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# Study Of Antibacterial Properties Of 3D Graphene Synthesized By Soft Template Mesitylene

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This paper reports the Template Assisted Synthesis of three dimensional graphene foam (3DGF). Various templates can be implemented for the synthesis of 3DGF. These templates can be categorized into two groups as soft template and hard templates. The soft templates act in the form of microemulsions. Mesitylene was employed as soft template in the form of emulsion. The method uses graphene oxide prepared by modified Hummers method as the substrate or precursor for the synthesis of 3DGF. The template assisted graphene foam was characterized by Ultraviolet-Visible (UV-Visible) spectroscopy, Fourier Transform Infra Red (FTIR) spectroscopy, powder X-Ray Diffraction (XRD), Raman spectroscopy and Field Emission Scanning Electron Microscopy (FESEM). These graphene foams were then screened for antimicrobial activity against two Gram negative (Escherichiia coli, Staphylococcus aureus) and two Gram positive Bacillus subtilis, Pseudomonas mirabilis strains. The well diffusion method was used for the testing of antimicrobial activity. This 3DGF synthesized by template assisted method shows moderate antimicrobial activity.

## Introduction:-

Three dimensional graphene structure is also known as graphene foam, graphene sponge, graphene hydrogel, graphene aerogel, graphene monolith, graphene network etc. Three dimensional graphene is unique due to its structure and properties like high surface area, light weight, electro-mechanical properties. Various methods have been developed for the synthesis of this three dimensional graphene. The methods can be divided into two classes template assisted and template free.They can be prepared by direct or solution based techniques. Each of which has template assisted and template free methods. Uniform pore size is obtained in direct synthesis whereas the solution based methods give higher yield and scalability. Carbonization of polymer network is the direct synthesis template assisted method for getting three dimensional graphene structures. In the template assisted synthesis, here we will study the soft template method for the synthesis of three dimensional graphene.

## MATERIALS AND METHODOLOGY:

Graphite powder, Trimethyl benzene (TMB-Mesitylene) were purchased from Aldrich Chemicals. The remaining chemicals sodium nitrate, potassium permanganate, conc.,

sulphuric acid, hydrogen peroxide, dil hydrochloric acid, potassium dioxide were of analytical grade and used as it is without further purification. Deionized water was used wherever required.

**Synthesis of Graphene Oxide by Modified Hummers Method (GOh):**
46 ml of cold conc sulphuric acid ($H_2SO_4$) αwas added into a mixture of graphite powder and sodium nitrate. Potassium permanganate $KMnO_4$ (6 g) was added gradually with continuous stirring and cooling, to maintain the temperature of the mixture below 20°C. The mixture stirred at 35 °C for 30 min, followed by slow addition of distilled water (75 ml) which increases the temperature to 85-90°C. After one hour, the reaction is terminated by the addition of distilled water (100 ml) and 30% $H_2O_2$ solution (20 ml). The color of the suspension changes to bright yellow. Washing of the suspension with 5% HCl solution three times (300 ml) followed by washing with deionised water. It is followed by sonication for the formation of graphene oxide. The product is then filtered. The paste collected from the filter paper is dried at 60°C. This is **graphene oxide**, abbreviated as **GOh.**

**Synthesis of Graphene Oxide by Modified Hummers Method by using Potassium Dioxide :**(The GOk used is synthesized by Modified Hummers method.)
Same method as above is followed except, in the method the reagent hydrogen peroxide, used for quenching the oxidation reaction is replaced by potassium dioxide. The product formed is named as **GOk.**

