



# PHOTOCATALYTIC, ANTIBACTERIAL ACTIVITY, ELECTROCHEMICAL AND IMPEDANCE STUDIES OF RHODAMINE-B DOPED REDUCED GRAPHENE OXIDE NANOMATERIAL SYNTHESIZED FROM CITRUS SINESIS FRUIT EXTRACT VIA GREEN SYNTHESIS APPROACH

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## ABSTRACT

Green synthesis was used to create rhodamine-B doped reduced graphene oxide (RhB-rGO) nanomaterial. A range of analytical and spectroscopic methods were employed to characterise the synthesised material. The electrochemical behaviour of RhB-rGO nanomaterial was investigated using cyclic voltammetry. The zone of inhibition of the prepared sample and antibacterial activity were examined. Under sun light, the synthesised sample's photocatalytic activity was examined and more than 90% of the methylene blue (MB) dye was eliminated from the dye solution using photocatalytic testing. The sample's impedance analysis helped to understand its electrical behaviour, and a HRTEM analysis was used to determine the sample's particle size.

**Keywords:** Green synthesis; reduced graphene oxide; fruit extract; rhodamine-B; characterization; cyclic voltammetry; photocatalytic activity; HRTEM

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## 1. Introduction

Geim and K. Novoselov were awarded the 2010 Nobel Prize in Physics for their discovery of graphene [1]. Condensed six-membered rings make up graphene, a sheet of carbon one atom thick. Graphene's hexagonal two-dimensional (2D) lattice is made up of carbon atoms bound by  $sp^2$  bonds [2]. With amazing electrical, mechanical, thermal, and optical capabilities, graphene is a two-dimensional substance in the shape of a honeycomb [3]. It is said to have good thermal conductivity ( $5000 \text{ Wm}^{-1} \text{ K}^{-1}$ ) [4], good charge carrier mobility ( $200,000 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) because of the  $\pi$ -conjugation structure [5], and good optical transparency (97.7%) in practice [6]. These characteristics show that graphene has potential applications in nanoelectronics [7], Li-ion batteries [8], supercapacitors [9], solar cells [10] and photocatalysis [11].

Several graphene preparation methods have been proposed to date and one of the most promising methods is the reduction of graphene oxide (GO). GO material can be prepared by deep oxidation of natural graphite, typically by Modified Hummers method and there is currently no unique formula of GO because the composition of this compound depends on synthesis conditions and the nature of the parent graphite. It is often thought to have the formula  $\text{C}_8\text{O}_2(\text{OH})_2$ , with the oxygen present in carboxyl, hydroxyl, ketone, epoxy, and other oxygen containing groups, which determine its acid-base properties and hydrophilicity [12].

While chemical synthesis methods that use dangerous chemical reagents lead to pollution issues, physical methods of synthesising reduced graphene oxide are very expensive. Even after thorough purification, any traces of these compounds in graphene will impart a toxic nature, which would limit its usefulness in biological applications. Green synthetic approaches, therefore, have emerged as a practical way to reduce pollution by minimising the use of expensive and dangerous reagents in industry and research. Over the past ten years, there have been numerous reports on the utilisation of plant extracts (leaf, fruit, and stem) in the reduction of graphene oxide (GO) to reduced graphene oxide (rGO) [13, 14]. The electrical, mechanical, optical, and thermal properties of reduced graphene oxide are found to be unique, according to measurements. Simultaneously, large volumes of reduced graphene doped other materials of suitable quality must be able to be produced for practical applications [15]. In this instance, rhodamine-B dye doping is used to modify the

properties of the reduced graphene oxide for the intended uses.

