INTERNATIONAL JOURNAL OF RESEARCH AND INNOVATION IN APPLIED SCIENCE (IJRIAS)
ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue XI November 2025

Hydroxyapatite-Graphene Oxide Nanocomposite for Wastewater Treatment

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DOI: https://dx.doi.org/10.51584/IJRIAS.2025.101100018

Received: 21 November 2025; Accepted: 28 November 2025; Published: 04 December 2025

ABSTRACT

This study presents an eco-friendly and cost-effective approach for the synthesis of hydroxyapatite (HAp) from quail eggshell waste, further functionalized with graphene oxide (GO) nanoparticles, for the treatment of industrial wastewater. Quail eggshells, rich in calcium carbonate, serve as a sustainable calcium source for hydroxyapatite production via a wet chemical precipitation method. The synthesized HAp was subsequently combined with GO to enhance its surface area, adsorption capacity, and reactivity toward pollutants. The composite material (HAp/GO) was characterized using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) to evaluate its morphology, functional groups, and crystalline structure, respectively. SEM images revealed a porous structure with uniform dispersion of GO sheets on the HAp surface. FTIR spectra confirmed the presence of characteristic phosphate, hydroxyl, and carbonyl groups, indicating successful synthesis and functionalization. XRD analysis showed well-defined peaks corresponding to the crystalline phases of hydroxyapatite and GO. The HAp/GO composite demonstrated effective adsorption and removal of heavy metals and organic pollutants from industrial wastewater samples, highlighting its potential as a novel, sustainable adsorbent material for environmental remediation applications.

INTRODUCTION

The growing consumption of eggs and egg-derived products, particularly quail eggs due to their high nutritional value, has resulted in the accumulation of large quantities of eggshell waste from hatcheries, households, and the food industry. These eggshells, primarily composed of calcium carbonate, are often discarded in landfills without proper treatment, leading to environmental pollution, unpleasant odors, and soil degradation. Given their rich calcium content, quail eggshells offer a sustainable and cost-effective raw material for synthesizing hydroxyapatite (HA), a calcium phosphate compound with wide applications in biomedicine and environmental remediation. Utilizing waste eggshells for HA production not only addresses waste management challenges but also contributes to the development of green materials, reducing the reliance on mined calcium sources and minimizing ecological impact.

To further enhance the functionality of hydroxyapatite, it can be combined with graphene oxide (GO) nanoparticles, which are known for their exceptional surface area, mechanical strength, and antibacterial properties. The resulting HA/GO nanocomposite presents a promising solution for the treatment of industrial wastewater, particularly in removing heavy metals and organic pollutants. In this study, hydroxyapatite was synthesized from quail eggshells using the wet chemical precipitation method and then integrated with graphene oxide to form a hybrid adsorbent. The synthesized materials were characterized using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) to investigate their morphology, chemical functionality, and crystallinity. This research aims to explore the potential of a low-cost, sustainable HA/GO nanocomposite for environmental cleanup and to contribute to waste-to-resource technology in line with circular economy principles.

MATERIALS AND METHODOLOGY

Materials: Quail eggshell, absolute ethanol (99.9%), deionized water, Graphene Oxide.

Glassware: Conical flask, beaker, glass pipette, micropipette, petri dish, test tubes, micropipette tips. Equipment: Hot air oven, Magnetic stirrer, weighing balance.

Methodology:

Collection and preparation of Hydroxyapatite from Quail eggshell:

The sample was prepared from a quail eggshell got from the local market. After being treated with 4% sodium hypochlorite and rinsed with distilled water, the powdered sample is filtered through filter paper. The sample is then allowed to air dry, yielding a powder. After adding the sample to the contaminated dye water, it took nearly ten days for the contaminated water to be completely clean. Hydroxyapatite was successfully prepared from the quail eggshells by following the method mentioned in the research paper (Anchana devi.C & Priya Perumal 2016).

