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Synthesis, characterization and antimicrobial activity of multifunctional Graphene oxide - Curcumin scaffold.

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Abstract: *Graphene oxide plays a vital role in recent trends of chemical reactions thanks to the consisting of multifunctional groups such as COOH, C=O, CHO, C_6 H_5, OH and Epoxy groups. Graphene oxide has been synthesized from graphene powder by modern hummer's method. Curcumin was synthesized from turmeric powder. The compounds were purified by column chromatographic technique. Dimethoxy curcumin was bind with the graphene oxide by autoclave method. The structures were determined by UV and IR spectral studies. Anti-bacterial activity of the gram negative bacteria which affects the Urinary tract of the human was carried out by Disk diffusion method. Antibacterial activities of the newly prepared scaffold were compared with the individual curcumin and Graphene oxide. Novel Go- Cur scaffold showed higher efficient its parent compounds.*

Keywords: *Graphene oxide, curcumin, Graphene oxide loaded curcumin scaffold, Antibacterial activity on UTI Bacteria*

I. Introduction

1.1 Graphene Oxide

During the last half decade, chemically modified graphene (CMG) has been studied in the context of many applications, such as polymer composites, energy-related materials, sensors, 'paper'-like materials, field-effect transistors (FET), and biomedical applications, due to its excellent electrical, mechanical, and thermal properties. Due to its excellent physical and mechanical properties, it is a good candidate for various applications such as nanoelectronic sensor, catalysis and photovoltaic[1-5].

Recently, graphene oxide (GO) an oxidized form of graphene, has gained more attention because it is easy to functionalize, able to label with fluorescent probe and compatible with most biomolecules. These unique properties of GO make it a promising carbon compound for bio application. This critical review will focus on the chemistry of graphene oxide, including its preparation, structure, and reactivity. In this study, GO sheets were synthesized from graphite powder by chemical exfoliation method. Antimicrobial activities were investigated. The structure was characterised by IR and UV spectroscopic methods [6-8].

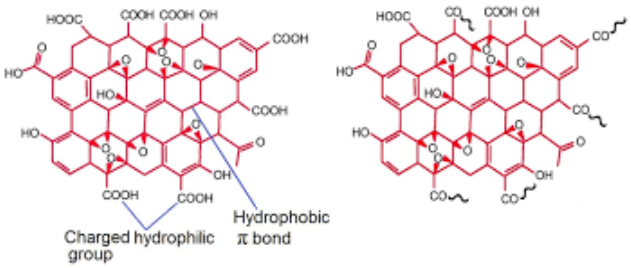

Figure1.1 Structure Of Graphene Oxide

1.2 Curcumin

Curcumin is an orange–yellow crystalline powder practically insoluble in water. The structure of curcumin (C_{21} H_{20} O_6) is widely used in traditional Indian medicine to cure biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis. Turmeric paste in slaked lime is a popular home remedy for the treatment of inflammation and wounds.Curcumin (diferuloylmethane), the main yellow bioactive

component of turmeric has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. Its anticancer effect is mainly mediated through induction of apoptosis[9-14].

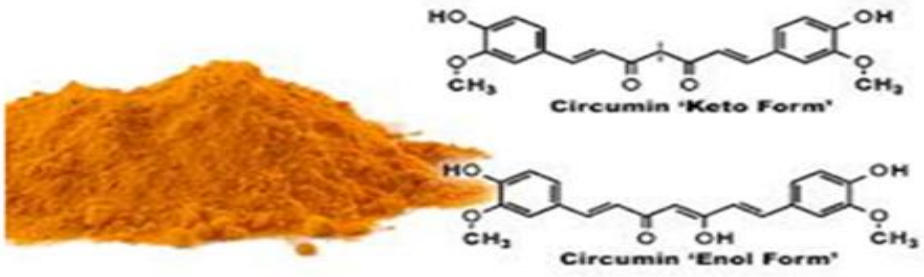

Figure 1.2 structure of curcumin

1.3 Curcumin loaded on Graphene oxide

Literure survey showed the novel idea about the GO derivatives. The addition of other functional groups directly onto GO, to form either covalent or non-covalent attachments, falls into another big category of GO chemistry. According to the widely accepted *Lerf-Klinowski*model, reactive functional groups on GO are epoxy, hydroxyl, carbonyl, carboxyl and ester moieties. Most of the functionalization occurred on more than one type of oxygenated groups and resulted in very complicated products Additionally, non-covalent functionalization of GO has also been demonstrated. Non-covalent interactions such as π-π stacking, cation-π, or van der Waals interactions mainly happen on sp2 carbon domains. For instance, a GO based biosensor has been demonstrated utilizing the GO-protein/DNA π-π interactions . This research discussed the Curcumin loaded on GO and its medicinal applications [14-18].

