RESEARCH
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SYNTHESIS OF Ag@$Cu_3(PO4)_2$ -g-C_3N_4 FOR ELECTROCHEMICAL BIOSENSING OF BOVINE SERUM ALBUMIN

Running Title: Analyzing the biosensing activity of the fabricated Ag@$Cu_3(PO4)_2$ -g-C_3N_4 compound using bovine serum albumin assay

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Abstract
INTRODUCTION: Electrochemical biosensing is essential for identifying and measuring biomolecules. Proteins, such as bovine serum albumin (BSA), are particularly interesting among these biomolecules due to their importance in a variety of biological processes like their potential as biomarkers for different diseases,etc.
The superior conductivity and catalytic qualities of the Ag NPs make them the perfect choice for increasing the electrochemical response by providing a conductive substrate for electron transfer. Due to their porous structure and distinctive electrical properties, the $Cu_3(PO4)_2$ have garnered a lot of attention and aid in the immobilization and identification of BSA molecules.The C_3N_4 matrix, also known as carbon nitride, acts as a protective layer and improves the overall stability and selectivity of the biosensing platform thanks to its exceptional stability and biocompatibility. It can be used in a variety of applications, including fluorescent probes, electrochemical sensors, FETs, etc.
AIM: The aim of this study is to develop and characterize a novel Ag@$Cu_3(PO_4)_2$–g-C_3N_4 nanocomposite for electrochemical biosensing of bovine serum albumin (BSA).
MATERIALS AND METHODS: Synthesis of Ag@$Cu_3(PO_4)_2$–g-C_3N_4 Nanocomposite: The Ag@$Cu_3(PO_4)_2$–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 $Cu_3(PO_4)_2$ matrix, which is further combined with C_3N_4 to form the final nanocomposite.
RESULTS: XRD pattern typically exhibits characteristic peaks that correspond to the arrangement of atoms within the crystal lattice. EDS analysis shows that Ag@$Cu_3(PO_4)_2$–gC_3N_4 consists a composition (37.62 % - carbon, 23.03% - nitrogen, 31.94% - oxygen, 1.93% - phosphorous and 5.48% - copper) of various components. The Fluorescence intensity shows the degree of quantification of Bovine serum albumin by the electrochemical biosensor. At a BSA concentration of (1.0, 0.8, 0.6, 0.4 and 0.2) the corresponding fluorescence intensity values are (3500 nm, 2900 nm, 2250 nm, 1750 nm and 800 nm).
CONCLUSION: 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@$Cu_3(PO_4)_2$–gC_3N_4 biosensor for BSA
detection provides a foundation for potential forensic applications in various areas of analysis and investigation.

KEYWORDS: Biomolecular detection, Ag@Cu3(PO4)2–g-C3N4, Composite material, Electrochemical biosensing, Bovine serum albumin, Surface morphology, Sensor development.

INTRODUCTION

When analytes respond, electrochemical sensors create an electrical signal that is proportional to the concentration of the analyte. A sensing electrode (working electrode) and a reference electrode are typically found in an electrochemical sensor, which is divided by an electrolyte. The reference electrode is connected to a high-input-impedance potentiostat and a counterelectrode is utilized to complete the circuit for current flow in the majority of applications(1). One of the key advantages of electrochemical biosensors relies on their relative simplicity. Inexpensive electrodes can be easily integrated with simple electronics to perform rapid measurements in miniaturised easy-to-use portable systems. The ability to determine the concentration of an analyte within a complex sample at the point-of-care and in near real time is extremely attractive for medical diagnosis, monitor- ing of existing conditions and environmental monitoring(1,2).