Preparation of Hydroxyapatite/Graphene Oxide Nanocomposite:

Graphite powder was purchased commercially, and it was treated with sulfuric acid to prepare graphene oxide nanoparticles. Next, the nanoparticles of Hydroxyapatite and Graphene oxide nanoparticles were sonicated to prepare a nanocomposite. Following preparation, the sample and the contaminated dye water were combined. In contrast to the pure Hydroxyapatite nanoparticles, which took ten days to clear, the polluted dye water was cleaned in two days by Hap/GO nanocomposite (Mohamed A. Hassan et al., 2018).

Hydroxyapatite as Biofertilizer:

After combining soil and Hydroxyapatite fertilizer, mustard seeds were planted. In another pot, regular soil was used. Ten days later, the fertilizer-treated soil showed higher growth. The plant was longer than it was in the unfertilized soil (Madhavi Gaonkar & A.P.Chakraborty 2016).

Back Titration method:

After being heated for a few minutes, the sample was added to a conical flask containing a few drops of ethanol and hydrochloric acid, and it was titrated against standard sodium hydroxide. After determining the endpoint, the calcium content was known. The quail eggshell has a calcium content of roughly 69.63% (Anchana devi.C & Priya Perumal 2016).

Antimicrobial Activity by Well Diffusion Method:

Gram-positive and Gram-negative bacteria were used on the MHA medium plate to determine the sample's antibacterial activity. In the E. coli plate, the graphene oxide/hydroxyapatite sample had a larger zone than the pure hydroxyapatite sample. In contrast to the pure Hydroxyapatite sample, the Staphylococcus spp. plate showed a broad zone. Hydroxyapatite and Graphene oxide showed a zone of inhibition which was like the result from the research paper (NORSURIANI CHE HASHIM et al., 2019).

Antioxidant Assay:

1.5 ml of the sample was taken with 2 ml of distilled water, to the sample 3 ml of DPPH was added and read at 517nm. The findings show that because of its oxygen functional group, Graphene oxide considerably increases antioxidant activity. Superior antioxidant production was demonstrated by the Hydroxyapatite/Graphene oxide composite material, which may be advantageous for biomedical uses. The antioxidant value of the sample was found to be 0.0353. (Abdel Sattar O.E. Abdelhalim et al., 2022).

X-ray Diffraction:

The XRD pattern shows the crystalline nature of the sample under analysis. While the background signal points to a potential amorphous phase, most likely from bioactive glass, the existence of sharp peaks indicates a highly crystalline phase, with a dominating peak of 30° 2θ, which is indicative of hydroxyapatite. The sample composition matches that of hydroxyapatite when compared to reference data, suggesting the presence of a composite biomaterial.

With a prominent peak at about 30° 2θ, the image's XRD pattern shows a highly crystalline structure, suggesting the existence of a distinct phase. This peak's strength is noticeably greater than the other peak, indicating that the material is mainly made up of one crystalline phase. The presence of Graphene Oxide in the graph suggests that the sample might be a composite. Other peak's comparatively modest intensity points to the possibility of an amorphous component of subsequent phases. Similar diffraction peaks were found at 2θ as in the research paper (Mohamed A. Hassan et al., 2018).

FTIR:

The graph shows the presence of multiple carbonyl peaks, this suggests the presence of compounds such as carboxylic group, ketones and aldehydes (Mohamed A. Hassan et al., 2018).

SEM:

At 10,000x magnification, the surface morphology of Hydroxyapatite and Graphene oxide nanocomposite is seen in the accompanying SEM image. A heterogeneous structure with unique characteristics of both graphene oxide and hydroxyapatite is seen in the image. The composite was successfully formed, as evidenced by the existence of porous structures, rough surface textures, and irregularly shaped particles. Graphene oxide is seen as layered, sheet-like formations incorporated into the composite, whereas hydroxyapatite manifests as agglomerated clusters with crystalline structures. Graphene oxide's even dispersion throughout the hydroxyapatite matrix points to increased structural reinforcement, which could improve mechanical strength and have antibacterial effects (NORSURIANI CHE HASHIM et al., 2019).