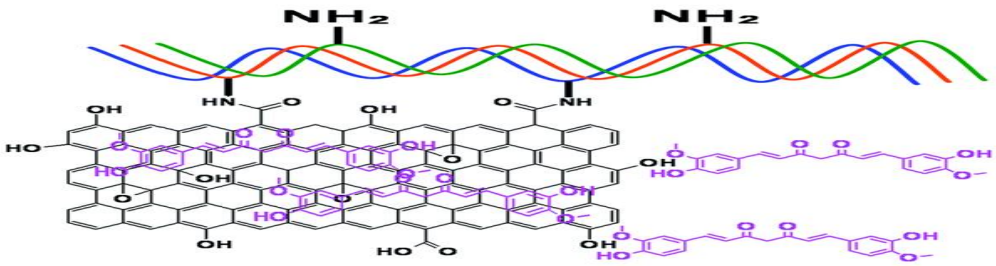

Figure 1.3 Curcumin loaded on GO and interact with DNA

II. Methods

2.1 Synthesis of Graphene Oxide by Hummers Method and Its Modifications

Graphene Oxide was prepared by a modified Hummers method. Typically1.0 g natural graphite powder and 0.5 g sodium nitrate were mixed with 23 mL, sulfuric acid in a 500 mL, and flask place in an ice bath. The obtained solution was stirred and slowly added 3 g potassium permanganate, the stirring was continued for 2 h. The mixture solution was transferred to a 35 °C water bath and stirred for 30 min. After that 46 mL of deionized water was slowly added into the solution and the solution temperature mentioned was about 98 °C. The mixture solution was maintained at this temperature by heating for 30 min. Then, 140 mL.deionized water 10 mL of hydrogen peroxide was added sequentially to the mixture solution to terminate the reaction. The observed color of mixture was dark yellow. The resulting product was filtered and rinsed with 5% HCl solution followed by distilled water for several times. Graphite oxide powder was obtained after drying in vacuum at 60 °C for 12 h. The powder was then dispersed in distilled water to make concentration of 0.5 mg/ml. and exfoliated by microwave for 30 min to generate GO sheets, followed by centrifugation at 4000 rpm for 30 min to remove unexfoliated graphite oxide. Finally, the stable suspension of brown GO sheets was obtained. A water-free mixture of concentrated sulfuric acid, sodium nitrate and potassium permanganate was prepared and maintained below 45 °C for graphite oxidation. The most common source of graphite is a naturally occurring mineral which has been purified to remove heteroatomic contaminations such as sulfur and iron. As for the second combination $KMnO_4$ and H_2SO_4, permanganate ion is also a typical oxidation reagent.

2.2. Preparation of Curcumin

Fresh rhizomes were cleaned washed with deionized water, sliced and dried in the sun for one week and again dried at 50 °C in a hot air oven for 6 hours. Dried rhizomes were cut in small pieces, powdered.

Approximately 20gm of sample were taken and set up with various solvent from nonpolar to polar. 150ml of solvent was added and extracted according to their boiling point for 6 hours. The solvents used were Hexane (b.pt = 69° C), Chloroform (B.P = 61°C), Ethyl acetate (B.P=77°C), Methanol (B.P=65°C), and Acetone (B.P=56.53°C). And one sample was extracted with hexane for 2 hours and hexane extract was discarded and the powder was re-extracted with methanol for 6 hours. After completion of extraction the dark brown extract was then cooled, filtered, concentrated using rotary evaporator, and finally by vaccum suction to get a crude dried extract which was black orange in color. Each raw sample of turmeric was extracted by the same method and yield was calculated.*Curcuma longa* (Turmeric) rhizome were collected from Assam - Lakhadong variety. All solvents / Chemicals used were of AR / HPLC grade and obtained from E-Merck. The reference standard of Curcumin was purchased from Sigma Chemicals Co. U.S.A.

