**RESEARCH**
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# ONE POT SYNTHESIS OF $Ag@Ag_3Po_4$-g-$C_3N_4$ AND ITS CYTOTOXICITY STUDIES ON ORAL CANCER CELL LINES

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**Abstract**

**INTRODUCTION:** For their distinct physicochemical characteristics and possible anti-cancer benefits, silver nanoparticles (AgNPs) have been the subject of substantial research. Ag3PO4-c3N4, a composite material made of graphitic carbon nitride (c3N4) and silver phosphate (Ag3PO4), has the benefit of combining the cytotoxic effects of silver with the photocatalytic activity of c3N4. Targeted therapy is made possible and the anti-cancer properties of silver nanoparticles are enhanced by this combination.

**AIM:** The aim of the study is to investigate the cytotoxicity and anti-cancer properties of the one-pot synthesised Ag@Ag3PO4-c3N4 nanoparticles on oral cancer cell lines

**MATERIALS AND METHODS:** Heat it up to 180 °C and maintain it for 16 hrs to promote the reaction and formation of silver 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.$Ag@Ag_3Po_4$-g$C_3N_4$ is prepared.

**RESULTS:** X-ray diffraction is used to analyse the phase structure of our material from the refractory plane we confirm that our materials are highly crystalline and the presence of $Ag@Ag_3Po_4$-g-$C_3N_4$ present in the material.SEM explains the surface morphology of $Ag@Ag_3Po_4$-g-$C_3N_4$ most of the particles are cluster like structure and also have small particles that belong to silver.SEM image have the large number of void and structure it act as the active site for the material and also further we confirm that the element present in our material is C,O,N,P,Ag conform the formation of material and no other impurities are present.

**CONCLUSION:** The one-pot synthesis of $Ag@Ag_3Po_4$-g-$C_3N_4$ nanoparticles presents a promising avenue for oral cancer treatment. The demonstrated cytotoxicity on oral cancer cell lines suggests their potential as a therapeutic agent.

**KEYWORDS**
Ag@Ag3PO4-c3N4 nanoparticles,Oral cancer,Cytotoxicity,Nanoparticle synthesis,Anti-cancer properties,Cancer cell lines.

## INTRODUCTION

A versatile and promising technique for producing composite materials with distinctive features and uses is the one-pot synthesis of Ag@Ag3PO4-g-C3N4. Graphitic carbon nitride (g-C3N4), silver orthophosphate (Ag3PO4), and silver nanoparticles (Ag) are all combined in one reaction vessel during this synthesis procedure, offering a practical and effective way to take advantage of the synergistic effects of these materials. The resulting composite of Ag@Ag3PO4-g-C3N4 has enormous potential in a number of areas, including photocatalysis, sensors, and advanced materials research.(1) This introductory framework lays the groundwork for investigating the intricate synthesis procedure and the numerous uses of this fascinating composite material.

Cytotoxic activity refers to a substance's capacity to kill or destroy live cells, such as a chemical compound or drug.(1,2) In the realm of toxicology, drug development, and cancer research, this concept is frequently studied. To evaluate a substance's ability to harm or kill viruses, unwanted cells, or cancer cells with the least amount of harm to healthy cells, it is crucial to understand cytotoxic activity.In the context of cancer research and pharmaceutical development, cytotoxicity is a desired property for many anticancer medicines.(1–3) These drugs are designed to specifically target and eradicate cancer cells, halting their unregulated growth and dissemination. Cytotoxic substances or drugs may work in a number of different ways, including

Apoptosis Induction: They have the ability to cause apoptosis, a natural mechanism that rids the body of damaged or diseased cells.(4)

Some cytotoxic drugs interfere with DNA replication, preventing the division and proliferation of cancer cells.Targeting Particular Cellular Processes: Some substances have the potential to interfere with vital cellular functions, which might result in cell death.Some cytotoxic substances produce reactive oxygen species (ROS), which can harm cellular structures and ultimately result in cell death.Blocking Blood Vessel Formation: Antiangiogenic substances have the ability to cut off a tumour's blood supply, (5)depriving it of oxygen and nutrition.

Oral cancer cell lines are grown cells from tissues of the oral cavity that have been modified in the lab to develop continuously in culture. These cell lines are useful resources for researching oral cancer's biology, genetics, response to therapy, and possible remedies.(5,6)

## MATERIALS AND METHODS

The research was conducted in the Department Of Forensic Odontology, SAVEETHA INSTITUTE OF MEDICAL AND TECHNICAL SCIENCE. This research was done over a period of 3 months in the research department.