Anti-cancer activity:

The prepared Hydroxyapatite-Graphene oxide nanocomposite sample has a low anticancer activity which was performed in the MCF-7 Breast cancer cell line. The anticancer activity of the sample is 71%. The percentage of cancer cell viability is high, which shows that the nanocomposite has only minimal anticancer properties (Ali Shafiee et al., 2022).

RESULT AND DISCUSSION:

Collection and preparation of the sample

Fig.1 Collected Quail shells Fig.2 Powdered shell sample

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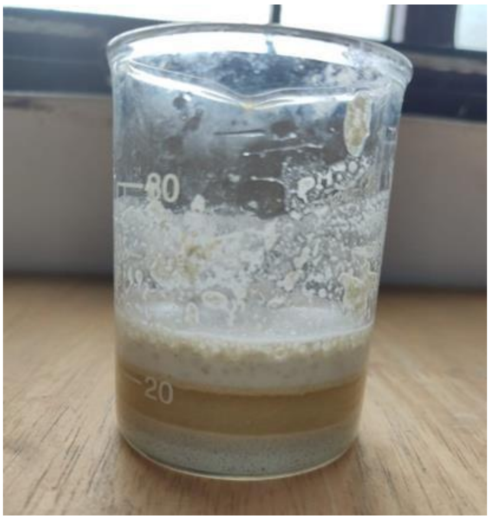

Fig.3 Sample treated with sodium hypochlorite

An eggshell from a fowl that was purchased at the neighborhood market was used to prepare the sample. The powdered sample is filtered through filter paper after being treated with 4% sodium hypochlorite and washed with distilled water. After that, the sample is set to air dry, turning into a powder. Following the addition of the sample, the tainted coloring water took over ten days to become entirely clean. The quail eggshells were effectively converted into hydroxyapatite by using the procedure outlined in the research report.

Preparation of Hydroxyapatite/Graphene Oxide Nanocomposite:

Fig.4 Preparation of Graphene oxide Nanoparticles

Fig.5 Filtration of Graphene Oxide

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Fig.6 Air-dried Hap/GO Nanocomposite

To produce graphene oxide nanoparticles, commercially available graphite powder was treated with sulfuric acid. A nanocomposite was then created by sonicating the hydroxyapatite and graphene oxide nanoparticles. After preparation, the sample was mixed with the tainted dye water. The HAp/GO nanocomposite cleared the contaminated dye water in two days, but the pure Hydroxyapatite nanoparticles took 10 days to clear.

Hydroxyapatite as Biofertilizer:

Fig.7 Soil without and with HAp DAY 1

Fig.8 DAY 2 - DAY 5

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Mustard seeds were sown following the addition of soil and hydroxyapatite fertilizer. They used ordinary dirt in another pot. The soil treated with fertilizer grew more after ten days. Compared to the unfertilized soil, the plant was longer.

Back Titration method:

Fig.9 Back titration method

The sample was placed in a conical flask with a few drops of ethanol and hydrochloric acid after being heated for a few minutes. It was then titrated against standard sodium hydroxide. The calcium content was known once the endpoint was established. The calcium content of quail eggshells is approximately 69.63%.

Antimicrobial Activity by Well Diffusion Method:

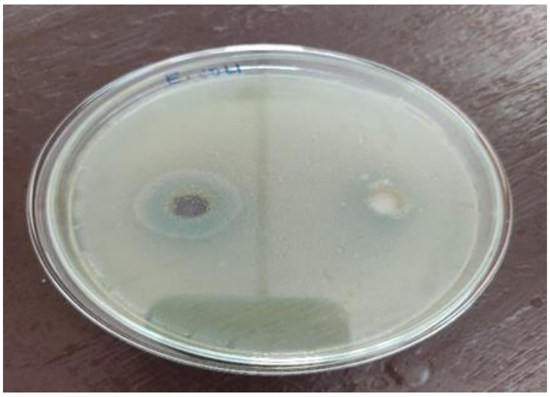

Fig.10 E. coli in the MHA media plate

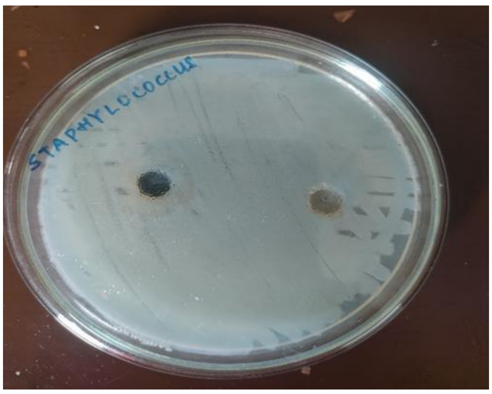

Fig.11 Staphylococcus spp. In the MHA media plate

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ORGANISM	HYDROXYAPATITE (mm)	HYDROXYAPATITE/GRAPHENE OXIDE NANOCOMPOSITE (mm)
Staphylococcus spp	10mm	15mm
E. coli	11mm	20mm

The antibacterial activity of the sample was assessed using both Gram-positive and Gram-negative bacteria on the MHA medium plate. The graphene oxide/hydroxyapatite sample had a bigger zone in the E. coli plate than the pure hydroxyapatite sample. A wide zone was visible on the Staphylococcus spp. plate as opposed to the pure Hydroxyapatite sample. A zone of inhibition was seen in hydroxyapatite and graphene oxide, which matched the findings of the study.

Antioxidant Assay:

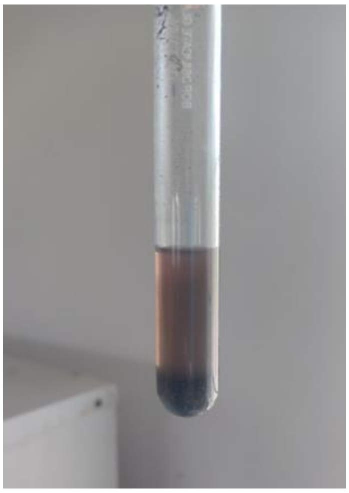

Fig.12 Anti-oxidant activity of HAp/GO Nanocomposite

After adding 3 ml of DPPH to 1.5 ml of the sample and 2 ml of distilled water, the sample was measured at 517 nm. The results demonstrate that graphene oxide significantly boosts antioxidant activity due to its oxygen functional group. The Hydroxyapatite/Graphene Oxide composite material showed superior antioxidant production, which could be useful for biomedical applications. The sample's antioxidant value was determined to be 0.0353.

Treating Dye water with the sample:

Fig.13 Treated sample in polluted dye water

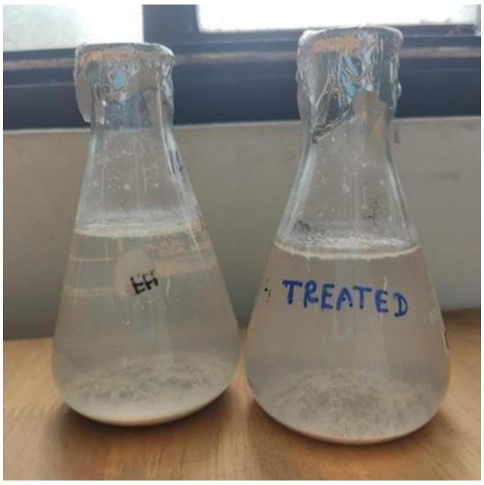

Fig.14 Treated Water

Waste dye water from the industry was treated using a hydroxyapatite sample made from quail eggshells, which eliminated the dye from the water.