Inorganic dyes have also been effectively doped with other materials to modify the characteristics, just as organic dyes. Eosin-Y and Eriochrome Black-T dye-doped poly (vinyl alcohol) composite film was created by Manikandan *et al.*, who also investigated its optical limiting characteristics [16]. In a publication by Yuezhong Xian *et al.*, silica nanoparticles produced by reverse microemulsion and doped with methylene blue (MB) were used as a new matrix for biological applications [17]. Optical waveguides, sensors, nonlinear optical devices, and light concentrators in solar cell systems all use organic and inorganic dyes as dopants [18]. In this work, structural and optical experiments have been carried out on rhodamine-B doped reduced graphene oxide (RhB-rGO) are given and discussed. With remarkable photophysical characteristics, rhodamine-B (RhB) is a significant laser dye. In addition to having a high extinction coefficient, long wavelength absorption and emission, and great light stability, it also has a high fluorescence quantum yield. Rhodamine-B is named N-[9-(ortho-carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-ylidene] diethyl ammonium chloride in the IUPAC classification and its chemical formula is  $\text{C}_{28}\text{H}_{31}\text{ClN}_2\text{O}_3$ . Rhodamine-B is a substance that is frequently employed as a dye laser source and fluorescent labelling reagent due to its excellent photophysical characteristics, which include long wavelength absorption and emission, a high fluorescence quantum yield, a large extinction coefficient, and good light stability. Research by Mukesh Kumar *et al.* characterised the thermal and optical properties of rhodamine-6G doped silica glasses were thermally and optically characterised [19]. The study conducted by Ibrayev *et al.* investigated the spectral and luminous characteristics of rhodamine dye within a porous aluminium anode oxide matrix [20].

With these details taken into account, it is decided to use RhB dye as the dopant for the first time in rGO material and the impact of RhB doping on rGO nanomaterial in terms of electrical, antibacterial and photocatalytic studies is discussed in this paper.

## 2. Synthesis of RhB-rGO nanomaterial

Graphite (99% acid treated), sodium nitrate (98%), potassium permanganate (99%), hydrogen peroxide (40% wt.), sulfuric acid (98%), hydrochloric acid (35%), and rhodamine-B were used to generate the rhodamine-B doped reduced

graphene oxide (RhB-rGO) sample. Throughout the entire experiment, double-distilled water was used. Initially, a material called graphene oxide (GO) was produced [21]. RhB-rGO nanomaterial was created by using the GO material in an environmentally friendly green approach. Effective bio-extracts have been used to create reduced graphene oxide from graphene oxide particles through a straight forward and environmentally benign experimental process.

Orange fruit extract (*Citrus sinensis* L.) was utilised in this investigation. The juice was bought from a nearby supermarket and then put into a sterilised polyethylene container. Following that, 100 millilitres of double-distilled water were added to dilute it. After extraction, the mixture was supplemented with precisely 100 mg of graphene oxide and 1 mole% of rhodamine-B. The black mixture was heated in an IFB 20SC2 20 L convection microwave oven at 4000 rpm for 30 minutes and at 800 W for 10 minutes. As a result, the treated graphene oxide's original black tint changed to a pinkish-black colour. The material was filtered, and then dried at 50°C until it started to turn pink.

### 3. Results and discussion

#### 3.1 Cyclic voltametric (CV) study

Cyclic voltammetry is an electrochemical technique, which is used to analyse the chemical reactions that occur when molecules are activated by electron transport. An electrochemical cell fitted with the electrodes viz. reference electrode, working electrode, and counter electrode are used to carry out the cyclic voltametric study of RhB-rGO nanomaterial. The potential and current are measured for the sample and here the potential is measured between the working electrode and the reference electrode. The current is measured between the working electrode and the counter electrode. The solution used in the electrochemical cell consists of solvent, electrolyte and the sample

to be studied. The measurement was carried out at different scan rates such as 0.05 V/s, 0.1 V/s and 0.15 V/s. The electron transfer between the sample at the working electrode and counter electrode generates current that is carried through the solution by the diffusion of ions. The measured values of potential and current for the sample are given in the figures 1, 2 and 3. From the observed data, it is noticed that there are two electrochemical processes: (a) oxidation process and (b) reduction process and this forms a complete cycle of voltammetry which indicates the electrochemical process of the sample. The excellent electrical double-layer capacitance behaviour is indicated by the rectangular and symmetric shape of the RhB-rGO cyclic voltametric curves. In particular, the CV curves of the synthesised RhB-rGO material furnishes valuable insights into the reaction mechanism, the kinetics and thermodynamics of electron transfer, the consequences of electron transfer, such as the production of functional groups, and it also facilitates the computation of the capacitor value. On the RhB-rGO surface, oxidation and reduction processes are assumed to be responsible for the anodic peaks and reduction processes, respectively. The oxygen-containing functional groups on the sample's surface enable to produce the pseudo capacitance behaviour to be seen in the cyclic voltammetry of RhB-rGO material [22]. The specific capacitance ( $C_s$ ) of the sample can be computed by the formula  $C_s = A / mK\Delta V$  where A is area under the CV curve, m is mass dipped in the electrode, k is the scan rate and  $\Delta V$  is the difference of potential. The calculated values of specific capacitance for RhB-rGO nanomaterial at different scan rates are provided in the table 1. It is observed that the specific capacitance of the sample is high and hence RhB-rGO nanomaterial could be used as the promising electrode material for high-performance capacitors [23].