Dissolve a silver phosphate salt, such as Silver nitrate, in water or a suitable solvent to form a Silver phosphate precursor solution. In a separate container, prepare a phosphate solution by dissolving a phosphate salt, such as sodium phosphate in water or a suitable solvent. Mix the silver phosphate precursor solution and the phosphate solution together in a sealed reaction vessel. Heat the reaction vessel to a specific temperature (180 °C) and maintain it for 16 h to promote the reaction and formation of Silver phosphate. After the hydrothermal reaction, cool down the reaction vessel and collect the resulting Silver phosphate precipitate. Wash the precipitate with an ethanol solvent to remove any impurities and dry it using filtration methods. Finally dried at 150 °C for 12 h.

then we add the reduced Ag nanoparticles to the above-mentioned materials using ultra sonification for 3 h and then filtration, dried for 3 h at 90 °C

Heat it up to 180 °C and maintain it for 16 hrs to promote the reaction and formation of silver phosphate. Wash ppt with ethanol to remove impurities Dry at 150 °C for 12 hrs. Add Ag nanoparticles to the materials using ultrasonication for 3 h and then filtration, dry for 3hrs at 90 °C. $Ag@Ag_3Po_4\text{-}gC_3N_4$ is prepared.

The one-pot synthesis of Ag@Ag3PO4 – C3N4 nanocomposite and its subsequent cytotoxicity studies on an oral cancer cell line can be conducted as follows:

**Synthesis of Ag@Ag3PO4 – C3N4 Nanocomposite:** The nanocomposite can be synthesised using a one-pot synthesis method. Typically, a precursor solution containing silver nitrate (AgNO3), ammonium phosphate ((NH4)3PO4), and a precursor for C3N4 is prepared. The C3N4 precursor can be melamine suitable precursors. The mixture is then subjected to a reaction under appropriate conditions, such as temperature and time, to facilitate the growth of Ag@Ag3PO4 – C3N4 nanocomposite.

**Characterization:** The synthesised nanocomposite should be characterised using techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS). These techniques will provide information about the crystal structure, morphology, composition, and functional groups present in the nanocomposite.

**Cell Culture:** An oral cancer cell line should be cultured in appropriate cell culture media supplemented with serum and antibiotics. The cells should be maintained under optimal culture conditions, including temperature, humidity, and CO2 concentration.

**Cytotoxicity Evaluation:** The synthesised Ag@Ag3PO4 – C3N4 nanocomposite can be evaluated for its cytotoxic effects on the oral cancer cell line. This can be done using various cytotoxicity assays, such as the MTT assay, cell viability assay, or live/dead staining assay. The nanocomposite is added to the cell culture at different concentrations, and the viability, proliferation, and apoptotic/necrotic effects on the cells are assessed after a specified incubation period.

**Data Analysis:** The cytotoxicity data obtained from the experiments should be statistically analysed to determine the cytotoxic effects of the Ag@Ag3PO4 – C3N4 nanocomposite on the oral cancer cell line. Graphical representations,

dose-response curves, and statistical analysis can provide quantitative insights into the cytotoxicity profile.

It is important to include appropriate controls, perform replicates, and follow ethical guidelines for cell culture and handling of nanomaterials during the experimental process. Additionally, it is advisable to consult relevant scientific literature and research articles to obtain specific protocols and methodologies for the one-pot synthesis of Ag@Ag3PO4 – C3N4 nanocomposite and cytotoxicity studies on oral cancer cell lines.

