

## Synthesis and Characterization of Hydroxyapatite/Graphene Oxide for Biomedical Applications

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**Abstract** - The well resemblance of the mineral phase of human hard tissue and excellent bioactivity of Hydroxyapatite makes it as an essential candidate in biomedical field, especially in orthopedics. The utility of composites of hydroxyapatite is tremendous in hard tissue implants as the properties can be tailored to meet specific physical, chemical and biological needs. In this study, Hydroxyapatite/Graphene oxide composite (HA/GO) was synthesized by co-precipitation method and Graphene Oxide was prepared by a modified Hummers' method. The samples were subjected to XRD, FTIR, SEM and Raman analyses. SEM images revealed crumpled morphology and exfoliated form of GO. And also FTIR analysis confirmed HA/GO composite formation. Antibacterial studies were carried out using *Enterococcus faecalis* (gram positive) and *Pseudomonas aeruginosa* (gram negative). The antimicrobial test revealed that the composite inhibited both Gram positive and negative bacteria.

**Key Words:** hydroxyapatite, graphene oxide, coprecipitation, antibacterial, biomedical

### 1. INTRODUCTION

There is a rapid growing demand for bio-engineered materials for biomedical applications. Efforts are being made to produce improved the quality of the bio implant materials. A wide variety of composite materials are being tailored with desired properties according to the physical, chemical and biological aspects in different fields of applications. Hydroxyapatite is an excellent candidate which is used widely in Orthopaedics as it is bioactive and replica of the humane bone mineral [1]. Graphene Oxide (GO) is one of the promising materials in biomedical application. It exhibits biocompatibility and antimicrobial effect. In addition to that, it enhances the mechanical properties of hard Tissue implants [2]. In this present study, Hydroxyapatite/Graphene Oxide composite was prepared by

co-precipitation method and the prepared samples were subjected to different characterization techniques to analyze the physical, chemical and biological properties. The structural and morphological properties were studied by X-Ray Diffraction and the Scanning Electron Microscope respectively. FTIR Spectrometer was used to analyze the functional properties. The antibacterial activity of the sample was screened by agar well diffusion method.

### 1.1 Materials and Methods

Graphite powder (APS 7-11 micron, 99%) was purchased from Alfa Aesar and Calcium Nitrate Tetra Hydrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ), Di-ammonium Hydrogen Phosphate ( $(\text{NH}_4)_2\text{HPO}_4$ ), Ammonium Hydroxide ( $\text{NH}_4\text{OH}$ ), Potassium Permanganate ( $\text{KMnO}_4$ ), Sodium Nitrate, Sulfuric acid ( $\text{H}_2\text{SO}_4$ ) and Hydrogen Peroxide were purchased from Merck Chemicals, India. All of the chemical reagents used in this experiment were analytical grade and used without further purification.

Graphene Oxide was synthesized by modified Hummers' method [3] and GO aqueous solution (10mg/ml) was prepared by ultra-sonication. Hydroxyapatite/Graphene Oxide composite (HA/GO) was prepared by co-precipitation method as follows. The prepared GO solution was dropped into 0.5 M  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  aqueous solution and stirred for 1 h. Then aqueous solution of 0.3 M  $(\text{NH}_4)_2\text{HPO}_4$  was added with the reaction mixture drop wise and stirred for 2h. The pH of the solution was adjusted to 11 with Ammonium hydroxide. After the addition of Ammonium hydroxide the composite was precipitated and it was aged for 24 h. The product was washed with deionized water and ethanol repeatedly. Then it was deep freeze at  $-80^\circ\text{C}$  followed by lyophilization.

The prepared samples were subjected to X-Ray diffraction analysis using X'pert Pro Panalytical Diffractometer in reflection mode with Cu K $\alpha$  ( $\lambda=1.5406 \text{ \AA}$ ) radiation, in the  $2\theta$  range from  $10^\circ$  to  $80^\circ$  at room temperature. Fourier transformed infrared (FTIR) spectroscopic technique was used for the functional analysis of the prepared samples. The composites were pelletized with KBr and the FTIR spectra were obtained using Perkin Elmer-Spectrum RXI over the wavenumber range from  $4000$  to  $400 \text{ cm}^{-1}$ . The morphological studies were carried out by Scanning electronic microscopy. The composites were gold sputtered and subjected to the morphological characterization using VEGA3 TESCAN SEM.