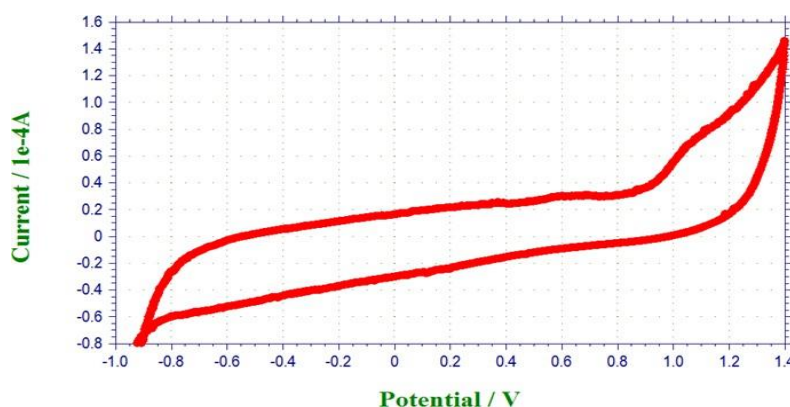


Fig 1: CV curve for RhB-rGO nanomaterial at the scan rate of 0.05 V/s

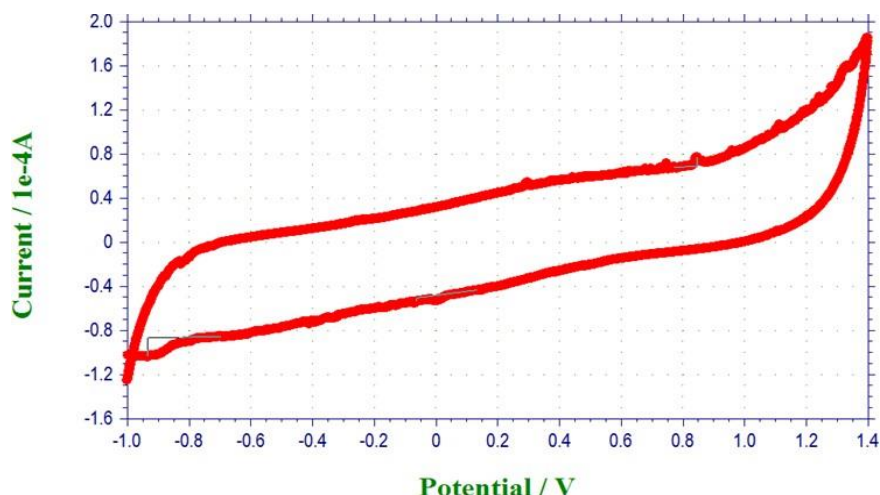


Fig 2: CV curve for RhB-rGO nanomaterial at the scan rate 0.1 V/s

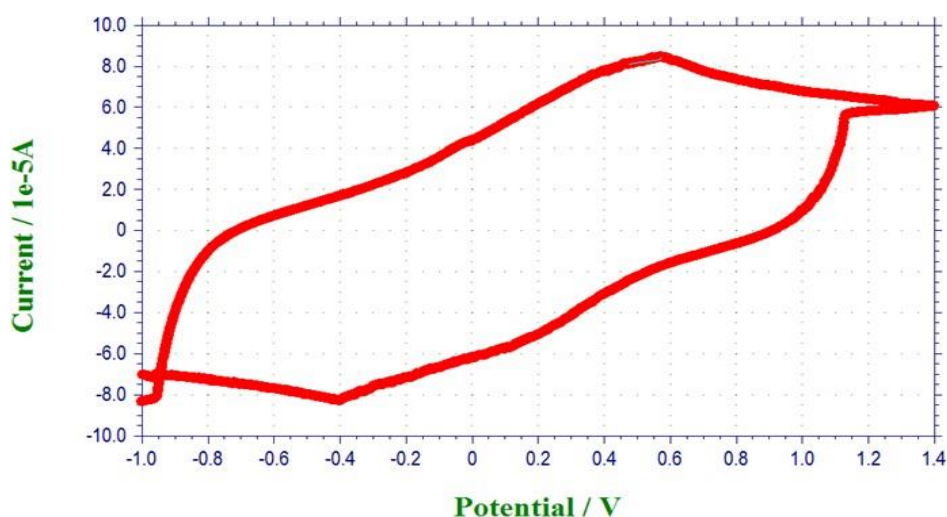


Fig 3: CV curve for RhB-rGO nanomaterial at the scan rate of 0.15 V/s

Table 1: Values of scan rate and specific capacitance of RhB-rGO nanomaterial

Scan rate (V/s)	Specific capacitance (F/g)
0.05	173.92
0.1	168.38
0.15	98.66

### 3.2 Impedance study

Utilising complex impedance spectroscopy to examine the electrical characteristics of materials is an effective method. This method is based on determining the impedance and associated parameters as a function of frequency by first analysing the AC response of a system to a sinusoidal perturbation. The complex impedance  $Z^*$  was measured using an impedance analyzer (IM 6 ZAHNER/Germany-Electrochemical Workstation with Galvanostat) at a frequency ranging from 0.1 Hz to  $1 \times 10^5$  Hz. It can be expressed as a function of resistance and capacitance using the equation  $Z^* = Z' - jZ''$  where  $Z'$  and  $Z''$  are the real and imaginary parts of the impedance, respectively. The nanomaterial of

RhB-rGO was pelletized using a pelletizer and the surfaces of the sample were electroded with the silver paste to get good ohmic contact. With the use of equivalent circuit models, impedance spectroscopy is a valuable instrument for researching the conductivity behaviour of the sample as well as the dynamics of charge carriers, interfacial features, and