# Graphene oxide and Reduced graphene oxide reinforced hydroxyapatite based nano composites for biomedical application

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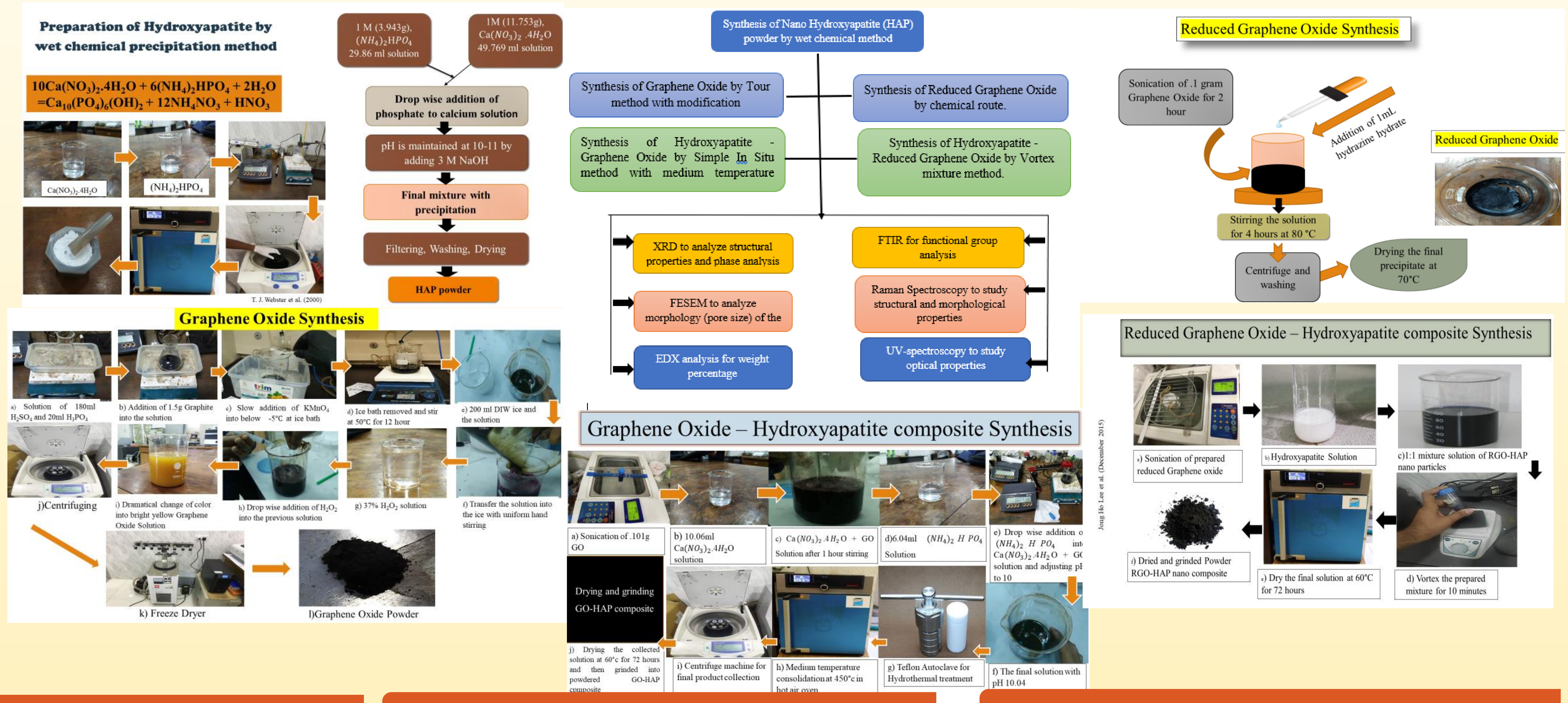
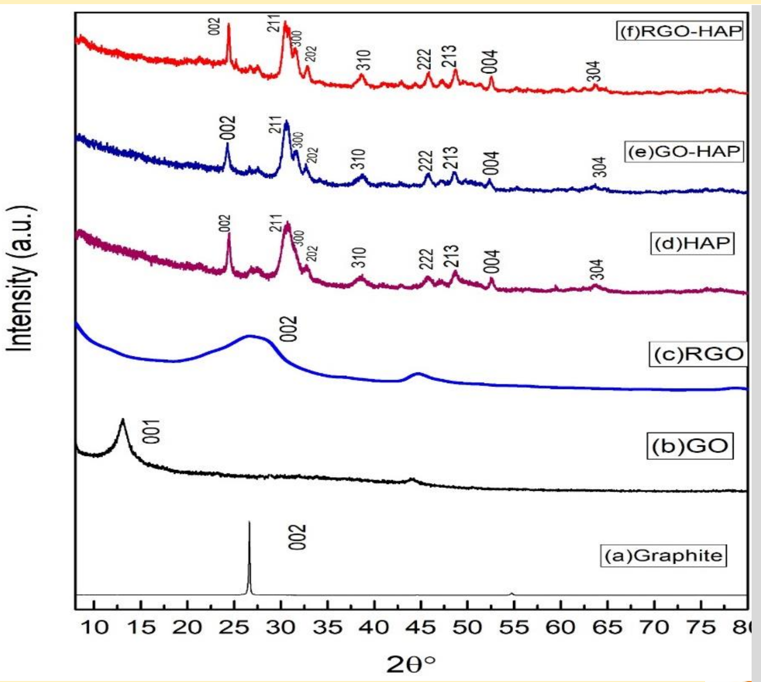
## Abstract