### 1.2 In Vitro Antibacterial Assay

The agar well diffusion method [4] was used to screen the antibacterial activity of Hydroxyapatite and Hydroxyapatite/Graphene Oxide composite against the Gram-positive (*Enterococcus faecalis* (HQ693279.1) and Gram negative (*Pseudomonas aeruginosa* HQ116441) bacteria. In vitro anti microbial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared pouring 15ml of molten media into sterile petri plates. The plates were allowed to solidify for 5min and 0.1% inoculums suspension of bacterial strains was swabbed uniformly and the inoculums was allowed to dry for 5min. Then, wells were made on the plate using well puncher for loading Hydroxyapatite and Hydroxyapatite/Graphene Oxide composite. 50  $\mu\text{L}$  of different concentrations of Hydroxyapatite and Hydroxyapatite/Graphene Oxide composite ( $25, 50$  and  $75 \mu\text{g mL}^{-1}$ ) was loaded on the wells. The compound was allowed to diffuse for 5min and the plates were incubated at  $37^\circ \text{C}$  for 24h. The antibacterial efficacy of Hydroxyapatite and Hydroxyapatite/Graphene Oxide composite was compared with Dimethyl sulfoxide (DMSO). After incubation, the inhibition zones formed around the wells were measured with transparent ruler in millimeter.

### 2. Results and Discussions

It is evident from the recorded X-Ray diffraction pattern that the synthesized specimen comprises both Hydroxyapatite and Graphene Oxide. The observed reflections at  $25.85^\circ, 31.74^\circ, 32.16^\circ$  and  $32.90^\circ$  for the planes (002), (211), (112) and (300) respectively corresponding to HA, are in good agreement with JCPDS Card no. 09-0432. The peaks corresponding to GO at  $10.86^\circ$  and  $43^\circ$  were also observed.

Fig-2 shows that the synthesized GO has a range of oxygen functionalities such as hydroxyl, carbonyl and epoxide. The band at  $3415 \text{ cm}^{-1}$  is assigned to the OH stretching vibration due to the existence of surface hydroxyl groups.

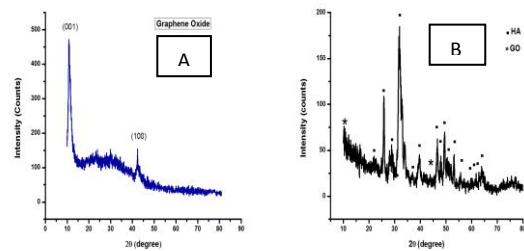


Fig-1: XRD Patterns of Graphene Oxide (A) and Hydroxyapatite/Graphene composite (B)

The band at  $1726 \text{ cm}^{-1}$  is the characteristic of the C=O stretch of the carboxylic acid group. The absorption at  $1625 \text{ cm}^{-1}$  corresponds to the C=C bond. The C-O bonds are attributed by  $1393 \text{ cm}^{-1}$  and  $1071 \text{ cm}^{-1}$ , confirming the presence of oxide functional groups in the synthesized GO. In the FTIR spectrum of HA/GO composite (Fig-2), the observed absorption at  $3583 \text{ cm}^{-1}$  is corresponding to stretching vibration of O-H present in HA [5] and the strong absorption peaks at  $1421 \text{ cm}^{-1}$  corresponds to carbonate ions present in  $\text{PO}_4$  site in HA [6]. The strong absorption band at  $1034 \text{ cm}^{-1}$  confirms the stretching vibration of  $\text{PO}_4$  while the bending vibrations of  $\text{PO}_4$  were confirmed by intensive peaks at  $604$  &  $567 \text{ cm}^{-1}$ . There is also observed the existence of C-O and hydroxyl functional groups in the composite which occurred at  $1071 \text{ cm}^{-1}$  and around  $3000-3500 \text{ cm}^{-1}$  respectively, which confirms HA/GO composite formation.