dielectric properties. Drawing the real part of the impedance ( $Z'$ ) along the X-axis and the imaginary part ( $Z''$ ) along the Y-axis at different frequencies results in a Nyquist plot and it is presented in the figure 4. The result indicates that as the real part of impedance increases, the imaginary part of impedance increases reaching the maximum value of 4750 ohm and this value is equal to the grain boundary

resistance. Since the semi-circular arc is not produced in the figure 4, the sample's electrical

characteristics are mostly caused by grain boundary effects [24].

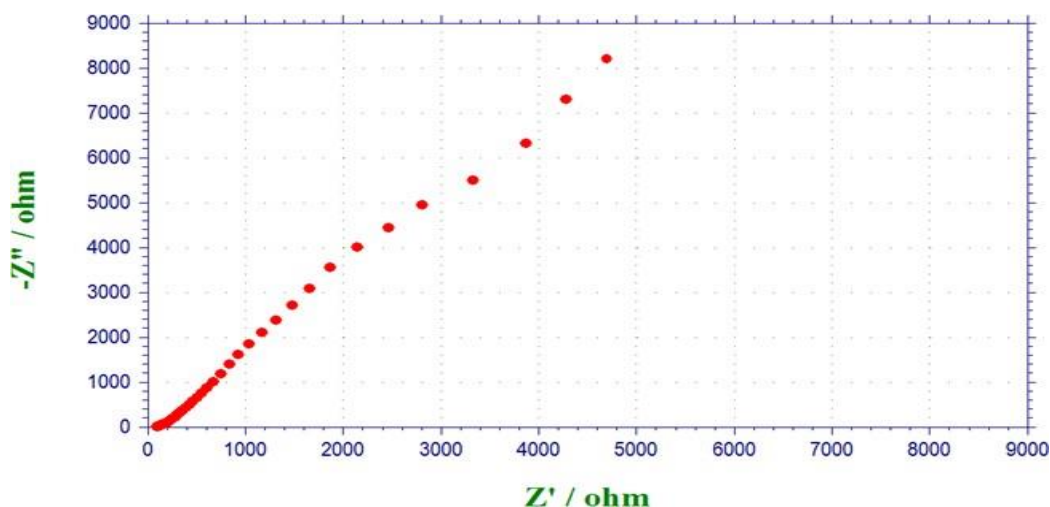


Fig 4: Impedance characteristics of RhB-rGO nanomaterial

### 3.3 Antibacterial activity

The effectiveness of the RhB-rGO nanoparticles against the bacteria is demonstrated by the bacterial evaluation in Fig.5. In the plates, the inhibitory zone is obvious. Table 2 gives the zone of inhibition that were obtained for three gram-positive bacteria, namely Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus and for one gram-negative bacterium, Escherichia coli. The findings indicate that the RhB-rGO sample exhibits very high antibacterial activity against the gram-positive Bacillus cereus (B.cereus) bacteria and very poor activity against the remaining three micro pathogens. B.cereus is well-known for its symptoms in the gastrointestinal tract and eyes, but little is known about how important a role it plays in serious systemic infections. According to environmental literature, the seasonal, widely dispersed environmental bacterium Bacillus cereus