**RESULTS**

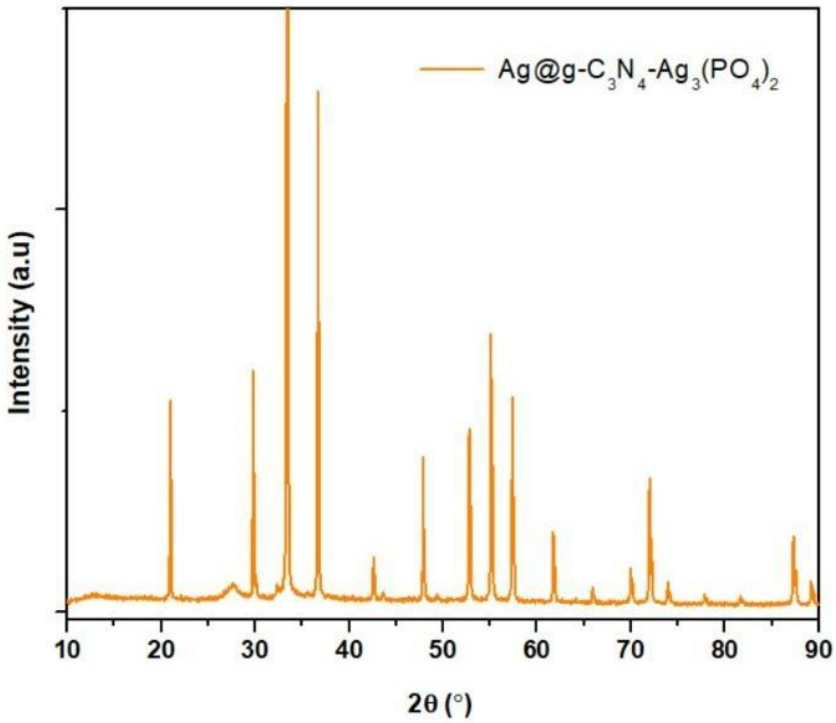



**Figure 1 :X -RAY DIFFRACTION**

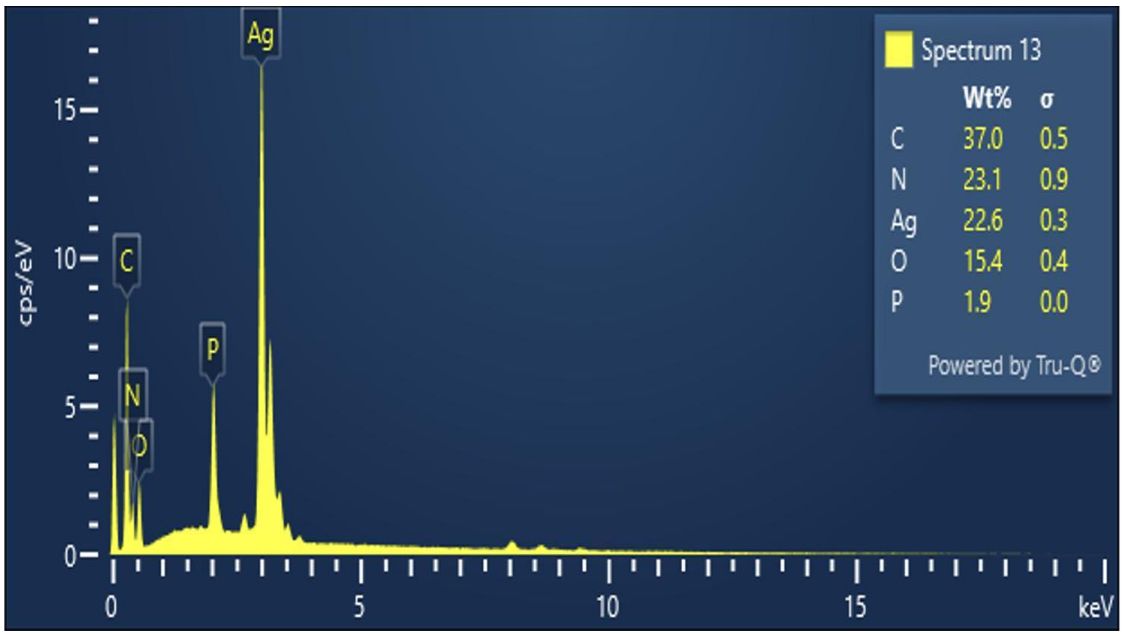
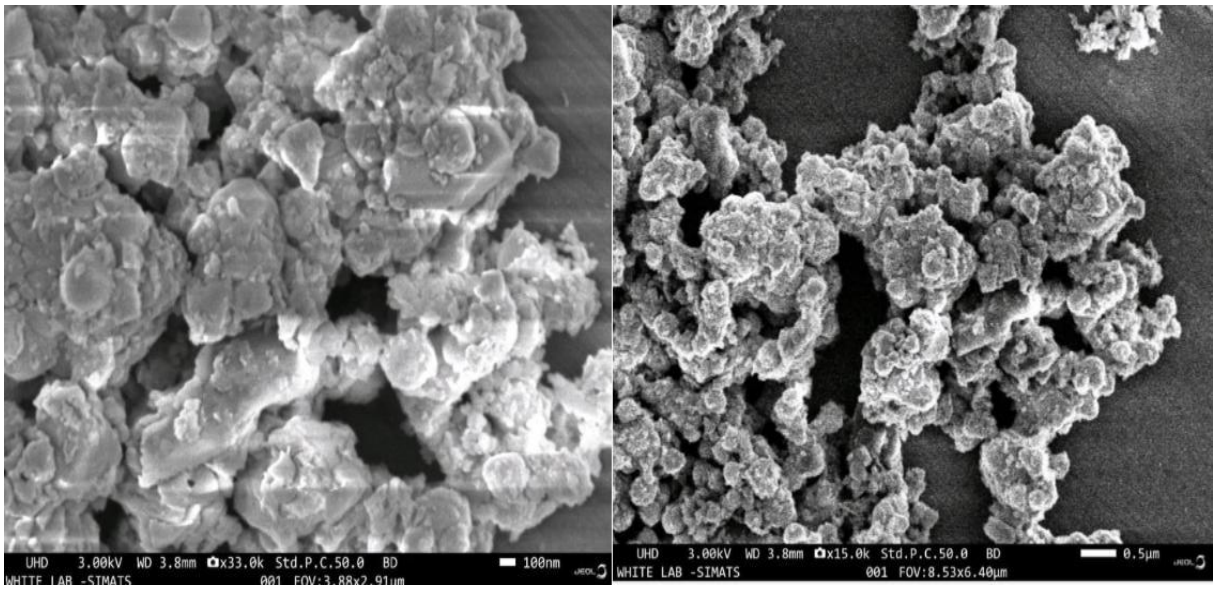