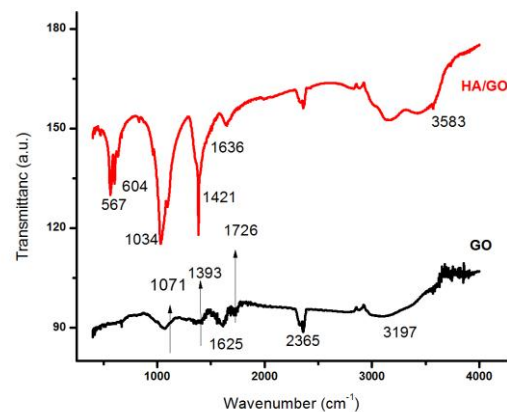


Fig-2: FTIR spectra of HA/GO composite and GO

Fig-3 shows the Raman spectrum of Graphene oxide synthesized by modified Hummers' method. Basically G-band in the range of  $\sim 1500-1600\text{ cm}^{-1}$  signifies  $sp^2$  hybridization (graphitic signature of carbon) and D-band in the range of  $\sim 1300-1400\text{ cm}^{-1}$  denotes disorder due to the defects involved in  $sp^3$  hybridized carbon. The Raman spectrum of the synthesized GO showed a shift at  $1591\text{ cm}^{-1}$ , also called G band, was attributed to the first-order scattering of the  $E_{2g}$  phonons of  $sp^2$ -hybridized carbon atoms, while Raman shift at  $1352\text{ cm}^{-1}$ , i.e., D band ascribed to the breathing mode of the  $\kappa$ -point phonons of the  $A_{1g}$  symmetry, originated from defects involved in  $sp^3$ -hybridized carbon bonds (e.g., hydroxyl and epoxide bonds) [7].

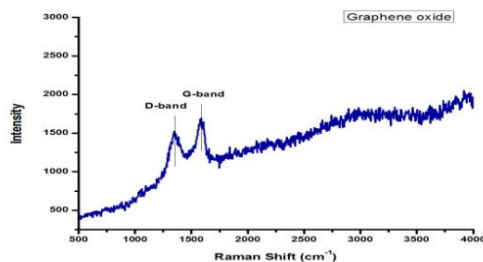


Fig-3: Raman Spectra of Graphene Oxide

In the Raman spectrum corresponding to the composite, the peaks at  $969\text{ cm}^{-1}$  &  $1039\text{ cm}^{-1}$  are corresponding to  $PO_4$  in Hydroxyapatite. Also, there is the presence of D-band, G-band and 2D band of GO which was recorded at  $1347$ ,  $1680$  and  $2970\text{ cm}^{-1}$  respectively.

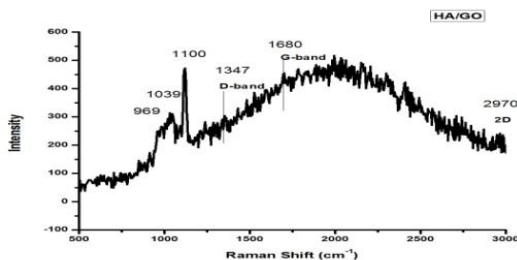


Fig-4: Raman Spectra of Hydroxyapatite/Graphene Oxide Composite

SEM images of GO and HA/GO are shown in the Fig-5A & B respectively. In Fig-5A the exfoliated transparent Graphene oxide sheets were seen. Besides it is evident from the SEM analysis that the synthesized GO sheets possess irregular edges and the surfaces of GO sheets have a lot of crumplings which come from the scrolling of GO sheets. Fig-5B depicts the uniform morphology of HA/GO composite.

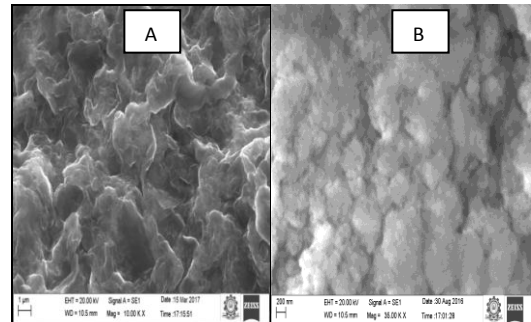


Fig-5: SEM images of Graphene Oxide (A), Hydroxyapatite/Graphene Oxide Composite (B)

HA/GO composite showed antimicrobial activity against both Gram positive and Gram negative bacteria (*Enterococcus faecalis* and *Pseudomonas aeruginosa* respectively). The composite inhibited *Enterococcus faecalis* around 6mm, 10mm & 12mm for the concentrations  $25$ ,  $50$  and  $75\text{ }\mu\text{g mL}^{-1}$  respectively. There were the inhibition zones of diameter 2, 4 & 8 mm for the concentrations  $25$ ,  $50$  and  $75\text{ }\mu\text{g mL}^{-1}$  respectively.