**Hydroxyapatite (HAP) is applied in medical applications for repair or replacement of bone tissues in body system. The constituent and structure of HAP is similar to the bone and teeth. Pure HAP has been synthesized by wet chemical precipitation method by using calcium nitrate $Ca(NO_3)_2.4H_2O$ and diammonium hydrogen phosphate $(NH_4)_2HPO_4$. However, HAP shows comparatively inferior mechanical properties than natural bone and as such, improvement of mechanical properties is necessary. One of the best candidate materials for the reinforcing phase to HAP is Graphene Oxide (GO) and Reduced Graphene Oxide (RGO), which exhibit excellent biocompatibility. Graphene Oxide have been synthesized by Tour method with modification. And the synthesized GO is reduced to Reduced Graphene Oxide (RGO). In this study, GO-HAP nano-composites have been synthesized by using In-situ method and RGO-HAP nanocomposites have been synthesized by vortex mixture method. GO-HAP and RGO-HAP bio-composites were fabricated with the objective to improve the mechanical, morphological and structural properties of the biomaterial and future biomedical uses. The X-ray diffraction (XRD) of GO-HAP and RGO-HAP nanocomposite indicates that the incorporation of a certain amount of GO and RGO does not affect the crystallinity and phase stability of HAP. Fourier transforms infrared spectroscopy (FTIR) confirms the presence of functional groups in HAP, GO, RGO and their composites. The morphology of the synthesized composites is investigated by FESEM, which predicts that the pore size of the composites is in the range of 100 nm, which is good for tissue ingrowth. Raman spectroscopy predicts that the amount of disorder of graphene layers increases due to the functionalization in GO-HAP and RGO-HAP composites. And also predicts that the bond strength of GO-HAP is greater than synthesized RGO-HAP. EDX analysis verifies the formation of nano-HAP particles on the GO and RGO flakes as the Ca/P ratio was close to standard 1.67 exists in natural bone tissue. UV-vis spectra show the strong interaction between graphene sheets and HAP nanoparticles. And also predicts lower chance of bonding between RGO and HAP nanoparticles. Eventually the prepared GO-HAP and RGO-HAP nano-composites will be a good alternative option in the bone tissue engineering, bone regeneration or any other biomedical applications.**

