**RESEARCH**
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# PREPARATION OF AG@NI$_3$(PO$_4$)$_2$-G-C$_3$N$_4$ FOR ELECTROCHEMICAL BIOSENSING BOVINE SERUM ALBUMIN

Running Title: Analyzing the biosensing activity of the fabricated compound Ag@$Ni_3(PO_4)_2$-g-$C_3N_4$ using bovine serum albumin assay

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**Abstract**
**INTRODUCTION:** The development of sensitive and selective biosensors has become a crucial area of research in fields, including healthcare, environmental monitoring, and food safety. Particularly electrochemical biosensors have many benefits, including high sensitivity, quick response, and ease of miniaturization. Composites based on nanomaterials have drawn a lot of attention recently for improving the functionality of electrochemical biosensors. One such material is Ag@$Ni_3(PO_4)_2$-g-$C_3N_4$ , which combines the special qualities of nickel phosphate, graphitic carbon nitride, and silver nanoparticles (AgNPs).
**AIM:** The aim of this study is to prepare the Ag@$Ni_3(PO_4)_2$-g-$C_3N_4$ nanocomposite and immobilize bovine serum albumin (BSA) on its surface for the development of an electrochemical biosensor. The synthesized composite will be assessed for its potential use in the sensitive and specific detection of particular infections, contaminants, or biomarkers.
**MATERIALS AND METHODS:** Synthesis of Ag@$Ni_3(PO_4)_2$-g-$C_3N_4$ Nanocomposite: The Ag@$Ni_3(PO_4)_2$-g-$C_3N_4$ nanocomposite is synthesized through a suitable method, such as a one-pot hydrothermal or co-precipitation process. The Ag nanoparticles are incorporated into the $Ni_3(PO_4)_2$ matrix, which is further combined with $C_3N_4$ to form the final nanocomposite.
**RESULTS:** Ag compound is highly sensitive, highly compatible, high in strength, and also highly selective. The composite material demonstrates positive results even at low concentrations of BSA, indicating its effectiveness for detecting and quantifying BSA in various samples.
**CONCLUSION:** The research on Ag@$Ni_3(PO_4)_2$-g-$C_3N_4$ biosensors for BSA detection provides a foundation for potential forensic applications in various areas of analysis and investigation

**KEYWORDS:** Ag@$Ni_3(PO_4)_2$-g-$C_3N_4$, biosensors, bovine serum albumin, nanocomposite, forensic

## INTRODUCTION

Healthcare, environmental monitoring, and food safety are just a few of the industries that place a high priority on the development of sensitive and selective biosensors. Due to the direct conversion of a biological event to an electrical signal, electrochemical biosensors offer an appealing way to analyze the content of a biological sample (1). Many sensing concepts and associated technologies have been developed over the past few decades. To enhance the performance of electrochemical biosensors, nanomaterial-based composites have gained significant attention (2).

Due to their distinctive physical and chemical characteristics, silver nanoparticles (AgNPs) are being employed more and more in a variety of industries, including medicine, food, and health care. These include biological properties, strong electrical conductivity, optical, electrical, and thermal properties (3). In order to fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Among the various synthetic techniques for AgNPs, biological techniques appear to be the most straightforward, quick, safe, dependable, and environmentally friendly ways that can generate well-defined size and morphology under the ideal circumstances for translational research. In the end, the production of AgNPs using green chemistry has great potential (4).

Nickel phosphate ($Ni_3(PO_4)_2$) is a compound that has been investigated for various applications, including biosensors. Its unique properties make it suitable for sensing applications, particularly in the detection of biomolecules and analytes. In enzymatic biosensors, nickel phosphate can be employed as a support material for the immobilization of enzymes (5). The nickel phosphate matrix can contain connected or encapsulated enzymes, creating a catalytically active and stable environment. The immobilized enzymes can subsequently be used to catalyze bioanalytical reactions or detect certain substrates. The enzymatic nickel phosphate-based biosensors have improved selectivity and sensitivity for identifying target analytes. Nickel phosphate can be utilized as an electrode material in electrochemical biosensors. Its excellent conductivity allows for efficient electron transfer, enabling sensitive and rapid electrochemical detection (6).