X-ray Diffraction:

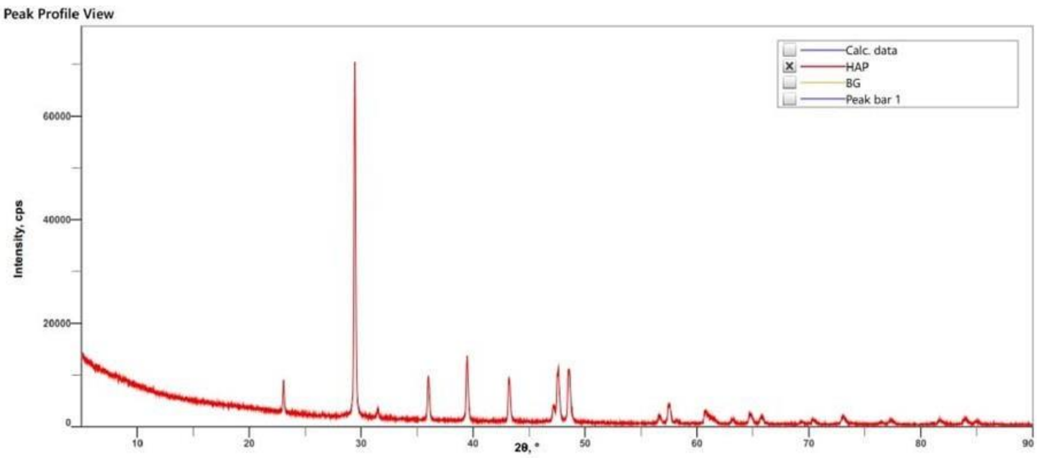

Fig.15 Peaks obtained for HAp

The XRD pattern demonstrates the sample's crystalline nature. The presence of sharp peaks implies a highly crystalline phase, with a prominent peak of 30° 2θ, which is indicative of hydroxyapatite, while the background signal suggests a possible amorphous phase, most likely from bioactive glass. When compared to reference data, the sample composition is consistent with that of hydroxyapatite, indicating the presence of a composite biomaterial.

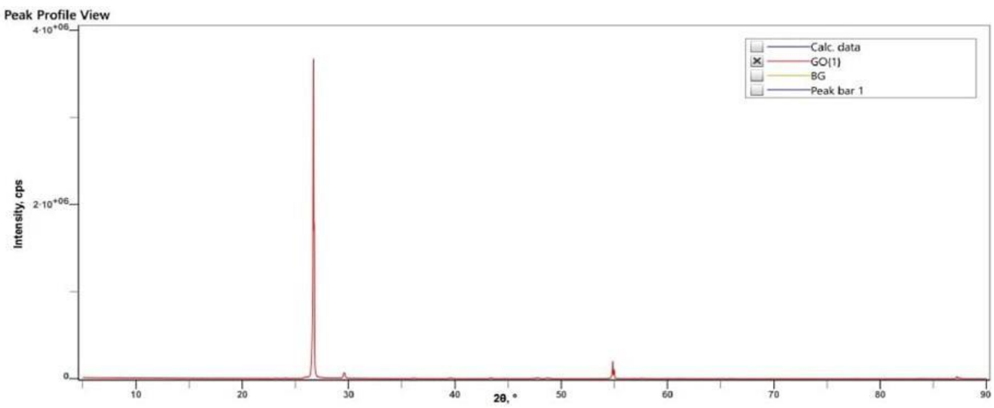

Fig.15 Peaks obtained for GO

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The XRD pattern of the image reveals a highly crystalline structure with a strong peak at around 30° 2θ, indicating the presence of a separate phase. The strength of this peak is significantly higher than that of the other peak, suggesting that the material is mostly composed of a single crystalline phase. The graph's graphene oxide content raises the possibility that the sample is a composite. The relatively low intensity of other peaks suggests that later phases may have an amorphous component. Similar diffraction peaks to those in the research report were discovered at 2θ.