**Synthesis of 3DGraphene using Trimethylbenzene. (Mesitylene) :**

For the synthesis, trimethyl benzene (TMB, 6.0 ml) was added to hydrochloric acid solution (HCl 2.0 M, 100 ml) and stirred continuously for sixty minutes at room temperature (25-20°C). The mixture was sonicated for the formation of a cloudy suspension for few more hours. This trimethyl benzene emulsion was mixed with graphene oxide suspension (60 ml) and distilled water (40 ml) at room temperature. Stirring was continued and the precipitate filtered by vacuum filtration and calcined at 350°C and the final product **a.1**was obtained. [*15]

| Product code | Soft template Used | Carbon sources |
|---|---|---|
| a.1 | Trimethyl benzene | Graphene oxide (GOh) |
| a.2 | Trimethyl benzene | Graphene oxide (GOk) |

**Table 1 Reactants Involved for the soft template assisted synthesis**

**Photo 1 Reactions for the formation of the products a.1**

## 4 CHARACTERIZATION:

The product obtained is characterised by methods like, FTIR, XRD, RAMAN and FE-SEM.FTIR data collected using PXRD patterns were obtained using a Bruker D8 Advanced X-ray diffractometer with a CuKα X-ray source (1.5418 A). Raman spectroscopy was obtained by applying a laser wavelength of 532 nm (RENISHAW) from the department of Physics, SPPU, Pune. The crystal structure of template assisted graphene was studied by XRD and RAMAN spectroscopy. The surface of the powdered materials was examined on field emission scanning electron microscopy (FE-SEM, FEI NOVA Nano SEM 450) at an acceleration voltage of 10 kV. at the CIF, SPPU, Pune. Origin 2021 software was used wherever required for plotting the spectra.

**FTIR Spectrum of the Product by Soft Template Method**

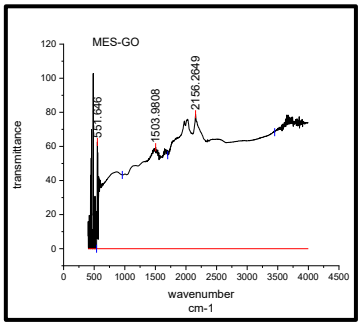



**Fig 1 FTIR spectrum of a.1**

**XRD Spectrum of the Product by Soft Template Method**

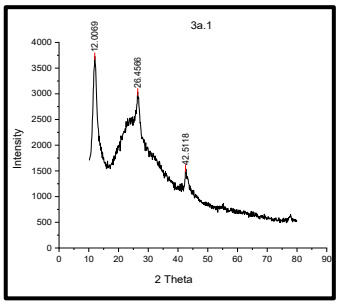



**Fig 2 XRD spectrum of a.1**

**Raman Spectra of the Product by Soft Template Method:**

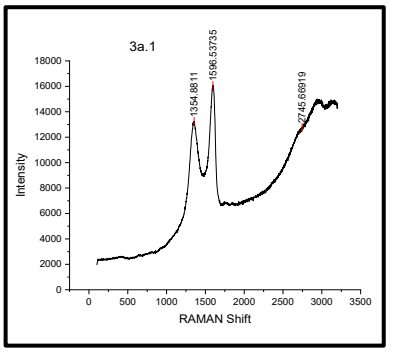
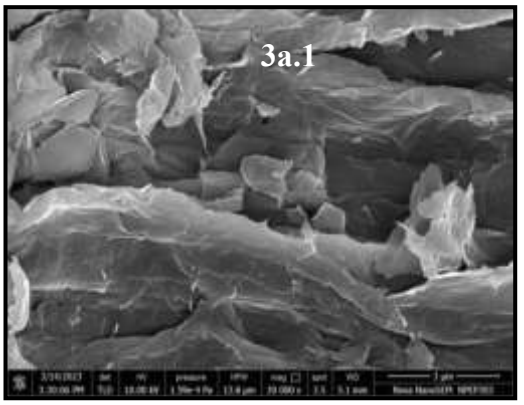



