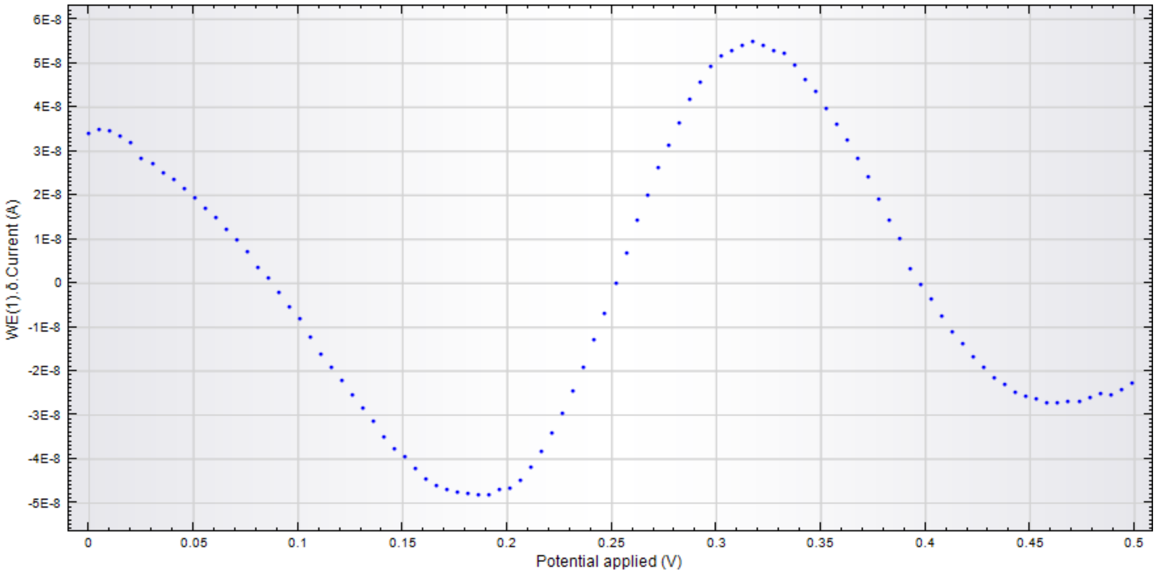


Figure-10.3: DPV for 1mM sunset yellow prepared in buffer used on silk screen electrode (with new RGO pasted electrode).

Figure 10.3 is outcome of the the graphene oxide dopping on silkscreen electrode. 1 milligram of graphene oxide is prepared in 1 ml of Phosphate buffer solutions of PH 7.46 is prepared and reduced graphene oxide is prepared from graphene oxide after repeated cycling of CV operation.

After doping the electrode and the sensor with graphene oxide is converted into reduced graphene oxide by repeated cyclic process. Moreover, one mili mole sample of sunset yellow was added on electrode by a micropipette then DPV was done and the smooth regular graph with behaving 1 oxidation peak and one production peak was obtained which was done by using Nova software 1.1.

IV.B Graphical Representation of CV and DPV using sunset yellow

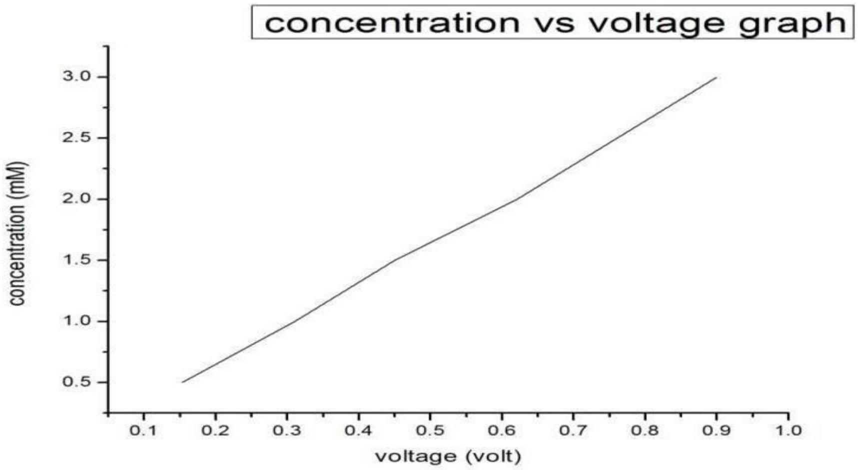


Figure 11.1: Variation between Concentration and Voltage Graph.

