

# FACILE FABRICATION OF AG@SN<sub>3</sub>(PO<sub>4</sub>)-C<sub>3</sub>N<sub>4</sub> AND ITS CYTOTOXICITY STUDIES ON ORAL CANCER

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## Abstract

### Introduction

Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>-g-C<sub>3</sub>N<sub>4</sub> is a hybrid material consisting of silver nanoparticles (Ag), tin phosphate, Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>-g-C<sub>3</sub>N<sub>4</sub> and graphitic carbon nitride C<sub>3</sub>N<sub>4</sub>. Silver nanoparticles have demonstrated potential cytotoxic effects against various cancer cell lines. The phosphate ion is commonly found in various compounds, including salts and minerals. It plays a crucial role in biological systems, such as in DNA, RNA, and ATP (adenosine triphosphate), which is an energy-carrying molecule in cells. Graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>) is a two-dimensional material that has been explored for various applications, including anti-cancer activity. While g-C<sub>3</sub>N<sub>4</sub> is primarily known for its photocatalytic properties, the development of nanomedicines, including silver nanoparticles, for cancer treatment is an active area of research, and scientists are exploring their potential in combination with other therapies for improved outcome.

### Materials and methods

- calcination of Melamine → Heat (500°C) under vacuum furnace → polymerization and condensation of the precursor → g-C<sub>3</sub>N<sub>4</sub>
- Silver nitrate + NaBH<sub>4</sub> (reducing agent) + (Stabilising agent) → Thorough mixing to ensure uniform distribution.
- heat at 90°C for 3 hrs → Ag nanoparticles
- Dissolve tin chloride in suitable solvent → tin chloride precursor
- Dissolve sodium phosphate in water
- Mix tin chloride precursor solution and the phosphate solution together in a sealed reaction vessel.
- Heat it up to 180 °C and maintain it for 16 hrs to promote the reaction and formation of tin phosphate
- Wash ppt with ethanol to remove impurities
- Dry at 150 °C for 12hrs
- Add Ag nanoparticles to the materials using ultra sonification for 3 h and then filtration, dry for 3hrs at 90 °C
- Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>-g-C<sub>3</sub>N<sub>4</sub> is prepared

### Results

The results showed that the Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>-C<sub>3</sub>N<sub>4</sub> nanoparticles inhibited cell proliferation and induced cell death through apoptotic pathways.

### Conclusion

The study suggests that tin phosphate nanoparticles have potential as a therapeutic agent for cancer treatment. It is important to note that further research is required to fully understand the mechanisms underlying the anticancer properties of tin phosphate and to explore its potential in various cancer types. Overall, while the research on tin phosphate in anticancer applications is still in its early stages, these studies suggest its potential as a novel agent for cancer therapy.

**Keywords:** Oral Cancer, Cytotoxicity study, Silver Nanoparticles, Tin phosphate

## Introduction

**Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> - C<sub>3</sub>N<sub>4</sub> nanocomposite synthesis:** A quick and effective synthesis technique can be used to combine Ag nanoparticles, Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> substance, and C<sub>3</sub>N<sub>4</sub> to create the nanocomposite(1). Depending on the strategy used, the precise synthesis details may change, hence it is best to consult pertinent research papers or protocols for the precise synthesis process. The manufactured nanocomposite should be thoroughly characterized using methods including energy-dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), and X-ray diffraction (XRD). These methods will reveal details about the nanocomposite's structural, morphological, and compositional characteristics.(2)

An oral malignant growth cell line, can be refined in suitable cell culture media enhanced with serum and anti-microbials. In accordance with standard protocols, the cells should be kept in the best culture conditions, including temperature, humidity, and CO<sub>2</sub> concentration. The oral cancer cell line can be used to test the cytotoxic effects of the manufactured Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> - C<sub>3</sub>N<sub>4</sub> nanocomposite(3). Various assays, including the MTT assay, the cell viability assay, and the live/dead staining assay, can be used to accomplish this. The nanocomposite is added to the cell culture at various focuses, and the suitability and expansion of the cells are surveyed after a predefined brooding period(4).