**Fig 3 Raman spectrum of a.1**

**FESEM Images of Soft Template Assisted Graphene:**



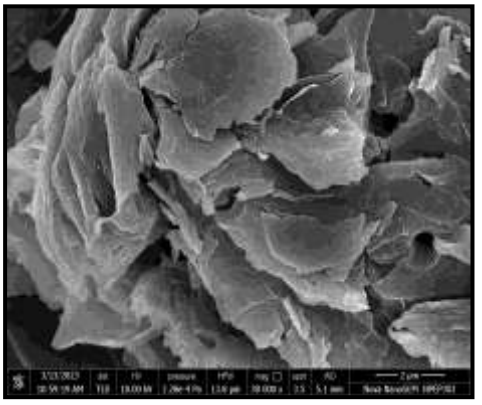



**Figure 4 and 5 FESEM images a.1**

## 5 Results and Discussions:

| samples | a.1 | a.2 |
|---|---|---|
| 2 Θ values | 12.00 | 11.90 |
| | 26.45 | 26.66 |
| | 42.51 | 42.71 |

**Table 2 XRD Diffraction Two Θ Values for Graphene Synthesised by Soft Template Method**

| | **D peak** | **G peak** | **2D peak** |
|---|---|---|---|
| a.1 | 1354.88 | 1596.54 | 2745.67 |
| a.2 | 1348.19 | 1593.29 | 2777.47 |

**Table 3 The Raman Shifts for graphene synthesised by soft template method**

**Figure 1** The FTIR spectrum of a.1 exhibit a strong peak at 2156 $cm^{-1}$. This peak is due to C=C stretching, It confirms the presence of C=C bonds in the graphene structure. Similarly absence of peaks at 1720, 1400 $cm^{-1}$ in the spectrum indicate reduction in the oxygen functionalities in the structure. In XRD plot the peak at 2 Θ =12 corresponds to a (002) basal plane with d-spacing at 0.737 nm. It is due to the large interlayer spacing of GO due to presence of oxygenated functional groups and intercalated water molecules. The peak at 2Θ = 23.8 corresponds to interlayer distance of 0.37nm and shows the reduction in oxygen functionalities corresponding to reduced graphene oxide. The peak at two Θ =43(001) exhibits orientation due to turbostatic band of disordered carbon materials.

**Figure 3 shows** the XRD peaks of. a.1 2 Θ values at 12, is due to the interlayer spacing of GO with d-spacing=0.737. The two theta peak at 26.45 corresponds to basal reflection (002) with d spacing 0.335 nm. From the **figure 4** Raman spectra a.1the peak obtained at 1354.88 corresponds to the D peak is related to the amount of disorder in the graphene, the peak at 1596 $cm^{-1}$ known as the G peak confirms the presence of carbon atoms. Whereas the shape and position of 2D peak at 2745.67 $cm^{-1}$ tells the number of layers. **FESEM** is useful to study the morphology of the substance**.** From FESEM micrograph the features like presence of impurities, folds on the graphene and discontinuities during the synthesis process can be investigated. From the **figures** the FESEM images of all the three a.1 and a.2 show the well exfoliated layers of graphene with porous nature corresponding to three dimensional graphene.

**APPLICATION: Antimicrobial Activity Test. ( Kirby-Bauer Method) :**

Antimicrobial activity test was performed by the commonly used Agar diffusion method which is designed to determine the smallest amount of the antibiotic needed to inhibit the growth of microorganism.

**Materials :**18 hrs Nutrient broth culture of Test organisms, Standard Chloramphenicol, Mueller –Hinton agar plates, Cork borer, Sterile std bioassay filter paper disc, Sterile cotton swabs, Alcohol, Ruler, Laminar flow chamber, Test samples.

**Procedure:** Take a sterile cotton swab and dip it into a culture of Test organism suspension. Inoculate the entire agar surface of each plate first in horizontal and then in vertical direction to ensure the even distribution of the organism over the agar surface using the swab. Allow the agar surface to dry for 5 min. Sterilize a cork borer by autoclaving or disinfect it by rinsing in alcohol followed by sterile water. Obtain a Mueller –Hinton agar plates and aseptically punch (4-mm) holes in the agar using a cork borer. Using a wax pencil, mark the underside of the Petri to label the wells. With the help of micropipette add test solution in the well. Repeat the procedure for all wells. Incubate all plates at 37°C for 24-48 hrs. in an incubator.