A globular protein called albumin is essential for maintaining both the dietary balance and plasma pressure. As different substances bind to albumin in the blood, they are transported. Additionally, the level of serum albumin in blood plasma or other biological fluids is intimately related to human health. Bovine serum albumin (BSA), which shares a high degree of structural similarity with human serum albumin (HSA), has received extensive research as a model protein in a variety of domains. It is also referred to as a carrier protein and an allergen. Determining bovine albumin is therefore crucial in a variety of fields, including medicine, pharmaceuticals, clinical testing, and food. Therefore, it is crucial to create novel, effective, quick, and simple techniques for the selective detection of BSA(3).

metal nanoparticles have been used in wide applications in electrochemical sensors. Among the metal nanoparticles, silver nanoparticles (AgNPs) are one of the most well-developed materials and have been used to modify the surface of working electrodes because they are inexpensive in relative comparison with those other materials, possess good chemical and physical properties, providing excellent electron transfer rates and greatly decrease the overpotential of oxidizing or reducing agents produced from enzymatic products(4). AgNPs shows excellent electrocatalytic activity for H_2O_2 and size distribution of AgNPs played an important role in their electrocatalytic activity(5). Silver nanoparticles (Ag NPs) have gained much research interest in biomedical applications due to excellent surface-enhanced Raman scattering (SERS), biocompatibility, high conductivity, amplified electrochemical signals, and catalytic activity. An optical fiber sensor based on both localized surface plasmon resonance (LSPR) and lossy-mode resonance (LMR) was demonstrated using Ag NPs. The devices showed high sensitivity (0.943 nm per RH %), a large dynamic range (42.4 nm for RH changes between 25% and 70%), and a fast response time (476 ms and 447 ms for rise and fall, respectively(6).

To improve the immobilization of recognition components (such as antibodies or aptamers) for BSA detection, $Cu_3(PO4)_2$ is used as a supporting substance or as part of the electrode surface modification. $Cu_3(PO4)_2$has a porous structure that can offer a large surface area, enhancing BSA or recognition element binding. Additionally, the electrochemical detecting procedure may benefit from its electrical features' signal amplification or effective electron transfer(7). C_3N_4 can be incorporated into electrochemical biosensors as a modified electrode material. Its excellent stability and conductive properties can improve the efficiency of electron transfer reactions involved in biosensing. This is especially useful in electrochemical detection of various biomolecules, including proteins like BSA(8).

MATERIALS AND METHODS

STEP1: g-C_3N_4 was synthesised by 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-C_3N_4.

STEP2: 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.

STEP3: Prepare a copper precursor solution by dissolving a copper salt, like copper 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 copper 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 copper phosphate precipitate.

STEP4: To eliminate any impurities, cleanse the precipitate using an ethanol solvent, and then employ filtration methods to dry it. Finally, subject the dried copper phosphate to a temperature of 150 °C for 12 hours to complete the process.

STEP5: Add Ag nanoparticles to the materials using ultra sonification for 3 h and then filtration, dry for 3 hrs at 90 °C.