2.3 Synthesis of curcumin loaded GO

5g of GO and 5 ml of curcumin are taken in Rb flask and dissolved in 50 ml of acetone and applied magnetics stirring for 20 hrs (1:2) ratio has been prepared by the same procedure[19-21].

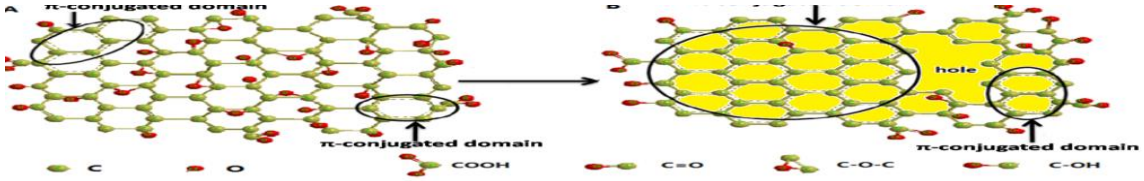

Figure-2.1 Curcumin loaded on GO

2.4Antibacterial assay of extracts:

The agar diffusion method was used to determine the antibacterial activity of the water extract of Cur-GO . 20 ml of Mueller Hinton agar was distributed into sterile Petridis 1 ml of e.*coli* organism was spread on Mueller Hinton agar. Two (or) three disc were made in agar layer of each Petridis . Extract of Cur-GO of 0.2 mg/ml was applied in the culture plates . The plates were left on the bench for 1 hour for adequate diffusion of the extracts & incubated as at 37℃ for 24 hours . After incubation the diameter of the zones of inhibition around each well were measured along two axis i.e. 90° zone of inhibition was recorded in millimeter.

As the extracts diffuse through the agar medium from the edge of the discs, its concentration diminishes to the point where it is no longer inhibitory to the organism.

III. Results And Discussion

3.1 Characterizations, Chemical Structure and Properties

FT-IR spectrum of GO is shown in Figure 3.1 Presence of the bands at 1049 (C–O stretching), 1224 (C–O–C stretching) and 1720 cm^{-1} (C=O stretching) with the broad band at around 3400 cm^{-1} (–OH stretching) confirmed the presence of carbonyl, carboxylic, epoxy and hydroxyl moieties in GO.

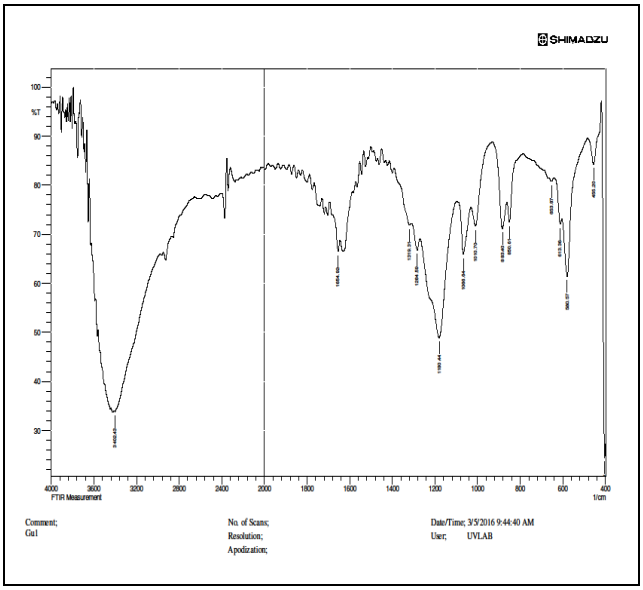

Figure3.1 FT-IR spectrum of GO

Figure.3.2 shows the FT-IR spectrum of curcumin, vital bands were observed at 3420, 1720, 1510, 1286, 1137 and 1033 cm^{-1}. These bands correspond to the –OH, –C=O, –C–H, C–O groups of curcumin. This confirms the successful extraction of curcumin from turmeric. All these bands are also present in the other spectra which confirmed the presence of curcumin in GO.