The 2D material graphitic carbon nitride (g-$C_3N_4$) is thought to be appropriate for photocatalytic water splitting because it has a conjugated structure, photoelectric activity, and good photochemical reaction stability (7). Precursors are typically substances rich in carbon and nitrogen components, such as melamine, urea, cyanamide, dicyandiamide, cyanuric acid, etc. Electrochemical deposition, thermal shrinkage polymerization, solid phase synthesis, gas phase synthesis, solvothermal synthesis, and electrochemical deposition were used to create graphitic carbon nitride materials (8).

Cows are the source of the serum albumin protein known as bovine serum albumin (BSA or "Fraction V"). It is frequently used as a benchmark for protein concentration in laboratory investigations. BSA is frequently used as a model for various serum albumin proteins, particularly human serum albumin, with which it shares 76% structural homology (9). BSA is essential for maintaining oncotic pressure inside capillaries, carrying fatty acids, bilirubin, minerals, and hormones, and acting as both an anticoagulant and an antioxidant (10). This study focuses on the synthesis of Ag@$Ni_3(PO_4)_2$–g-$C_3N_4$ nanocomposite and the immobilization of bovine serum albumin (BSA) to improve the biosensor's selectivity.

## MATERIALS AND METHODS

The prevailing approach to synthesizing g-C3N4 involves a straightforward method known as direct thermal polymerization. In this process, a specific precursor material is placed inside either a ceramic crucible or a quartz boat. Subsequently, the setup is subjected to high temperatures, typically ranging around 500 °C. To ensure the success of the reaction, this heating step is carried out under an inert atmosphere (such as nitrogen or argon) or in a vacuum furnace. As a result of this controlled heating, the precursor material undergoes polymerization and condensation, leading to the formation of g-C3N4.

In an appropriate reaction vessel, combine the silver precursor (for example, silver nitrate, $AgNO_3$), $NABH_4$ 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 nickel precursor solution by dissolving a nickel salt, like Nickel nitrate, 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 nickel 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 nickel phosphate. Once the hydrothermal reaction is complete, cool down the reaction vessel and collect the resulting nickel 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 cobalt 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

## RESULTS

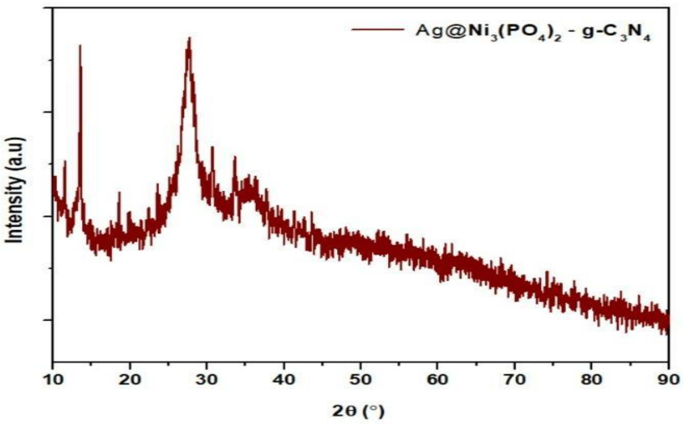


**FIGURE 1 : XRD (X-Ray Diffraction)**

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Figure 1 shows the XRD (X-Ray Diffraction) of compound $Ag@Ni_3(PO_4)_2$–$g\text{-}C_3N_4$. $Ni_3(PO_4)_2$ shows the strongest peak around 2θ = 16° - 18°