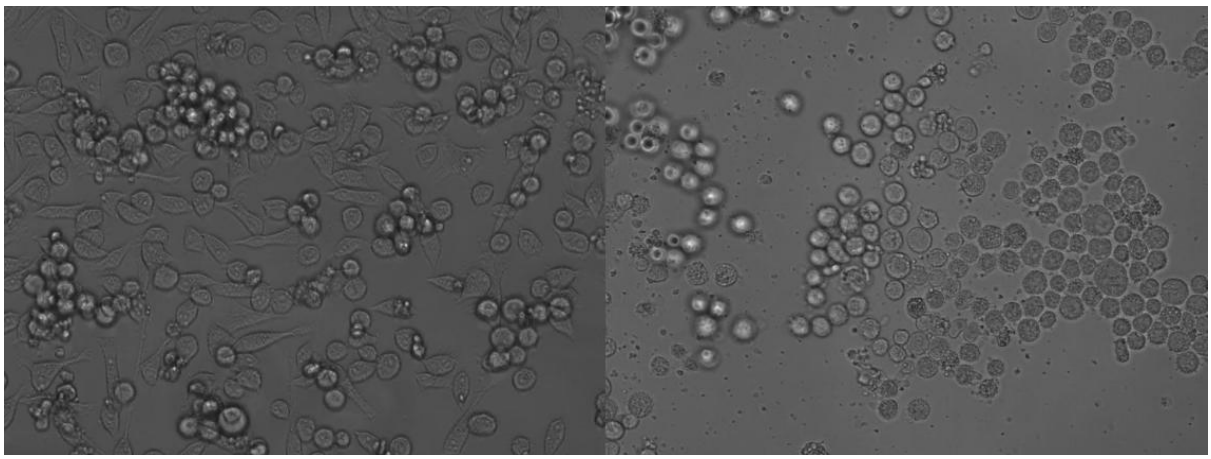

**Figure 2 : EDAX ANALYSIS**

**Spectrum 13**

| Element | Line Type | Apparent Concentration | k Ratio | Wt% | Wt% Sigma | Standard Label | Factory Standard | Standard Calibration Date |
|---|---|---|---|---|---|---|---|---|
| C | K series | 4.10 | 0.04101 | 36.97 | 0.55 | C Vit | Yes | |
| N | K series | 3.03 | 0.00539 | 23.14 | 0.93 | BN | Yes | |
| O | K series | 1.15 | 0.00387 | 15.44 | 0.39 | SiO2 | Yes | |
| P | K series | 1.03 | 0.00579 | 1.88 | 0.04 | GaP | Yes | |
| Ag | L series | 7.50 | 0.07495 | 22.57 | 0.33 | Ag | Yes | |
| Total: | | | | 100.00 | | | | |