In case of curcumin loaded GO, figure 3.3 showed the two characteristic bands were observed at 2871 and 2919 cm-^{1,} which are due to the symmetric and asymmetric vibrations of –CH groups of the polyphenolic compounds of the plant extract. In the region 3300–3450 cm^{-1}, a broad band was observed which is attributed to the –OH stretching of curcumin and other polyphenolic compounds.

Removal of oxygen-containing moieties of GO in curcumin loaded GO are clearly reflected by the absence of the aforementioned bands in Figure 3.3. Relative decrease in the intensity of the broad band due to O-H group at 3400 cm^{-1} is attributed to the successful reduction of GO

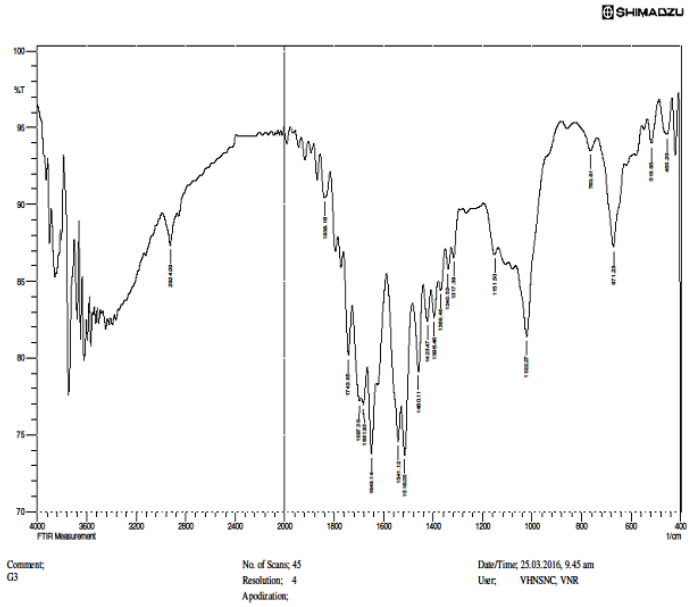

Figure.3.2 shows the FT-IR spectrum of curcumin

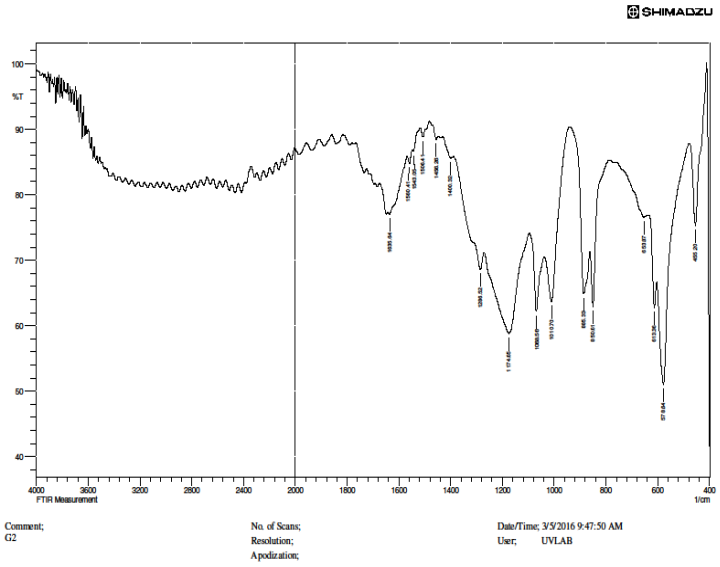

Figure 3.3 curcumin loaded GO

3.2 **UV-visible spectrum**

Figure 3.4 shows the UV-visible spectrum of curcumin loaded GO. A broad peak around 418 nm, which upon deconvolution split to three peaks at 400, 418 and 439 nm. Characteristic peaks at 408 and 330 nm were observed for curcumin loaded GO. The second peak is due to the n-π^* electronic transitions of the

aromatic C=C bond . In case of curcumin loadedGO the total peak area was broadened. This upon deconvolution showed four peaks at 388, 402, 422 and 441 nm. The surface peak of curcumin loaded GO showed a blue shift from 408 to 388 nm, which is because of the oxygeneous groups present in the curcumin structure .