**Observations and Results:**

Examine all the plates for the clear zone of inhibition surrounding the discs/ well. Measure the diameter of zone of inhibition in mm using a ruler on the underside of the plate. Record the zone size and prepare a graph comparing the zone obtained with the known concentration verses the zone of inhibition of std. antibiotic.

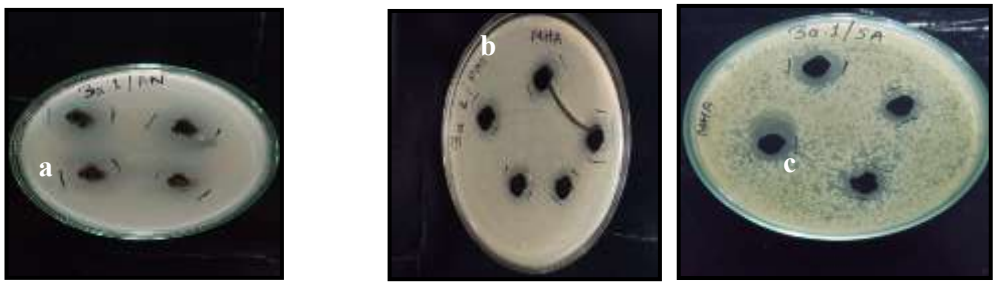



Image: Antimicrobial activity of grapheme

| Sr. No. | Sample Name* | Zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Microorganisms studied | | | | | |
| | | A* | Mean | B* | Mean | C* | Mean |
| 1 | a.1 | 13,14,14,16 | 13.75 | 15,15,15,12,14 | 14.2 | 18,17,17,18 | 17.5 |
| | | 24,24,25,25 | 24.5 | 14,14,14,14,13 | 14 | 15,15,15,15 | 15 |
| | | D* | Mean | E* | Mean | F* | Mean |
| | a.1 | 16,16,16,14 | 15.5 | 12,12,13,14 | 12.75 | 19,18,15,19,17 | 18.25 |
| | a.2 | 16,16,16,17,18 | 16.25 | 13,13,13,13 | 13 | 12,12,12,12,12 | 12 |

Table A. Antimicrobial Activity of the Graphene Synthesized by soft template method

**A: Candida albicans(NCIM 3100) B: Escherichia coli (2065) C: Aspergillus niger(ATCC504)**

**D: Staphylococcus aureus(NCIM2178) E: Bacillus subtilis (NCIM2063) F: P. mirabilis (NCIM2388)**

**DISCUSSION**:

The Image and tables A show the antimicrobial activity of the graphene synthesized by soft template method. The antimicrobial activity of all the seven products is different. From the data it can be concluded that they are active against all the Gram positive and Gram negative pathogenic bacteria and the two fungi selected. This is advantageous as the drugs made by including these products will show broad spectrum activity. The results from above tables show the efficiency of soft template and hard template assisted graphene against the microorganisms tested and listed above. The graphene a.2 exhibits highest antifungal activity for the fungus Candida albicans (24.5 mm). Product a.1 for P mirabilis (18.25 mm).

**SUMMARY:**

We have synthesized 3D graphene foam by using various combinations of the soft template and carbon sources and also by using various combinations of hard template and carbon sources. The characterisation of all of these graphene is done by FTIR, XRD, Raman and FESEM. Applications of these graphenes i.e. antimicrobial activity is studied by well diffusion method.

**CONCLUSION:**

Successful synthesis of the graphene was done by using trimethyl benzene, as the soft template in the form of microemulsions using graphene oxide as the carbon source. Similarly **successful** synthesis of the graphene was done by using in the form of microemulsions using graphene oxide as the carbon source. Both the products a.1 and a.2 were found to have better antifungal activity and antibacterial activity.

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