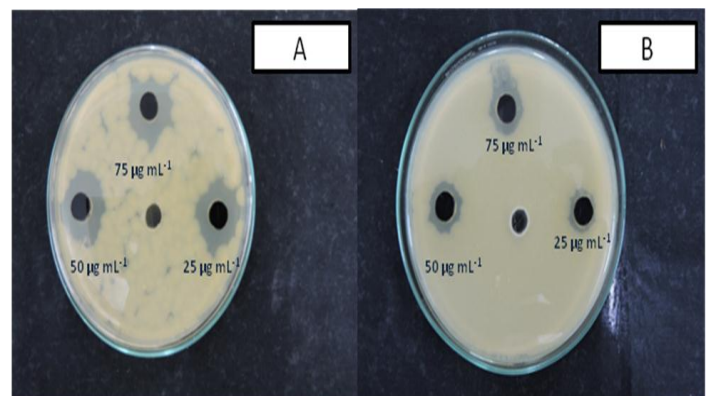


Fig-6: Antibacterial activity of HA/GO against *Enterococcus faecalis* (A) and *Pseudomonas aeruginosa* (B)

### 3. CONCLUSIONS

Hydroxyapatite/Graphene Oxide was synthesized successfully using co-precipitation method. XRD result revealed the prepared composite comprising Hydroxyapatite and Graphene Oxide. Besides, the functional groups of the composite had been confirmed through FTIR and Raman analyses. The prepared composite inhibited both Gram positive and negative bacteria which facilitates HA/GO composite to be a good candidate in biomedical application.

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**REFERENCES**

- [1] K. Rezwan, Q.Z. Chen, J.J. Blaker & Boccaccin, "Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering", *Biomaterials*, vol. 27, Feb. 2006, pp. 3413-3431, doi:10.1016/j.biomaterials.2006.01.039.
- [2] Yu, Peng., Bao, Rui-Ying., Shi, Xiao-Jun., Yang, Wei., & Yang, Ming-Bo., Self-assembled High-strength Hydroxyapatite/graphene oxide/chitosan Composite Hydrogel for Bone Tissue Engineering, *Carbohydrate Polymers*, vol. 155, Jan. 2017, pp. 507-515, doi: <http://dx.doi.org/10.1016/j.carbpol.2016.09.001>.
- [3] B. Paulcham, G. Arthi and BD. Lignesh, A Simple Approach to Stepwise Synthesis of Graphene Oxide Nanomaterial, *J Nanomed Nanotechnol*, vol. 6, Jan. 2015, pp. 253. doi: 10.4172/2157-7439.1000253
- [4] C. Perez, M. Paul and P. Bazerque, "Antibiotic Assay by Agar Well Diffusion Method," *Acta Biologiae et Medicine Experimentalis*, Vol. 15, 1990, pp. 113-135.
- [5] S.Ramesh, K.L. Aw, R. Tolouei, M. Amiriyani, C.Y. Tan, M. Hamdi, et al. Sintering properties of hydroxyapatite powders prepared using different methods, *Ceramics International* vol. 39, Jan 2013, pp. 111-119, doi: <https://doi.org/10.1016/j.ceramint.2012.05.103>
- [6] H.R. Ramay, M. Zhang, Preparation of porous hydroxyapatite scaffolds by combination of the gel-casting and polymer sponge methods, *Biomaterials*, vol. 24, 2003, pp. 3293-3302, doi: 10.1016/S0142-9612(03)00171-6
- [7] S. Baradaran, et al., Mechanical properties and biomedical applications of a nanotube hydroxyapatite-reduced graphene oxide composite, *Carbon*, vol. 69 Apr. 2014, pp. 32-45, doi: <https://doi.org/10.1016/j.carbon.2013.11.054>