## Methodology



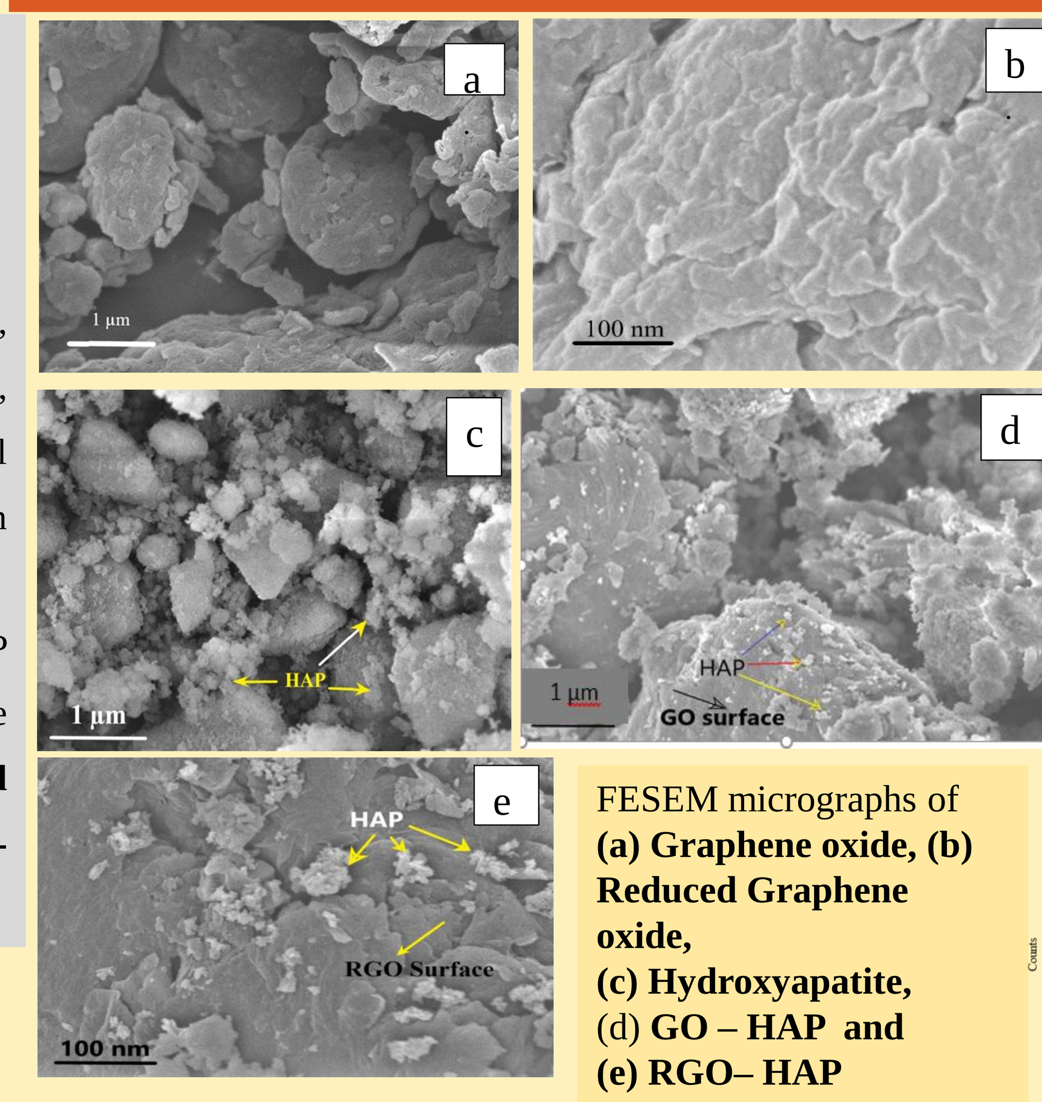

## XRD measurement




| Sample | a=b (Å) | c (Å) | Crystallite size(nm) |
|---|---|---|---|
| HAP | 9.36 | 6.88 | 46.3 |
| GO-HAP | 9.37 | 6.86 | 19.4 |
| RGO-HAP | 9.34 | 6.89 | 20.79 |