FTIR:

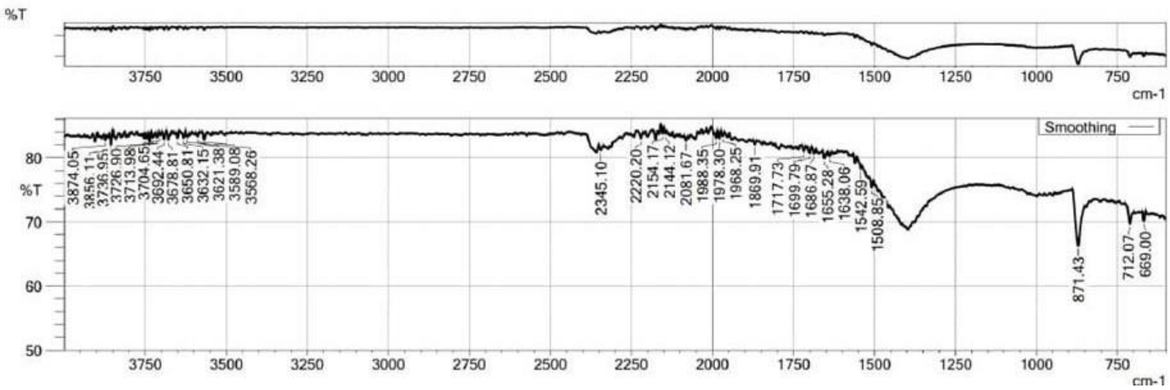

Fig.16 FTIR Graph

PEAK VALUE	FUNCTIONAL GROUPS	BOND TYPE
3847.05cm^{-1} – 3568.26 cm^{-1}	O-H (hydroxyl) or N-H(amine)	Free or hydrogen bond
234.10 cm^{-1}	C≡C (alkyne) or C≡N (nitrile)	Strong bonds
2220.07 cm^{-1}	C≡C (alkyne) or C≡N (nitrile)	Strong bonds
2141.72 cm^{-1}	C≡C (alkyne) or C≡N (nitrile)	Strong bonds
2081.42 cm^{-1}, 1983.15 cm^{-1}, 1888.35 cm^{-1}	Carbonyl or aromatic group	Weak bonds
1717.79 cm^{-1}, 1688.97 cm^{-1}, 1658.28 cm^{-1}	C=O(carbonyl)	Strong bond

Multiple carbonyl peaks are visible in this graph, indicating the existence of substances such aldehydes, ketones, and carboxylic groups.

SEM:

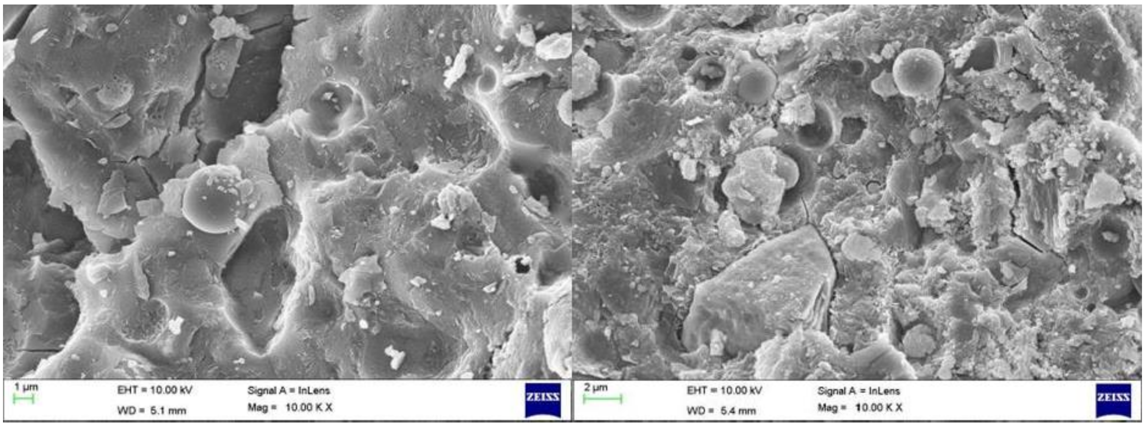

Fig.17 SEM image of Hydroxyapatite-Graphene oxide

The corresponding SEM image shows the surface morphology of the Hydroxyapatite and Graphene Oxide nanocomposite at 10,000x magnification. The image shows a heterogeneous structure with distinct properties of both hydroxyapatite and graphene oxide. The presence of porous structures, rough surface textures, and irregularly shaped particles all demonstrate that the composite was successfully created. In the composite, graphene oxide appears as layered, sheet-like structures, whereas hydroxyapatite takes the form of agglomerated clusters with crystalline structures. The uniform distribution of graphene oxide in the hydroxyapatite matrix suggests more structural reinforcement, which may enhance mechanical strength and have antimicrobial properties.