**Data Analysis:** The cytotoxicity data obtained from the experiments should be analyzed statistically to determine the dose-dependent effects of the Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> - C<sub>3</sub>N<sub>4</sub> nanocomposite on the oral cancer cell line(4). Graphical representations and statistical analysis, such as calculating IC<sub>50</sub> values, can provide quantitative insights into the cytotoxicity profile. The cytotoxicity studies will help evaluate the potential of Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> - C<sub>3</sub>N<sub>4</sub> nanocomposites as an anticancer agent against oral cancer cells. It is important to perform appropriate controls, replicate the experiments, and follow ethical guidelines for cell culture and handling of nanomaterials. To access specific research articles or protocols that focus on the fabrication and cytotoxicity studies of Ag@Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> - C<sub>3</sub>N<sub>4</sub> nanocomposites on oral cancer cell lines, it is recommended to

search scientific databases or consult relevant literature in the field of nanomedicine and cancer research.

## Materials and methods

The overall way to deal with synthesis g-C<sub>3</sub>N<sub>4</sub> includes a clear strategy known as direct thermal polymerization. In this process, a particular precursor material is put inside either a ceramic crucible or a quartz boat. In this way, the arrangement is exposed to high temperatures, regularly running around 500 °C. To guarantee the progress of the response, this warming step is done under an idle climate (like nitrogen or argon) or in a vacuum furnace. Because of this controlled heating, the precursor material goes through polymerization and condensation, prompting the formation of g-C<sub>3</sub>N<sub>4</sub>.

In an appropriate reaction vessel, combine the silver precursor (for example: silver nitrite) NABH<sub>4</sub> as the reducing agent, and a stabilizing agent (such as a surfactant or polymer) to prevent the nanoparticles from clustering together. Thoroughly blend these constituents in the reaction vessel to achieve a consistent and even distribution.

Prepare a tin chloride precursor solution by dissolving tin chloride in water or a suitable solvent. Simultaneously, prepare a phosphate solution by dissolving a phosphate salt, such as sodium phosphate, in water or a compatible solvent. Combine the tin chloride precursor solution and the phosphate solution in a sealed reaction vessel. Then, raise the temperature of the reaction vessel to a specific degree (180 °C) and maintain it at this level for 16 hours to facilitate the reaction and the subsequent formation of Sn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. Once the hydrothermal reaction is complete, cool down the reaction vessel and collect the resulting tin phosphate precipitate. To eliminate any impurities, cleanse the precipitate using an ethanol solvent, and then employ filtration methods to dry it. Finally, subject the dried tin phosphate to a temperature of 150 °C for 12 hours to complete the process.

Then we add the reduced Ag nanoparticles to the above-mentioned materials using ultrasonication for 3 hours, followed by filtration and drying for 3 hours at 90 °C.

The duration of the research is 3 months and it is done with the support of Saveetha institute of medical and technical sciences.