The above figure obtained by doped electrode of GO and convert it into RGO with repeated cyclic process and added with different concentration of sunset yellow solution ranging from 0.5 to 2.5 milli mole which was prepared from 1 milli molar stock solution(buffer). The concentration in millimole versus Peak voltage is plotted using origin software. The graph is very regular and very smooth.

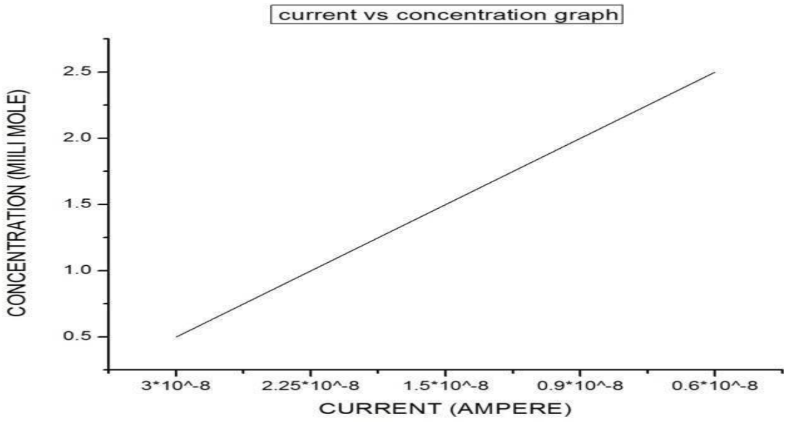


Figure 11.2: Variation between Concentration and Current.

The dye concentrations is 1mM and scan rate: 0.1v/s. Sample prepared in 7.46 pH phosphate buffer solution

The following figure is obtained by doped electrode of GO and convert it into RGO with repeated cyclic process and added with different concentration of sunset yellow solution ranging from 0.5 to 2.5 milli mole which was prepared from 1 millimolar stock solution(buffer). The concentration is in millimole versus peak current is plotted using origin software. The graph is very regular and very smooth.

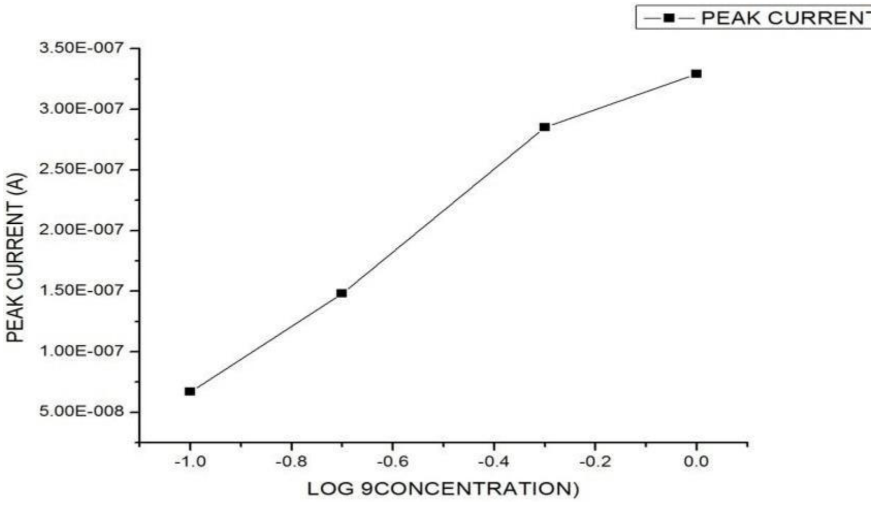


Figure 11.3: Variation between Peak Current and Conc.