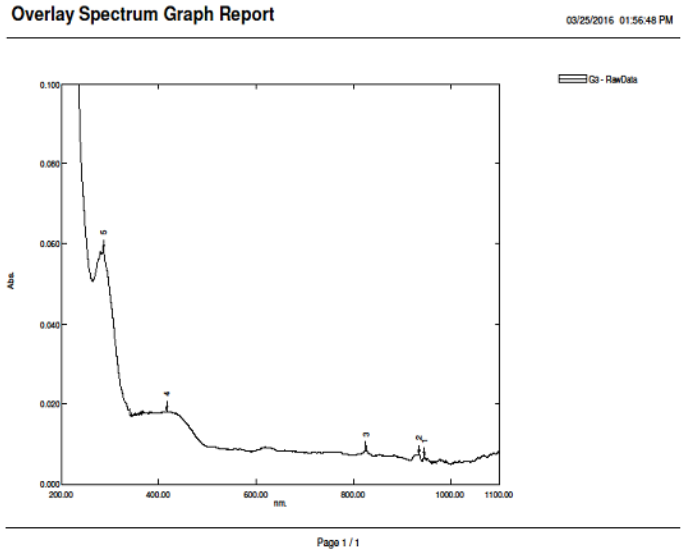

Figure 3.4 shows the UV-visible spectrum of curcumin loaded GO.

This clearly indicates the interaction of curcumin with GO Similarly the peaks at 338 and 419 nm indicates the presence of curcumin loaded GO.

3.3 Antimicrobial assays

The antimicrobial activity

The loading of Curcumin on GO showed the higher antimicrobial capability when compare to the parent compound. *In vitro* antibacterial activity of Curcumin loaded Graphene oxide extracts were assessed using the disc diffusion method by measuring the diameter of inhibition zones produced against by bacteria (*Escherichia coli).* Antimicrobial activity of each compound was evaluated, in which GOshowed 13 mmand Cur loaded GO showed 26 mm in the disc. Statistically significant differences were found and proved that our research work is beneficial to the society

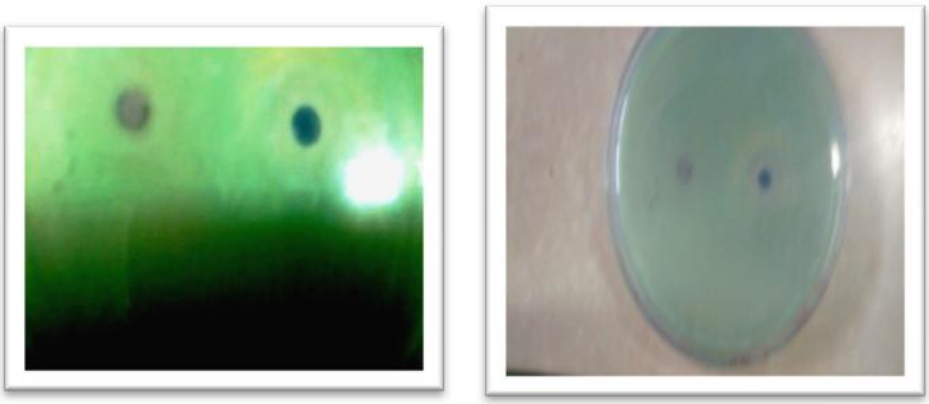

Figure 3.5 Antimicrobial activity inhibited zone of curcumin on *e.coli*.
Figure3.6 Antimicrobial activity inhibited zone of curcumin loaded GO on *e.coli*.

IV. Conclusion

Starting from natural graphitic material, GO was prepared in a large amount by hammers method which is a strong chemical oxidation process. Various characterizations proved GO to be an oxidized carbon compound with two dimensional structures, in other words a sheet of fused hexagonal rings attached with lots of oxygenated groups on both sides. Curcumin has extracted from natural plant. Then the novel Curcumin loaded Graphene oxide was synthesized. The structure of the products were characterized by using IR and UV spectral evidences. The applications of GO and its Cur-GO derivative have also been useful for future research in the

same field. Antimicrobial activity of each compound was evaluated, in which GOshowed 13 mmand Cur loaded GO showed 26 mm in the disc. Statistically significant differences were found and proved that our research work is beneficial to the society.

Review of literature

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