RESULTS

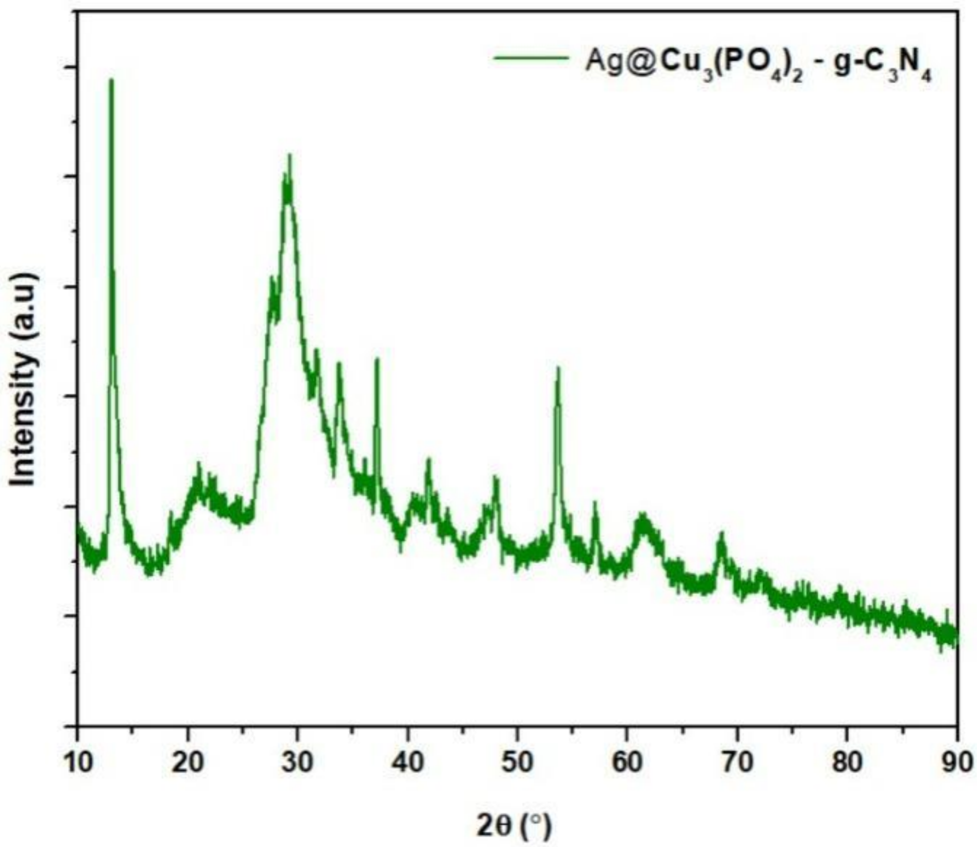

FIGURE 1: XRD (X-Ray Diffraction)

Figure 1 shows the XRD pattern which typically exhibits characteristic peaks that correspond to the arrangement of atoms within the crystal lattice of $Ag@Cu_3(PO_4)_2–gC_3N_4$

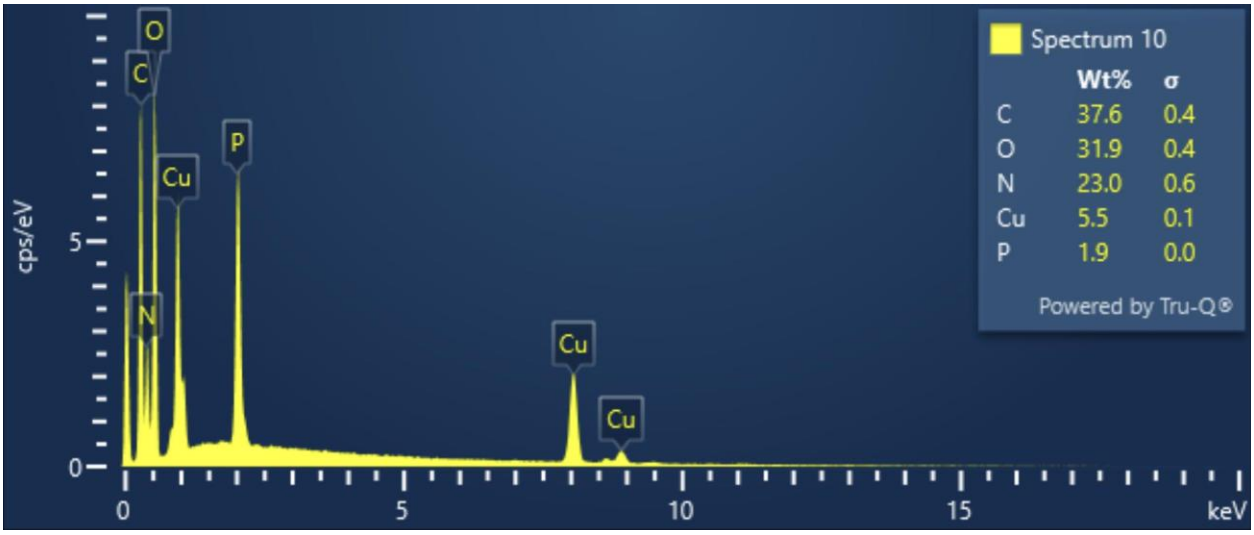

FIGURE 2 : EDS ANALYSIS

The graph shows the quantity of different components present in $Ag@Cu_3(PO_4)_2–gC_3N_4$

Spectrum 10

Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma	Standard Label	Factory Standard	Standard Calibration Date
C	K series	3.93	0.03926	37.62	0.41	C Vit	Yes	
N	K series	4.66	0.00830	23.03	0.57	BN	Yes	
O	K series	4.11	0.01382	31.94	0.36	SiO2	Yes	
P	K series	1.36	0.00759	1.93	0.03	GaP	Yes	
Cu	K series	2.30	0.02301	5.48	0.09	Cu	Yes	
Total:				100.00				