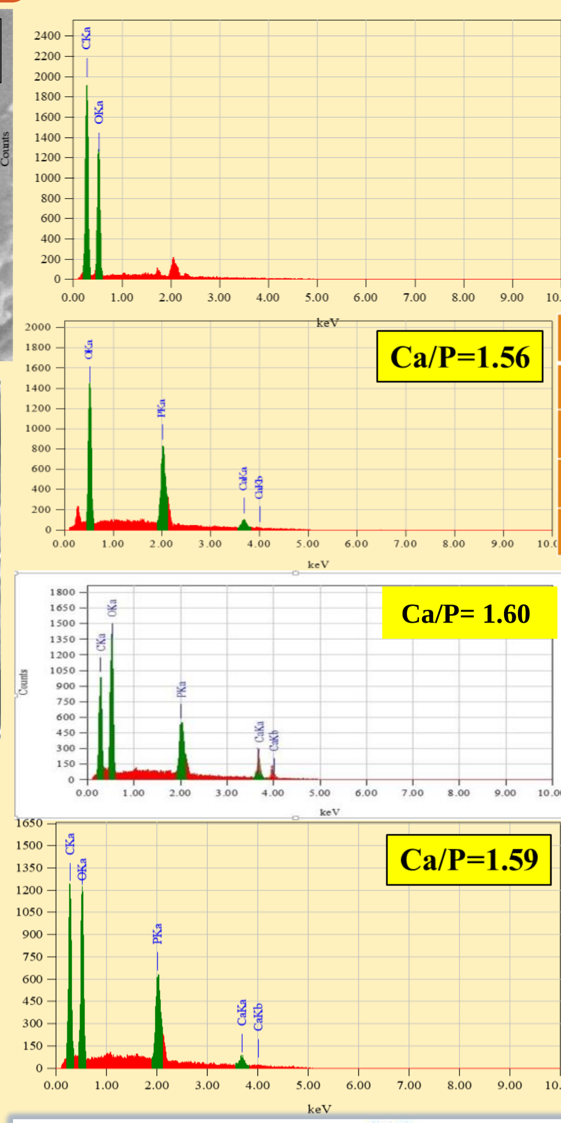
- Interlayer spacing **increased in GO**
- Interlayer spacing decreased after **reduction of GO**
- The existence of the (002), (211), (300), (202), (310), (222), (213), (004) and (304) crystal planes confirms the formation of **HAP nanoparticles.**
- The XRD patterns of GO-HAP and RGO-HAP composite were analogous to the **standard pattern of HAP (JCPDS 09-0432).**

## FESEM image




FESEM micrographs of **(a) Graphene oxide, (b) Reduced Graphene oxide,**
**(c) Hydroxyapatite,**
(d) **GO – HAP and**
**(e) RGO– HAP**

## EDX Analysis




| Element | Mass% | Atom% |
|---|---|---|
| C Ka | 49.82 | 56.94 |
| O Ka | 50.18 | 43.06 |
| TOTAL | 100.00% | 100.00% |