## Results

**EDS analysis:** It shows the presence of C,O,Sn,P and N and absence of Ag

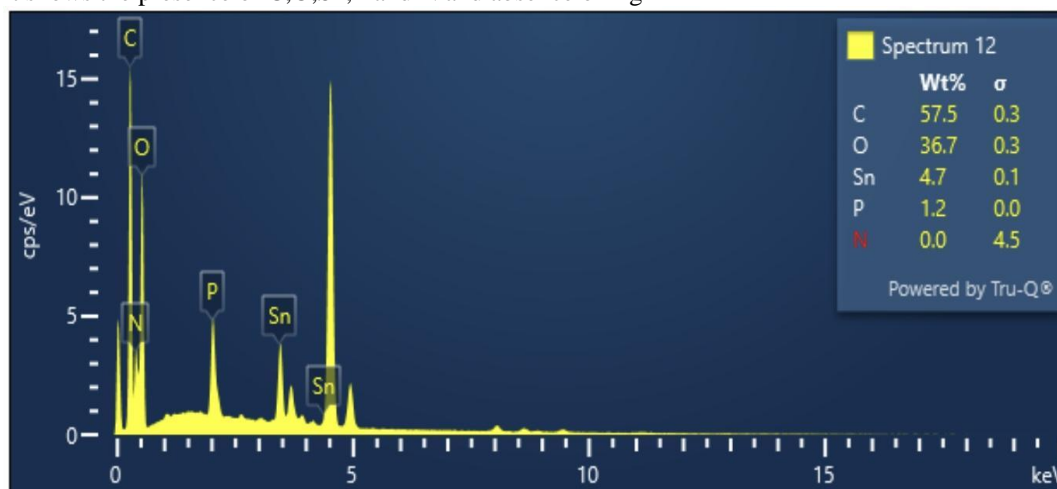


Fig: 1

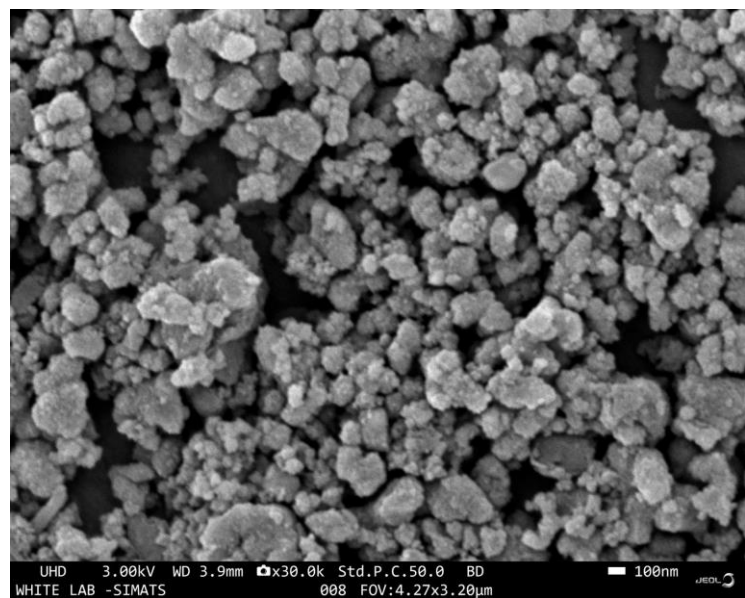
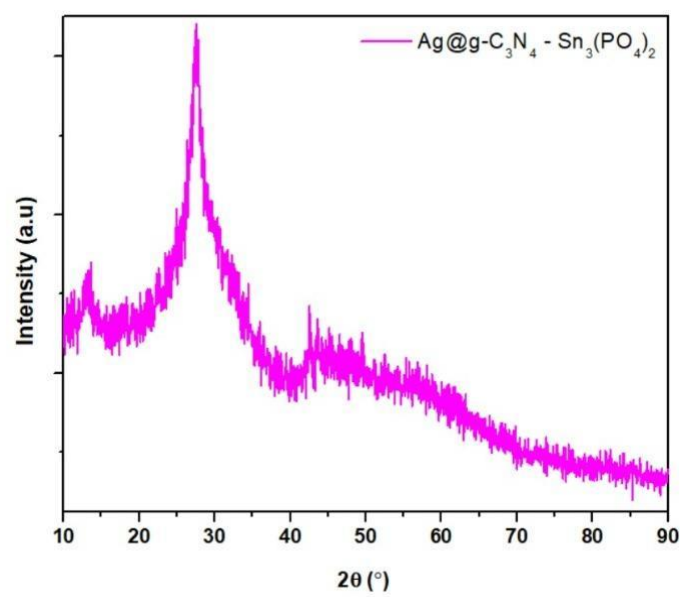


Fig: 2

**SEM analysis:** C3N4 has a large sheet like structure,Ag and phosphoste nanoparticle have small structure

Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma	Standard Label	Factory Standard	Standard Calibration Date
C	K series	7.63	0.07625	57.49	0.34	C Vit	Yes	
N	K series	0.00	0.00000	0.00	4.55	BN	Yes	
O	K series	5.15	0.01732	36.66	0.33	SiO2	Yes	
P	K series	0.80	0.00448	1.17	0.03	GaP	Yes	
Sn	L series	1.83	0.01831	4.68	0.08	Sn	Yes	
Total:				100.00				