can break down polyethylene polymers. Studies examining the significance of B.cereus in bloodstream infections observe a significant rise in the microbe's isolation throughout the summer. It generates biofilms and other toxins that are becoming more well acknowledged for their potential to cause serious harm and illness. The data obtained from the antibacterial activity study indicates adding fresh orange extract to the sample during preparation increases its effectiveness against the bacteria B.Cereus [25]. According to Arooj Naila *et al.*, nanoparticles with strong antibacterial activity against B. subtilis, S. aureus, and E. coli were created by combining extract from *Citrus sinensis* L. with silver nitrate solution [26]. Hence, a significant antibacterial activity against B.cereus was demonstrated in our study by *Citrus sinensis* L. fruit extract combined with RhB-rGO nanoparticles .

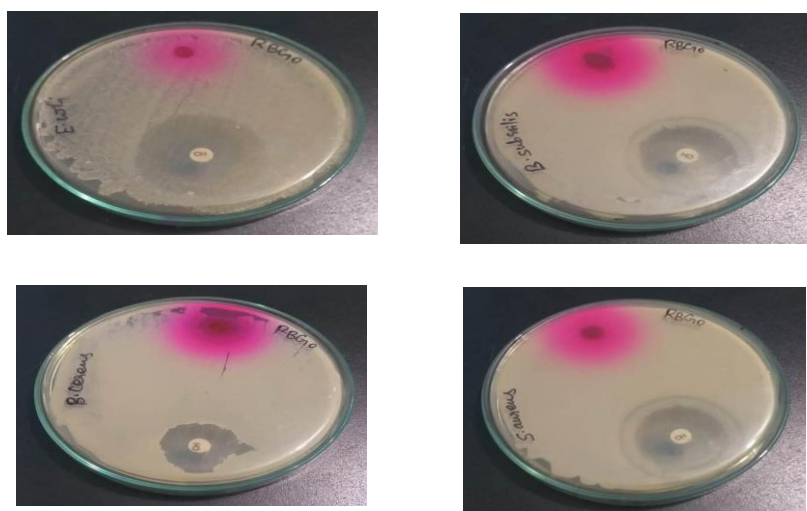


Fig. 5: Zone of inhibition for RhB-rGO nanomaterial against four bacterial specimens

**Table 2** Values of zone of inhibition for RhB-rGO nanomaterial against bacterial species

Bacteria	Zone of inhibition (mm)	
	RhB-GO material	Standard material
<i>E. coli</i>	15	35
<i>B.subtilis</i>	15	20
<i>B.cereus</i>	27	25
<i>S.aureus</i>	15	30

### 3.4 Photocatalytic activity study for dye removal

The photocatalysis process using the unique properties of nanomaterials has been applied in a wide range of applications such as degradation of pollutants from atmosphere and water. Some nanomaterials such as oxides, semiconductors, metals, and graphene have shown great effect on photo-catalysis processes, due to their enhanced and controllable optical properties, which makes them excellent photo-catalysts. The photocatalytic behaviour of the prepared RhB-rGO nanomaterial was tested through the degradation of methylene blue (MB) under visible light. To establish the equilibrium of adsorption and desorption of MB molecules on the catalyst surface, 10 mg of the catalyst (RhB-rGO nanomaterial) was suspended in 10 ml of the aqueous solution of MB (10 mg L<sup>-1</sup>). The suspension was then magnetically agitated in the dark for 30 minutes. The combination was then moved to a test tube and subjected to light. The degrade concentration of MB was determined by using a UV- vis spectrophotometer to measure the absorbance at 664 nm.