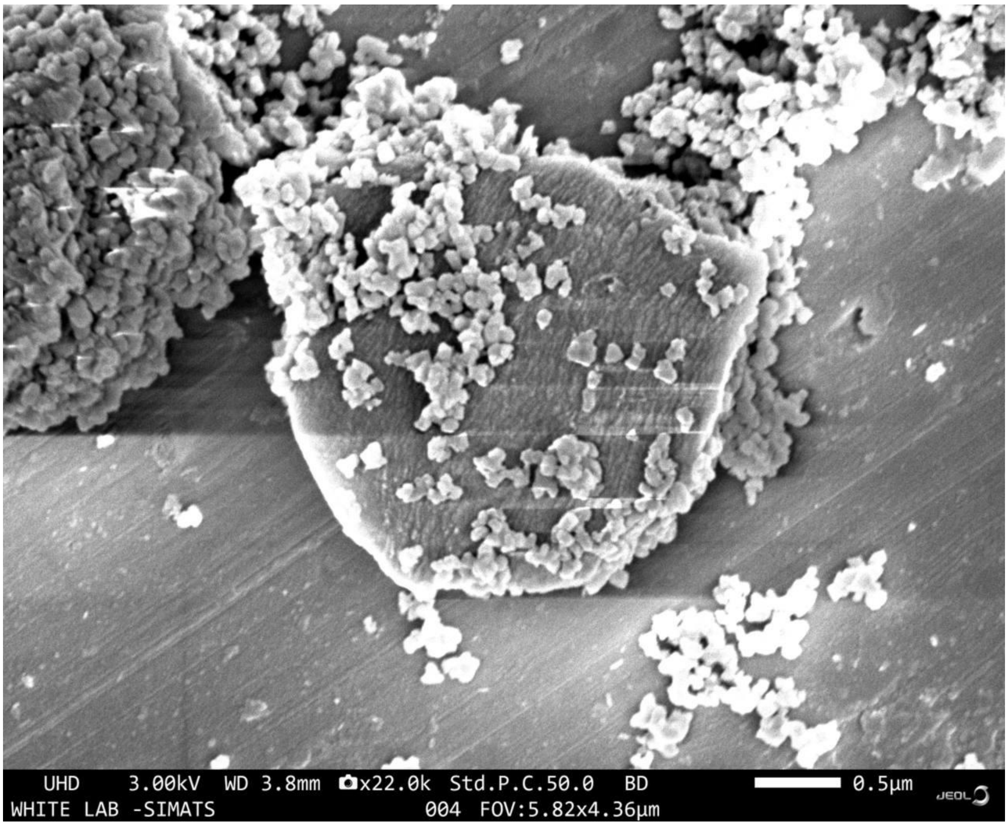


**FIGURE 2 : SEM IMAGE**

Figure 2 shows the SEM image of $g\text{-}C_3N_4$

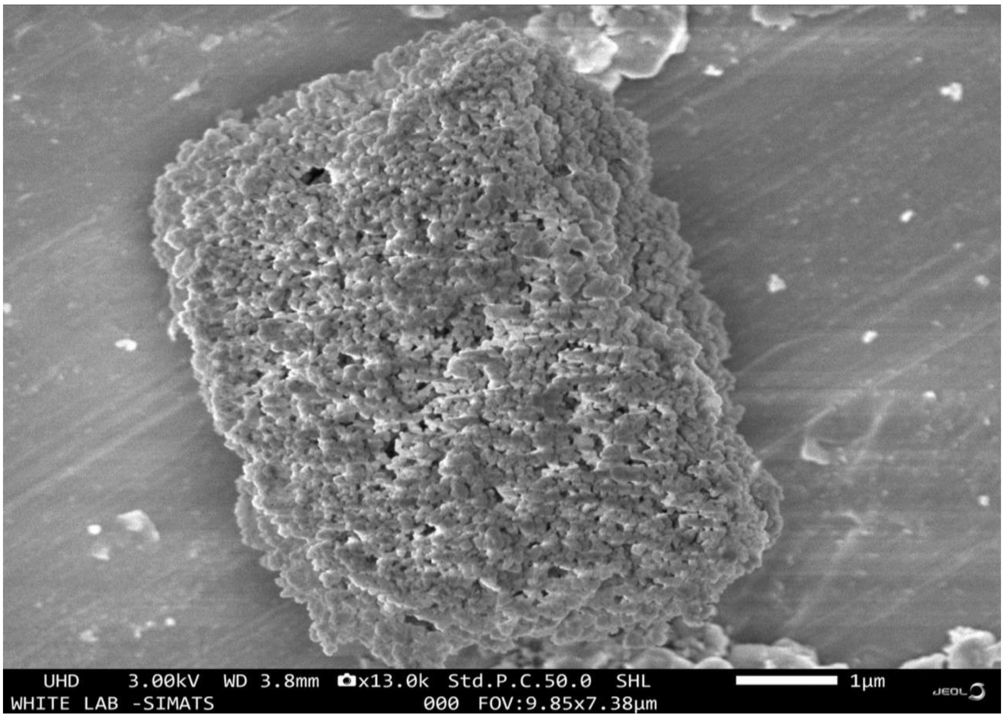


**FIGURE 3 : SEM IMAGE**

Figure 3 