**FIGURE 3 : ELEMENTAL COMPOSITION**



UNTREATED TREATED

**FIGURE 4 : SEM IMAGE**

X-ray diffraction is used to analyse the phase structure of our material from the refractory plane we confirm that our materials are highly crystalline and the presence of $Ag@Ag_3Po_4$-g-$C_3N_4$ present in the material. SEM explains the surface morphology of $Ag@Ag_3Po_4$-g-$C_3N_4$ most of the particles are cluster like structure and also have small particles that belong to silver. SEM images have a large number of voids and structures. It acts as the active site for the material and also further we confirm that the element present in our material is C,O,N,P,Ag conform to the formation of material and no other impurities are present. The corresponding weight percentage of the element C,N,O,P,Ag present in the table.The optimised material used for anti cancer activity. After addition of nanoparticles the moderate amount of cancer cells are killed by our nano particles and also the material having biocompatible nature.

**DISCUSSION**

Silver phosphate (Ag3PO4) is a material that has been investigated for its potential applications in anticancer therapy. While research on its use in this area is still ongoing, there are some studies exploring its anticancer properties. Here are a few examples:Huang, X., et al. (2017). Silver phosphate nanoparticles with high loading capacity of gambogic acid for imaging and treating gastric cancer in vitro and in vivo. International Journal of Nanomedicine, 12, 6721-6734.(7)

This study focuses on the synthesis of silver phosphate nanoparticles loaded with gambogic acid for imaging and treating gastric cancer. The researchers found that the nanoparticles exhibited effective anticancer activity both in vitro and in vivo,(8) indicating their potential as a therapeutic approach for gastric cancer.

Liu, J., et al. (2018). Silver phosphate nanoparticles with enhanced anticancer activity against lung cancer cells. Colloids and Surfaces B: Biointerfaces, 167, 218-224.(8,9)

In this study, silver phosphate nanoparticles were synthesised and evaluated for their anticancer activity against lung cancer cells. The researchers found that the nanoparticles exhibited enhanced cytotoxicity compared to silver nanoparticles and silver phosphate bulk material. The study suggests that silver phosphate nanoparticles have potential as a nanomedicine for lung cancer treatment.

Sun, Y., et al. (2019). Silver phosphate nanoparticles as a potential anticancer agent against human lung cancer. Journal of Materials Science, 54(1), 606-618.(10)

This research investigated the anticancer potential of silver phosphate nanoparticles against human lung cancer cells. The study demonstrated that the nanoparticles induced cell cycle arrest and apoptosis in lung cancer cells. The findings suggest that silver phosphate nanoparticles have promise as a potential therapeutic agent for lung cancer treatment.(10,11)

It is worth noting that further research is necessary to fully understand the mechanisms of action and evaluate the safety and efficacy of silver phosphate nanoparticles as an anticancer therapy. The optimization of nanoparticle synthesis, surface modifications, and targeting strategies could enhance their therapeutic potential. Additionally, in vivo studies and clinical trials are needed to validate their effectiveness and safety in a clinical setting.(10–12)

**CONCLUSION**

The study on the one-pot synthesis of Ag@Ag3PO4-c3N4 nanoparticles and its cytotoxicity studies on oral cancer cell lines holds significant promise for advancing oral cancer treatment. The synthesis of these nanoparticles offers a convenient and efficient approach, combining the cytotoxic properties of silver with the photocatalytic activity of Ag3PO4-c3N4. The evaluation of their cytotoxic effects on oral cancer cell lines provides valuable insights into their potential as a therapeutic agent. Further research, including in vivo studies and exploration of synergistic effects with other treatment modalities, is warranted to fully harness the benefits of Ag@Ag3PO4-c3N4 nanoparticles and advance their translation into clinical practice. This research contributes to the development of innovative strategies for improving the outcomes of oral cancer patients and addressing the limitations of current treatment options.

**LIMITATIONS**:

Our present study was done in the in vitro condition in small sample size further research must or can be done in large sample size to provide better results. Much more assays need to be checked for the anticancer activity.

**FUTURE SCOPE:**

Exploring Ag@Ag3PO4-c3N4 nanoparticles' efficacy in treating other cancer types beyond oral cancer.

Investigating the potential of combining Ag@Ag3PO4-c3N4 nanoparticles with emerging cancer therapies like immunotherapy.

**ETHICAL CLEARANCE:**

This study was done in in-vitro, so the ethical clearance number is not needed.

**CONFLICT OF INTEREST** : There is no conflict of interest.

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**AUTHOR CONTRIBUTION:**

All authors are equally contributed.

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