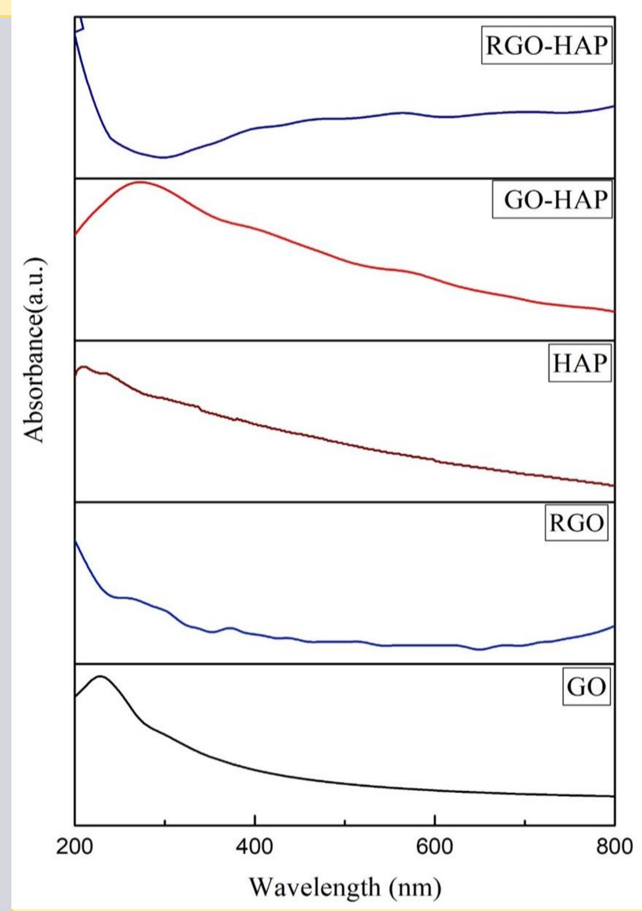
| Element | Mass% | Atom% |
|---|---|---|
| O Ka | 26.15 | 44.70 |
| P Ka | 24.52 | 21.65 |
| Ca K | 49.33 | 33.66 |
| Total | 100.00% | 100.00% |

| Element | Mass% | Atom% |
|---|---|---|
| C K | 10.51 | 26.40 |
| O k | 23.10 | 41.30 |
| P K | 15.71 | 12.40 |
| Ca K | 50.68 | 19.91 |
| Total | 100% | 100% |

| Element | Mass% | Atom% |
|---|---|---|
| C K | 16.74 | 31.22 |
| O k | 22.59 | 31.62 |
| P K | 19.81 | 14.33 |
| Ca K | 40.85 | 22.83 |
| Total | 100% | 100% |

## UV-vis Spectra

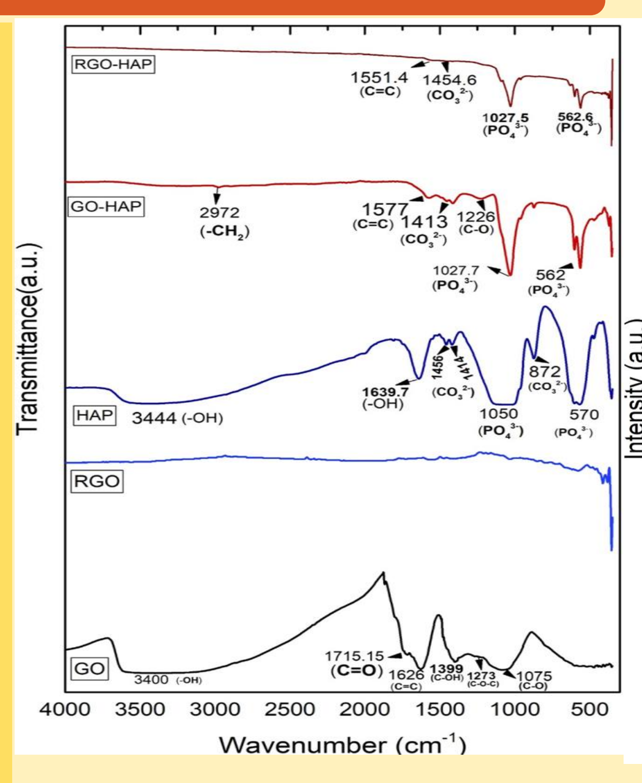
- Absorption peak **at 230 nm**, indicates **good oxidation** of graphene sheets.
- The shift from **230 nm to 296 nm** denotes the **reduction** of oxygen atom from the graphene layers.
- **The red shift** of the GO peak to 298 nm suggests **a strong interaction** between graphene sheets and Hydroxyapatite .
- **Disappearance** of peaks of RGO suggests a **significant** change in **RGO** structure **with HAP.**




- Due to the **reduction of oxygen sites** and tendency to go back to **inert graphitic structure**, there is **less chance** of **bonding** between RGO and HAP.