shows the SEM image of $Ag@Ni_3(PO_4)_2$

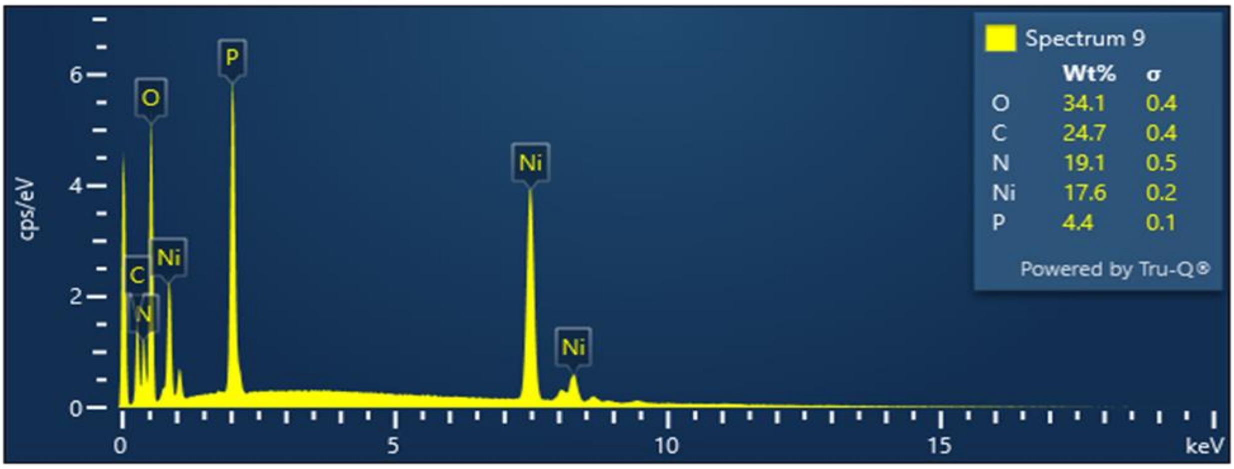


**FIGURE 4 : EDS ANALYSIS**

The graph shows the quantity of compounds present in $Ag@Ni_3(PO_4)_2$-g-$C_3N_4$