The percentage of degradation of the sample was evaluated using the following equation

Where

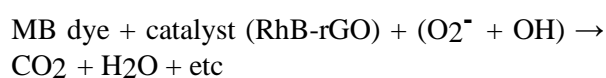
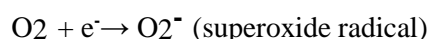
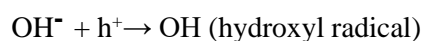
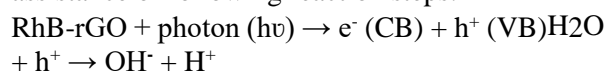
$$\text{Degradation efficiency} = \frac{(C_0 - C_t)}{C_0} \times 100\%$$

C<sub>0</sub> is the initial concentration of the dye at the time 0s and C<sub>t</sub> is the final concentration

of the dye at the time t. The obtained data of percentage of dye removal for the sample are put in the figure 6 and the corresponding data are provided in the table 3.

The electronic transition that occurs during photo-excitation from the valence band (VB) to the conduction band (CB) is primarily responsible for the photo-catalytic activity. Electrons are stimulated from VB to CB of the sample when the MB dye solution and photo-catalyst (RhB-rGO) are exposed with light. These electrons are then

received at the surface of the sample facilitating further electron transport. As a result, the dye solution includes mobile electrons that might combine with O<sub>2</sub> to create superoxide (O<sub>2</sub><sup>-</sup>) radical. Conversely, hydroxyl radicals (OH) are produced when the hole (h<sup>+</sup>) at the valence band was mixed with the OH<sup>-</sup> ions. The MB dye was oxidised into CO<sub>2</sub>, H<sub>2</sub>O, and other by products by these OH radicals, which were acting as oxidising agents. The complete mechanism for the degradation process by RhB-rGO might be clarified with the assistance of following reaction steps:



According to Xiong *et al.*, two hour exposure to sunlight can cause up to 81% of the MB dye in their CdS/rGO composite to breakdown [27]. Figure 6 shows the MB dye degradative percentage as a function of exposure time to sunlight irradiation during summer time up to maximum of 2 h. The experiments were done for different concentrations such as 10–100ppm MB dye and the results show that bio-reduced RhB-rGO sample could degrade more than 80% of MB molecules within the time of 120 min. Crucial factors influencing photocatalytic efficiency are concentration, surface features, and contact angle with the RhB-rGO photocatalyst. RhB-rGO has a greater concentration in self-oxidation sample, which encourages light absorption and increases photocatalytic efficiency. RhB-rGO sample that undergoes self-oxidation has superior photocatalytic efficacy. As a result, RhB-rGO nanomaterial may work effectively as a photo-catalyst to remove various organic dyes from waste water and hence this sample could be useful for dye removal from dye solution in textile industries.

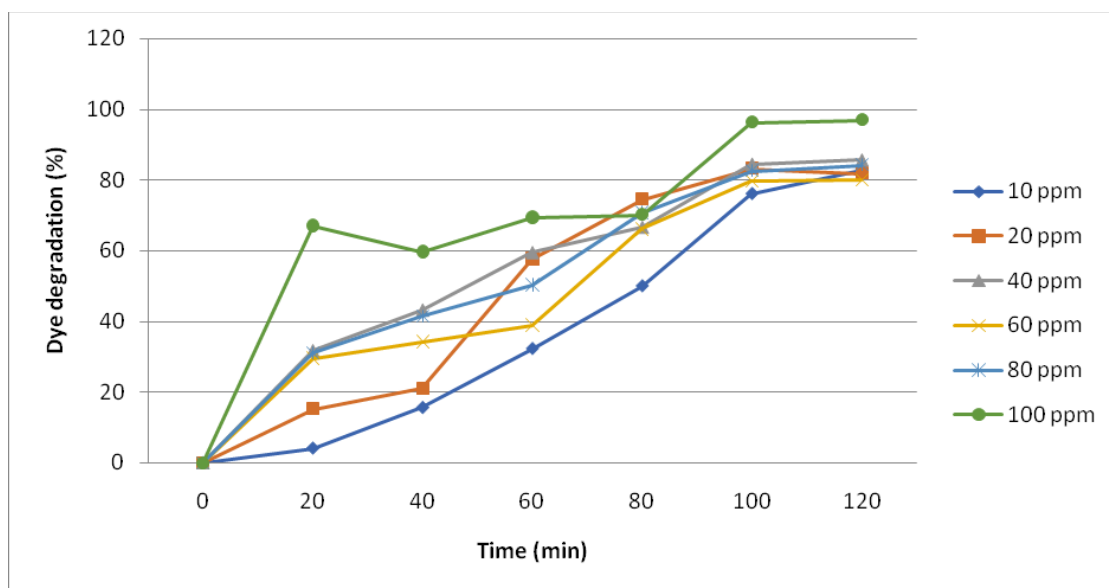


Fig 6: Percentage of dye removal by photocatalytic activity of RhB-rGO nanaomaterial