Anti-cancer activity:

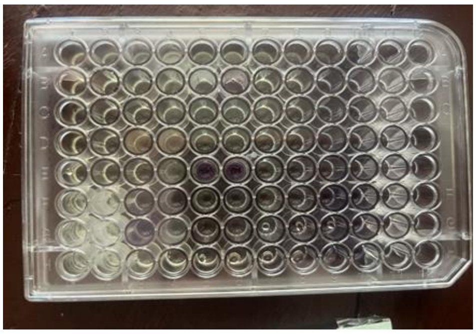

Fig.18 ELISA 96 well plate

The MCF-7 breast cancer cell line was used to test the generated Hydroxyapatite-Graphene Oxide nanocomposite sample, which showed a low level of anticancer activity. The sample has a 71% antitumor activity. The high proportion of cancer cell viability indicates that the nanocomposite's anticancer effects are somewhat limited.

CONCLUSION

The performance of the sample as a highly absorbent material is demonstrated by the investigation on the Hydroxyapatite/Graphene Oxide Nanocomposite in Polluted Dye Water. The high surface area of graphene oxide and the high absorption capacity of hydroxyapatite will enhance the removal of contaminants and purify the water. The sample's functional groups are crucial in eliminating the impurities from the water. The successful creation of hydroxyapatite/graphene oxide nanocomposites is demonstrated by the XRD result. The generated sample's antibacterial activity was demonstrated by the zone of inhibition. Because it is high in calcium and other minerals, the plants' growth indicated that it can also be utilized as a biofertilizer. Because graphene oxide possesses moderate to strong anti-cancer capabilities, it may also be used as a possible anti- cancer agent. Polluted water can be cleaned with this sustainable process, and eggshells that would otherwise be thrown out as waste can be repurposed. It is an economical and environmentally beneficial approach. In the future, hydroxyapatite can be made from other waste materials, such as fish bones and eggshells. Future studies might also concentrate on recycling the product, developing other synthesis techniques, and mass-producing it for the purpose of treating the contaminated water.

REFERENCE

1. Kareem, Z., & Eyiler, E. (2024). Synthesis of hydroxyapatite from eggshells via wet chemical precipitation: a review. RSC advances, 14(30), 21439-21452.
2. Kala, K. (2022). Evaluating synthesis of Novel Hydroxyapatite from Egg Shell Compared with Chemically Synthesized HAP for Orthopedic application. Journal of Pharmaceutical Negative Results, 13.
3. Pu'ad, N. M., Alipal, J., Abdullah, H. Z., Idris, M. I., & Lee, T. C. (2021). Synthesis of eggshell derived hydroxyapatite via chemical precipitation and calcination method. Materials Today: Proceedings, 42, 172-177.