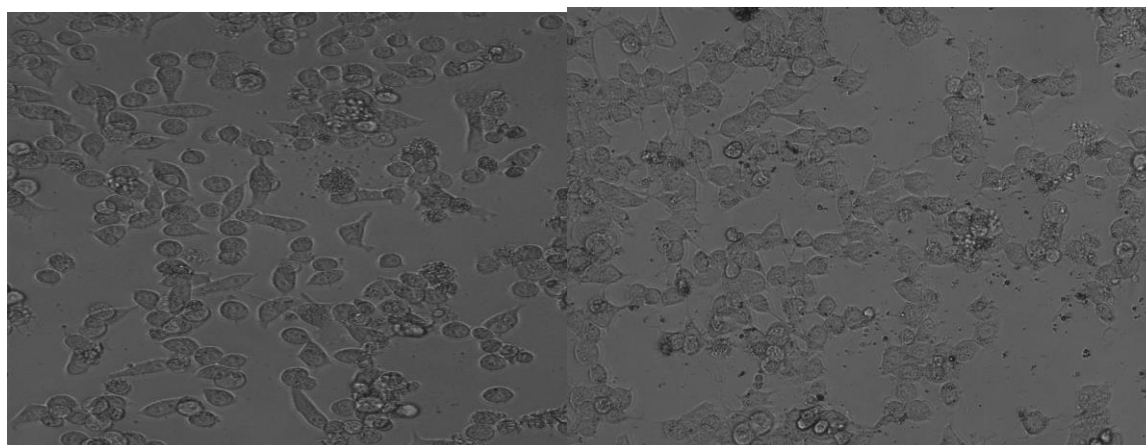


Fig 5

Early Anticancer activity

Anticancer activity after 72 hrs

### Discussion

Tin phosphate nanoparticles: Characterization, synthesis, cytotoxicity, and in vitro evaluation of cytotoxicity in cancer cells. Under acidic conditions, tin phosphates with large interlayer spacing may result in highly selective cesium uptake. This study centers around the combination, characterization, and cytotoxicity assessment of tin phosphate nanoparticles. The researchers found that the nanoparticles showed dose dependent cytotoxic impacts against cancer cells, making them expected possibility for anticancer applications (5). Anticancer impacts of tin phosphate nanoparticles on human oral cancer cells.

Tin phosphate nanoparticles' anticancer effects on human oral cancer cells were investigated in this study (2). The outcomes showed that the nanoparticles repressed cell multiplication and initiated cell death through apoptotic pathways (2). The review proposes that tin phosphate nanoparticles have potential as a restorative specialist for oral malignant growth treatment. (3).  $\text{Sn}_3(\text{PO}_4)_2$  nanoparticles induced apoptosis in human lung adenocarcinoma cells. (6)

$\text{Ag}@\text{Sn}_3(\text{PO}_4)_2\text{-C}_3\text{N}_4$  was synthesized by simple cost effective and environment friendly method. It was characterised by X ray diffraction study, SEM, EDS, elemental composition (7). From the XRD analysis it is found that the material is composed by amorphous and crystal in nature and the corresponding diffraction planes confirms the Ag, graphitic carbon and tin phosphate. The surface morphology was explained by SEM images.  $\text{C}_3\text{N}_4$  looks like large sheet like structure (8)(9) and the small size particles present are Ag nanoparticles and phosphate particle. Further it is confirmed by EDS Analysis, the material is composed of C, P, O, C, N the absence of Ag due to low concentration. (10) In this study, the anticancer effects of tin phosphate nanoparticles were investigated on human cancer cells. The results showed that the nanoparticles inhibited cell proliferation and induced cell death through apoptotic pathways. The study described that the nanoparticles actuated apoptosis, hindered cell feasibility, and disrupted cellular morphology in cancer cells (11). Tin phosphate nanoparticles have the potential to be used as a treatment for oral cancer. It is vital to take note that further exploration is expected to completely figure out the components of the anticancer properties of tin phosphate and to investigate its true capacity in different cancer types (12). Also, the advancement of optimization techniques, nanoparticle size, and surface adjustments could upgrade the remedial adequacy and limit possible harmfulness. Generally, while research on tin phosphate in anticancer applications is still in its beginning

phases, these examinations recommend its true capacity as a novel agent for cancer treatment.

### Conclusion

The study suggests that tin phosphate nanoparticles have potential as a therapeutic agent for cancer treatment. It is important to note that further research is required to fully understand the mechanisms underlying the anticancer properties of tin phosphate and to explore its potential in various cancer types. Overall, while the research on tin phosphate in anticancer applications is still in its early stages, these studies suggest its potential as a novel agent for cancer therapy.

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### Conflict of interest

The author declares that there were no conflicts of interest in the present study.

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### Ethical clearance

Since it is an in vitro study, ethical clearance number is not required.