## FTIR spectra

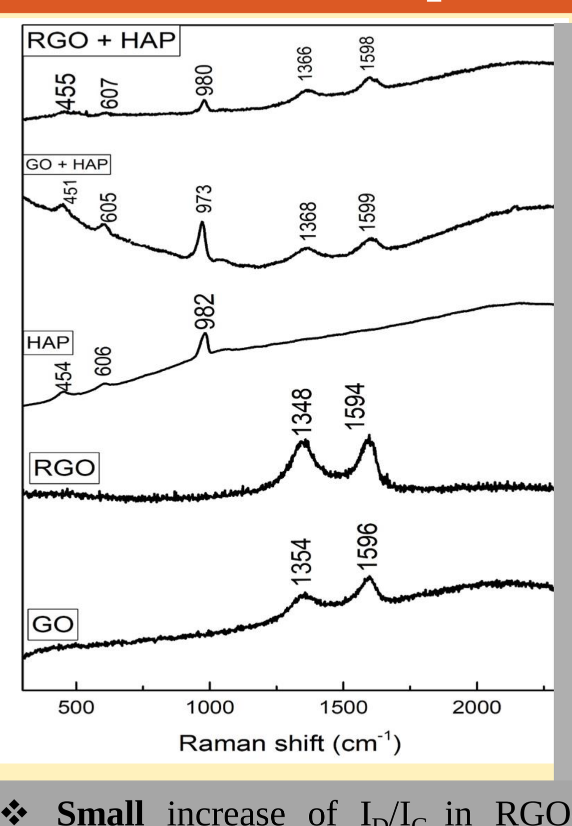
- In **GO** many oxygen groups are present and in **RGO** Oxygen functional groups are **decreased**
- In **GO-HAP** spectra Some of functional groups are **disappeared entirely** and some absorption bands **appeared** and **Hydroxyl** group is decreased and **shift of stretching band** of phosphate from 1050 $cm^{-1}$ in pure hydroxyapatite to 1027 $cm^{-1}$
- In **RGO-HAP** spectra **Oxygen functional groups** are **absent and** Clear absorption band for stressed vibration of graphene sheet **(C=C)** near 1551.3 $cm^{-1}$ and **increase** of **width** in phosphate group at 1027.5 $cm^{-1}$




| Wavenumber of the corresponding functional group (cm−1) | Stretching or bending mode | Functional group |
|---|---|---|
| 3400 | Stretching mode | OH− |
| 1725.15 | Stretching mode | C=O |
| 1626 | Stretching mode | C=C (Medium) |
| 1399 | Stretching mode | C-OH (Carboxyl) |
| 1273 | Bending | C-O-C (Epoxy) |
| 1075 | Stretching | C-O (Alcoxy) |

| Wavenumber of the corresponding functional group (cm−1) | Stretching or bending mode | Functional group |
|---|---|---|
| 3444 | Stretching mode OH− due to absorbed $H_2O$ | OH− |
| 1639.7 | Bending of OH- due to chemically absorbance of $H_2O$ | OH- |
| 1456, 1414 | Stretching mode | $CO_3^{2-}$ for dissolving $CO_2$ |
| 1050 | Asymmetric stretching | $PO_4^{3-}$ |
| 872 | Out of plane bending | $CO_3^{2-}$ |
| 570 | Asymmetric bending | $PO_4^{3-}$ |

## Raman spectroscopy




- Shifting of **G band** and **D band** is evident from **GO to RGO**, **GO** to **GO-HAP** and **RGO-HAP**
- Presence of **peaks of pure HAP** into the **GO-HAP** and **RGO-HAP** spectra
- **Large** increase of $I_D/I_G$ in GO-HAP indicates **strong bonding** in GO and HAP .
- **Small** increase of $I_D/I_G$ in RGO-HAP denotes bonding between RGO and HAP is evident but **week** .

| Sample | G band($cm^{-1}$) | D band($cm^{-1}$) | $I_D/I_G$ |
|---|---|---|---|
| Graphene Oxide | 1596 | 1354 | 0.54 |
| Reduced Graphene Oxide | 1594 | 1348 | 0.66 |
| GO - HAP | 1599 | 1368 | 0.84 |
| RGO - HAP | 1598 | 1366 | 0.68 |