4. Sujiono, E. H., Zurnansyah, Z. D., Dahlan, M. Y., Amin, B. D., & Samnur, A. J. (2020). Graphene oxide based coconut shell waste: synthesis by modified Hummers method and characterization. Heliyon 6: e04568.
5. Hashim, N. C., Frankel, D., & Nordin, D. (2019). Graphene oxide-modified hydroxyapatite nanocomposites in biomedical applications: A review. Ceramics-Silikaty.
6. Hassan, M. A., Mohammad, A. M., Salaheldin, T. A., & El-Anadouli, B. E. (2018). A promising hydroxyapatite/graphene hybrid nanocomposite for methylene blue dye's removal in wastewater treatment. International Journal of Electrochemical Science, 13(8), 8222-8240.
7. Ahmed, N., Kamil, F., Hasso, A., Abduljawaad, A., Saleh, T. & Mahmood, S. (2022). Calcium carbonate nanoparticles of quail's egg shells: Synthesis and characterizations. Journal of the Mechanical Behavior of Materials, 31(1), 1-7.
8. Nikam, A. P., Ratnaparkhiand, M. P., & Chaudhari, S. P. (2014). Nanoparticles–an overview. International journal of research and development in pharmacy & life sciences, 3(5), 1121-1127.
9. Rhazouani, A., Gamrani, H., El Achaby, M., Aziz, K., Gebrati, L., Uddin, M. S., & Aziz, F. (2021). Synthesis and toxicity of graphene oxide nanoparticles: A literature review of in vitro and in vivo studies. BioMed Research International, 2021(1), 5518999.
10. Miyah, Y., El Messaoudi, N., Benjelloun, M., Acikbas, Y., Şenol, Z. M., Ciğeroğlu, Z., & Lopez-Maldonado, E. A. (2024). Advanced applications of hydroxyapatite nanocomposite materials for heavy metals and organic pollutants removal by adsorption and photocatalytic degradation: a review. Chemosphere, 142236.
11. Hoa, N. V., Minh, N. C., Cuong, H. N., Dat, P. A., Nam, P. V., Viet, P. H. T., ... & Trung, T. S. (2021). Highly porous hydroxyapatite/graphene oxide/chitosan beads as an efficient adsorbent for dyes and heavy metal ions removal. Molecules, 26(20), 6127.
12. Yousefi, M., Dadashpour, M., Hejazi, M., Hasanzadeh, M., Behnam, B., de la Guardia, M., ... & Mokhtarzadeh, A. (2017). Anti-bacterial activity of graphene oxide as a new weapon nanomaterial to combat multidrug-resistance bacteria. Materials Science and Engineering: C, 74, 568-581.
13. Abdelhalim, A. O., Meshcheriakov, A. A., Maistrenko, D. N., Molchanov, O. E., Ageev, S. V., Ivanova,
14. D. A., ... & Semenov, K. N. (2022). Graphene oxide enriched with oxygen-containing groups: on the way to an increase of antioxidant activity and biocompatibility. Colloids and Surfaces B: Biointerfaces, 210, 112232.
15. Pulingam, T., Thong, K. L., Appaturi, J. N., Lai, C. W., & Leo, B. F. (2021). Mechanistic actions and contributing factors affecting the antibacterial property and cytotoxicity of graphene oxide. Chemosphere, 281, 130739.
16. Shafiee, A., Iravani, S., & Varma, R. S. (2022). Graphene and graphene oxide with anticancer applications: Challenges and future perspectives. MedComm, 3(1), e118.
17. Slama, H. B., Chenari Bouket, A., Pourhassan, Z., Alenezi, F. N., Silini, A., Cherif-Silini, H., ... & Belbahri, L. (2021). Diversity of synthetic dyes from textile industries, discharge impacts and treatment methods. Applied Sciences, 11(14), 6255.
18. Gaonkar, M., & Chakraborty, A. P. (2016). Application of eggshell as fertilizer and calcium supplement tablet. International journal of innovative research in science, engineering and technology, 5(3), 3520-3525.
19. Peşmen, G. (2023). Possibility of using eggshell in industry. International Journal of Computational and Experimental Science and Engineering, 9(4), 331-339.
20. Asghar, F., Shakoor, B., Fatima, S., Munir, S., Razzaq, H., Naheed, S., & Butler, I. S. (2022). Fabrication and prospective applications of graphene oxide-modified nanocomposites for wastewater remediation. RSC advances, 12(19), 11750-11768.
21. Anchana devi C, Perumal P. Synthesis & application of hydroxyapatite bioceramics from different marine sources. J Res Environ Earth Sci. 2016; 2(11): 7–15.
22. Osama, H. R., Mohamed, A. A., & Ashraf, A. M. (2021). AN EGGSHELL HYDROXYAPATITE-GRAPHENE OXIDE NANOCOMPOSITE FOR THE REMOVAL OF HEAVY METALS FROM WASTE WATER. Journal of Environmental Science, 50(2), 1-33.