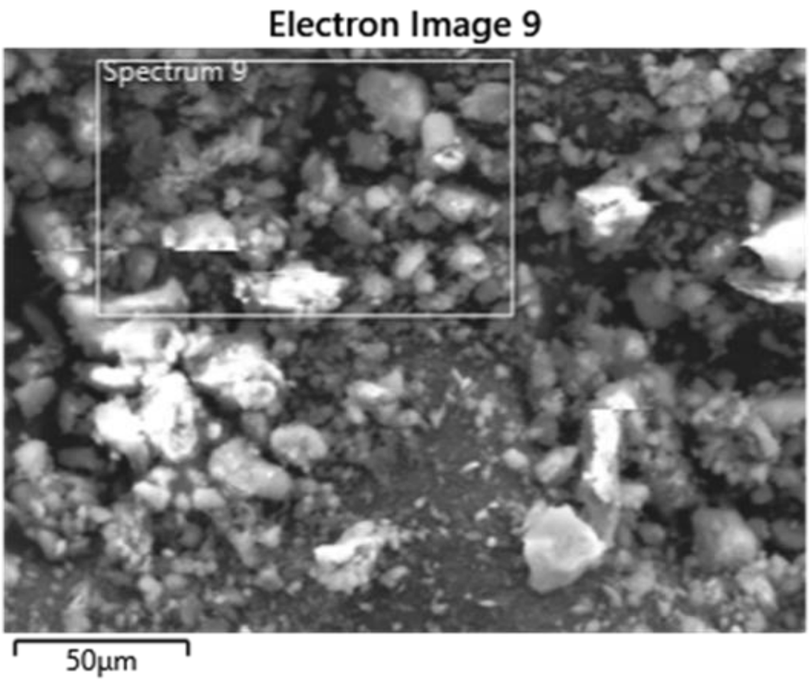


**FIGURE 5 : SEM IMAGE**

Figure 5 shows the SEM image of $Ag@Ni_3(PO_4)_2$-g-$C_3N_4$

| Spectrum 9 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Element | Line Type | Apparent Concentration | k Ratio | Wt% | Wt% Sigma | Standard Label | Factory Standard | Standard Calibration Date |
| C | K series | 0.73 | 0.00733 | 24.71 | 0.44 | C Vit | Yes | |
| N | K series | 2.24 | 0.00399 | 19.12 | 0.50 | BN | Yes | |
| O | K series | 2.56 | 0.00860 | 34.12 | 0.35 | SiO2 | Yes | |
| P | K series | 1.27 | 0.00710 | 4.40 | 0.05 | GaP | Yes | |
| Ni | K series | 3.58 | 0.03577 | 17.64 | 0.19 | Ni | Yes | |
| Total: | | | | 100.00 | | | | |

**FIGURE 6 : ELEMENTAL COMPOSITION**

The table shows the quantity of compounds present in $Ag@Ni_3(PO_4)_2$-g-$C_3N_4$