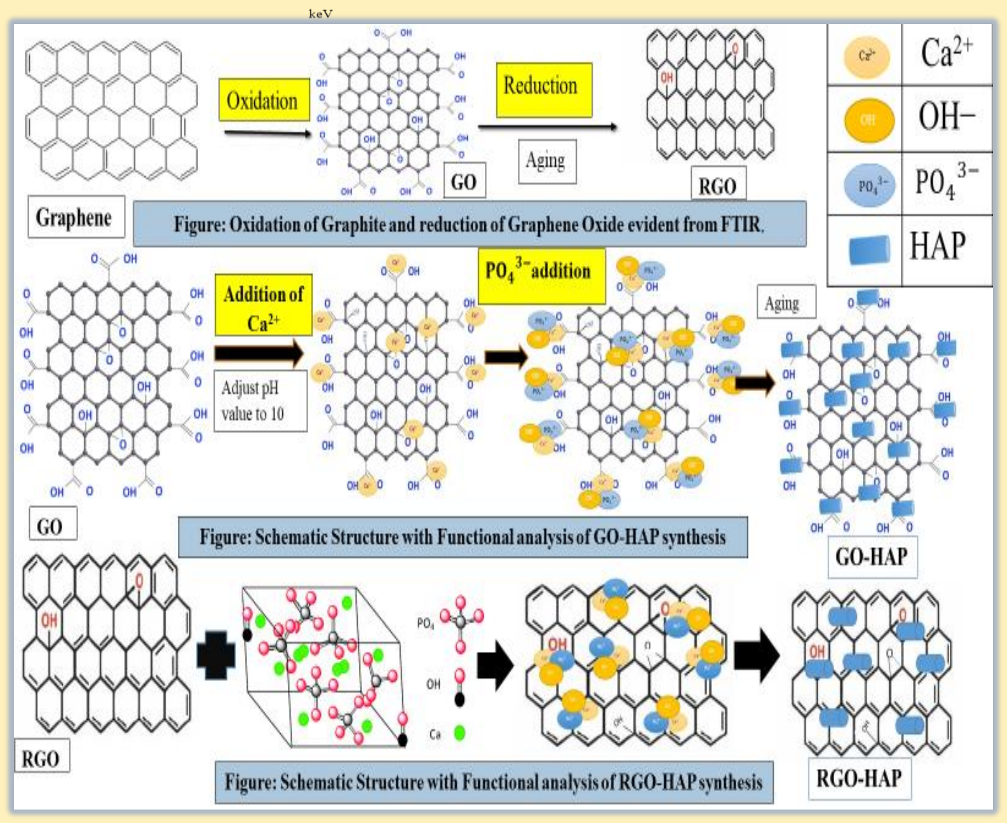



Figure: Oxidation of Graphite and reduction of Graphene Oxide evident from FTIR.

Figure: Schematic Structure with Functional analysis of GO-HAP synthesis

Figure: Schematic Structure with Functional analysis of RGO-HAP synthesis

## Conclusion

- **XRD** clearly predicted the **crystalline and phase purity** of prepared samples. And **introduction** of **GO** and **RGO** did not **disturb or alter** the crystalline structure of Hydroxyapatite.
- The successful **formation** of **GO-HAP** and **RGO-HAP** nanocomposite were confirmed by **chemical analysis** of **FTIR** and **morphological analysis** of **FESEM** .
- **EDX** confirmed the successful **elemental mapping** of prepared samples and composites.
- **Quality of bonding** and **amount of disorder** present into the composites were clearly predicted by **Raman** and **UV-Vis spectroscopy** analysis.

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## Suggestion for Future Works

1. **Cytotoxicity, Antibacterial activity , TEM** , **TGA** of **GO-HAP** and **RGO-HAP** composites can be checked.
2. Measurement of **mechanical strength** can be done.
3. This composites can be applied as **drug delivery carriers** and can be applied to the **Orthopedic implants** and **surgeries**.