Table 3 Data in connection with dye removal for RhB-rGO nanomaterial

Dye concentration	Time and % of degradation						
	0 (min)	20 (min)	40 (min)	60 (min)	80 (min)	100 (min)	120 (min)
10 ppm	0	4.16	15.63	32.29	50	76.04	82.81
20 ppm	0	15.23	20.99	57.62	74.48	83.12	81.89
40 ppm	0	31.74	43.25	59.52	66.67	84.52	85.71
60 ppm	0	29.53	34.23	38.92	66.11	79.53	79.86
80 ppm	0	31.09	41.46	50.3	70.73	82.62	84.14
100 ppm	0	67.09	59.64	69.4	70.17	96.4	96.91

### 3.5 HRTEM study

The morphology and particle size of RhB-rGO nanomaterial were investigated using a high resolution transmission electron microscope (HRTEM). Figure 7 shows the TEM images of the sample at different resolutions and the results indicate that RhB-rGO sample has nanostructures with average particle sizes ranging from 10 to 20

nm. The figure shows rGO sheets with some obvious surface wrinkles and additionally, random rhodamine-B anchors have been found on the rGO surfaces. The results of typical HRTEM pictures of the RhB-rGO show that the spherical rhodamine B particles are uniformly distributed on the surface of each individual rGO sheets with negligible aggregation.

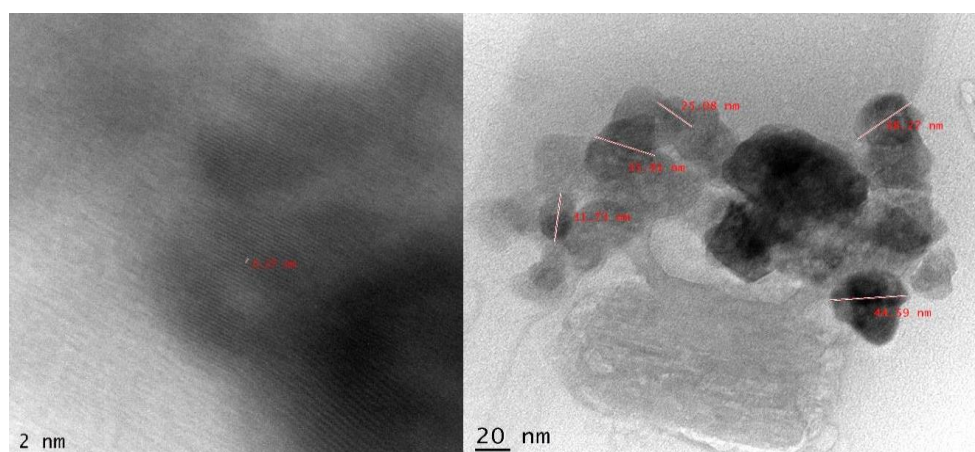


Fig.7: HRTEM images of RhB-rGO nanomaterial at the resolutions of 2 nm and 20 nm

#### 4. Conclusion

RhB-rGO nanomaterial was synthesized by green synthesis by using the extract of *Citrus sinensis* L (Orange fruit). Due to the presence of citric acid in *Citrus sinensis*.L juice, it may be used as a bio-reductant for the synthesis of RhB-rGO nanoparticles. Based on the analysis of four different bacterial specimens, the antibacterial activity of the RhB-rGO sample was determined to be higher for the *B.cereus* specimen. From the work on the dye degradation of methylene blue (MB), it confirms that the synthesised RhB-rGO nanomaterial functions as an effective photocatalyst, capable of breaking down up to 100 parts per million of MB dye in just two hours. It is observed that as the real part of impedance increases, the imaginary part of impedance increases for RhB-rGO nanomaterial and the grain boundary resistance of this sample was found to be 4750ohm. The results of HRTEM indicates that RhB-rGO sample has nanostructures with average particle sizes ranging from 10 to 20 nm.

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#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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