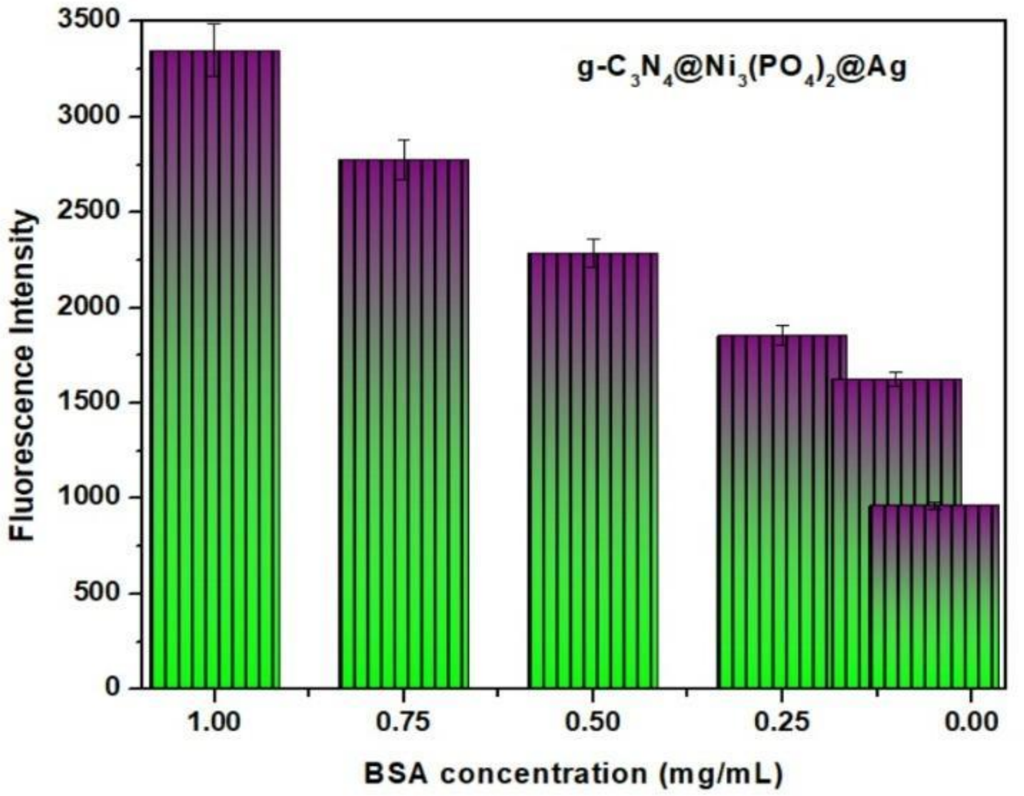


**FIGURE 7 : BSA CONCENTRATION**

Figure 7 shows the BSA concentration. In minimum BSA concentrations, the fabricated compound shows highest biosensing activity

## DISCUSSION

The arrangement of atoms within the crystal lattice is often represented by distinctive peaks in the C3N4 XRD pattern. These peaks' precise locations and intensities can reveal important details about the C3N4 crystal structure. $Ni_3(PO_4)_2$ crystallizes in a monoclinic crystal system, and its XRD pattern usually exhibits a strong peak around $2\theta = 16° - 18°$ ,Several medium to strong peaks in the range of $2\theta = 22°$ -additional peaks with lower intensities at higher $2\theta$ values, typically in the range of $2\theta = 35° - 60°$. These peaks correspond to the crystallographic planes within the material and can be used to determine the crystal structure (7).

Energy Dispersive X-ray Spectroscopy is referred to as EDS. It is an analytical method used to determine and quantify the sample's elemental composition (11). EDS is frequently used with scanning electron microscopy (SEM) in order to offer thorough details regarding the elemental composition and distribution of elements within a sample (12). Ag is not found in EDS analysis because it is added in very low concentration and confirmation of presence of other elements and the composition of the nanocomposite is obtained (13).

The X-ray diffraction (XRD) pattern of silver (Ag) typically exhibits a distinct pattern of diffraction peaks corresponding to its crystal structure (14). The general description of the XRD pattern of silver is face centered cubic structure, Miller indices and high intensity. In minimum BSA concentrations, the fabricated compound shows highest biosensing activity.

## CONCLUSION

In our study, Ag compounds are highly sensitive, highly compatible, high in strength and also highly selective. Because of using less Ag in results we didn't get EDX results positive. The composite material demonstrates positive results even at low concentrations of BSA, indicating its effectiveness for detecting and quantifying BSA in various samples. Nevertheless, the research on $Ag@Ni_3(PO_4)_2$-g-$C_3N_4$ biosensors for BSA detection provides a foundation for potential forensic applications in various areas of analysis and investigation. Future scope: optimization of synthesis parameters, characterization techniques, electrochemical performance evaluation, Biosensor optimization. The preparation of $Ag@Ni_3(PO_4)_2$-g-$C_3N_4$ nanocomposites holds significant potential for the future development of electrochemical biosensing, especially when combined with bovine serum albumin (BSA). Some future scope includes optimization of synthesis parameters, characterization techniques, electrochemical performance evaluation and biosensor optimization.

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## CONFLICT OF INTEREST

There is no conflicts of interests in the present study.

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This project is funded by KSB architects & builders, Anna nagar, Chennai-600040.

## ETHICAL CLEARANCE
Since it is an in vitro study, ethical clearance number is not required**.**

## DURATION OF STUDY
The study was done for a period of three months

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