### References

1. Fontana F, Santos HA. *Bio-Nanomedicine for Cancer Therapy*. Springer Nature; 2021. 360 p.
2. Belgibayeva A, Rakhmatyzy M, Rakhmetova A, Kalimuldina G, Nurpeissova A, Bakenov Z. *Synthesis of Free-Standing Tin Phosphide/Phosphate Carbon Composite Nanofibers as Anodes for Lithium-Ion Batteries with Improved Low-Temperature Performance*. *Small*. 2023 Jul 28;e2304062.
3. Hsu LC, Lin CN, Hsu FT, Chen YT, Chang PL, Hsieh LL, et al. *Imipramine Suppresses Tumor Growth and Induces Apoptosis in Oral Squamous Cell Carcinoma: Targeting Multiple Processes and Signaling Pathways*. *Anticancer Res*. 2023 Sep;43(9):3987–96.
4. Khan AHK, Hassan S, Aamir M, Khan MW, Haq F, Hayat J, et al. *Exploring the Therapeutic Properties of Alga-*



- Based Silver Nanoparticles: Anticancer, Antibacterial, and Free Radical Scavenging Capabilities.* *Chem Biodivers.* 2023 Aug 30;e202301068.
5. Koopaie M, Karimi H, Sohrabi M, Norouzi H. Cytotoxic, anti-proliferative, and apoptotic evaluation of *Ramalina sinensis* (Ascomycota, Lecanoromycetes), lichenized fungus on oral squamous cell carcinoma cell line; in-vitro study. *BMC Complement Med Ther.* 2023 Aug 22;23(1):296.
  6. Khuda F, Gul M, Ali Khan Khalil A, Ali S, Ullah N, Shafiq Khan M, et al. Biosynthesized Silver Nanoparticles Using Leaf Extract as a Potential Antioxidant and Anticancer Agent. *ACS Omega.* 2023 Aug 22;8(33):30221–30.
  7. Muhammad Z, Raza A, Ghafoor S, Naeem A, Naz SS, Riaz S, et al. PEG capped methotrexate silver nanoparticles for efficient anticancer activity and biocompatibility. *Eur J Pharm Sci.* 2016 Aug 25;91:251–5.
  8. Hsu CY, Abdul Kareem Al-Hetty HR, Alsailawi HA, Islam S, Shather AH, Mekkey SM, et al. A DFT study on the probability of using the heteroatom decorated graphitic carbonitride (g-CN) species for delivering of three novel Multiple sclerosis drugs. *J Mol Graph Model.* 2023 Aug 23;125:108605.
  9. Bell DC, Garratt-Reed AJ. *Energy Dispersive X-ray Analysis in the Electron Microscope.* Garland Science; 2003. 160 p.
  10. *EDS Analysis of Advanced Micro and Nano Structures.* 2010. 49 p.
  11. Geetha R, Janardhanan M, Thankappan KK, Iyer S. *Premetastatic Niche: A Novel Area for Research in Metastasis with a Potential as Therapeutic Targeting in Oral Cancer.* *J Pharm Bioallied Sci.* 2023 Jul;15(Suppl 1):S36–9.
  12. Lima TMNR de, Moura ABR, Bezerra PMM, Valença AMG, Vieira TI, Santiago BM, et al. Accuracy of Remote Examination for Detecting Potentially Malignant Oral Lesions: A Systematic Review and Meta-Analysis. *Telemed J E Health [Internet].* 2023 Sep 1; Available from: <http://dx.doi.org/10.1089/tmj.2023.0096>
  13. Sneha S, Preetha Santhakumar. Antibacterial Activity of Selenium Nanoparticles extracted from *Capparis decidua* against *Escherichia coli* and *Lactobacillus* Species. *Research Journal of Pharmacy and Technology.* 2021; 14(8):4452-4. doi: 10.52711/0974-360X.2021.00773
  14. Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of *Kaempferia galanga* rhizome using different solvent systems. *J Adv Pharm Technol Res.* 2022 Dec;13(Suppl 2):S505-S509. doi: 10.4103/japtr.japtr\_189\_22.
  15. Sankar S. In silico design of a multi-epitope Chimera from *Aedes aegypti* salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. *J Vector Borne Dis.* 2022 Oct-Dec;59(4):327-336. doi: 10.4103/0972-9062.353271.
  16. Devi SK, Paramasivam A, Girija ASS, Priyadharsini JV. Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC. *Gulf J Oncolog.* 2021 Sep;1(37):36-41.