The dye concentrations is 1mM and scan rate: 0.1v/s. Sample prepared in 7.46 pH phosphate buffer solution

This figure was done using same procedure as above but here for different concentration of sunset yellow ranging (0.5 to 2.5) mili mole produce different current and voltages using CV operation biy autolab with Nova 1.1 software and the log concentrations with current graph was plotted using origin software and we observed Linear graph passed through origin. There is some change observed at higher region. The scan rate was fixed at 0.1 volt per second and the stock solution (buffer) was 1 milli mole. From that different concentration (0.1 to 1.0) mili mole with constant pH value of 7.46 are carried throughout.

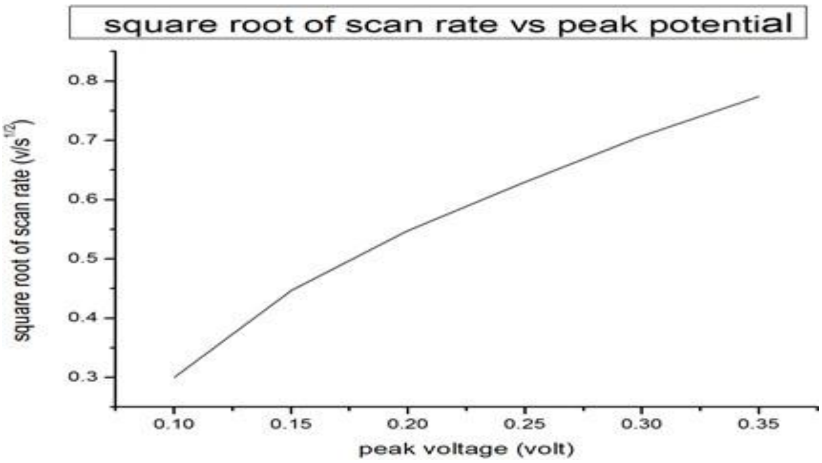


Figure 11.4: Variation between Peak Potential and Sq.Root of Scan Rate.

The dye concentrations is 1mm and scan rate: 0.1v/s Sample prepared in 7.46 pH phosphate buffer solution

The above figure depicts a plot between square root of scan rate versus peak voltage. It means this time scandid was not fixed but varying from (0.1 to 0.5) volt per second and the raw sample prepared in phosphate buffer solution thus the pH is always constant at 7.46 and the concentration of the sample was 1 milli mole.

Note: We have prepared the stock solution with buffer solution with constant value of 7.46 as checked in the laboratory.

CONCLUSION AND FUTURE PLAN

Through our experimental research we have used GO based system for detection of azo dyes namely Tartrazine and Sunset Yellow. We have used GO based system for detection of azo-dyes namely Tartrazine and Sunset Yellow. These dyes are found in breakfast cereals, candy, snacks, beverages etc. We have done several analysis for two set of GO after reducing electrochemically into RGO using Auto lab electrochemical analyzer for detecting various concentration of Tartrazine and Sunset yellow prepared in Phosphate buffer solution. Various comparative plots were done to understand the effect of RGO pasted electrodes in known dyes. So our future goal is to make a sensor to detect the presence of these azo dyes in processed foods.

So our next objective is to make a sensor to detect the presence of these azo-dyes in processed food.

- To standardize our sensor in terms of certain parameters.
- To use both GO and GO-PANI and compare the results.
- To do real sample analysis.
- To develop some MIP based sensor for detection of toxic food colors.
- To conduct statistical analysis for exposure assessment of food colors among consumers of various age groups.
- To predict the interaction of synthesized graphene based nanostructures and dyes by various theoretical models.

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