FIGURE 3 : ELEMENTAL COMPOSITION

The table shows the percentage by weight of various elements in $Ag@Cu_3(PO_4)_2–gC_3N_4$

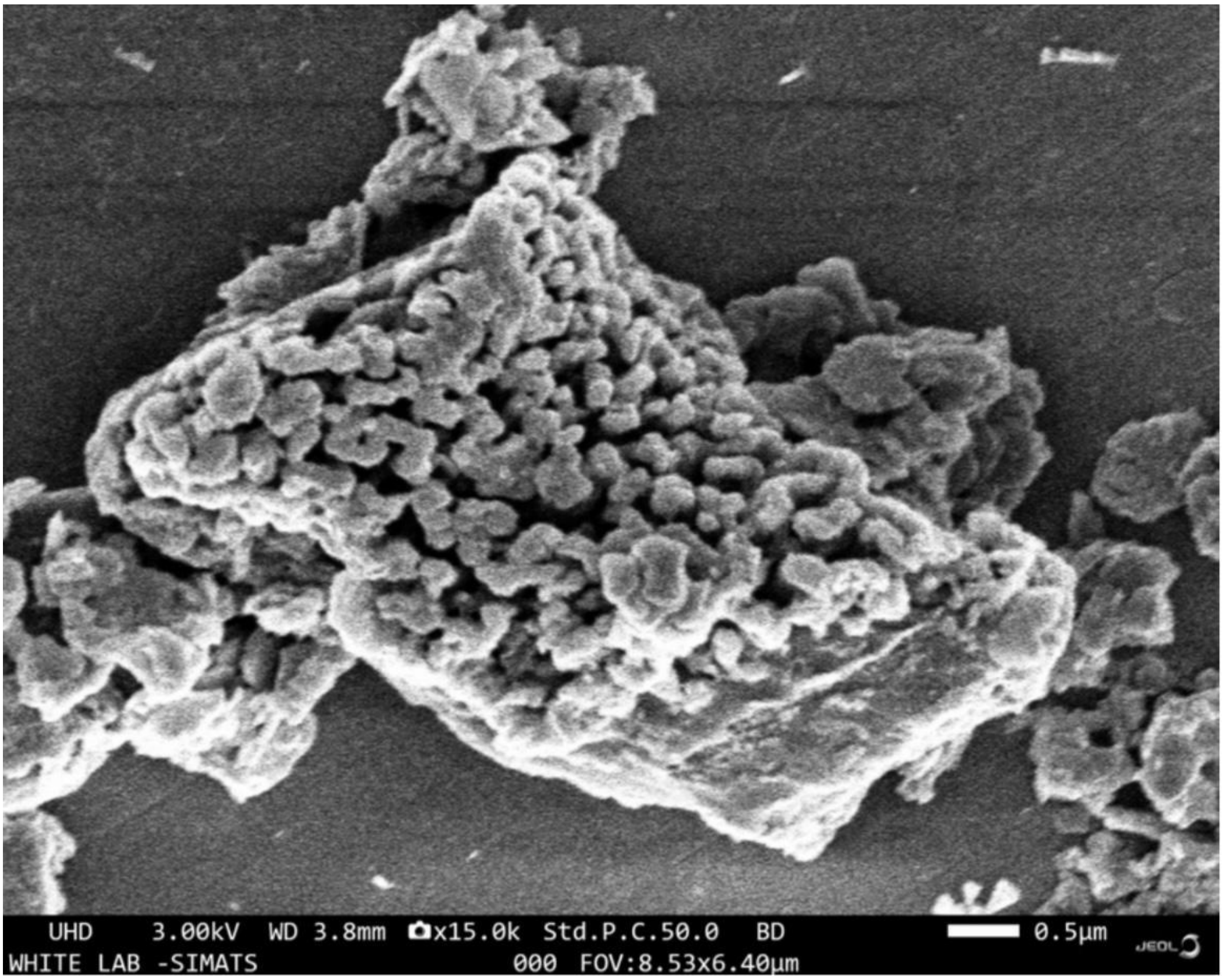

FIGURE 4 : SEM IMAGE

Figure 4 shows the SEM image of $g-C_3N_4$

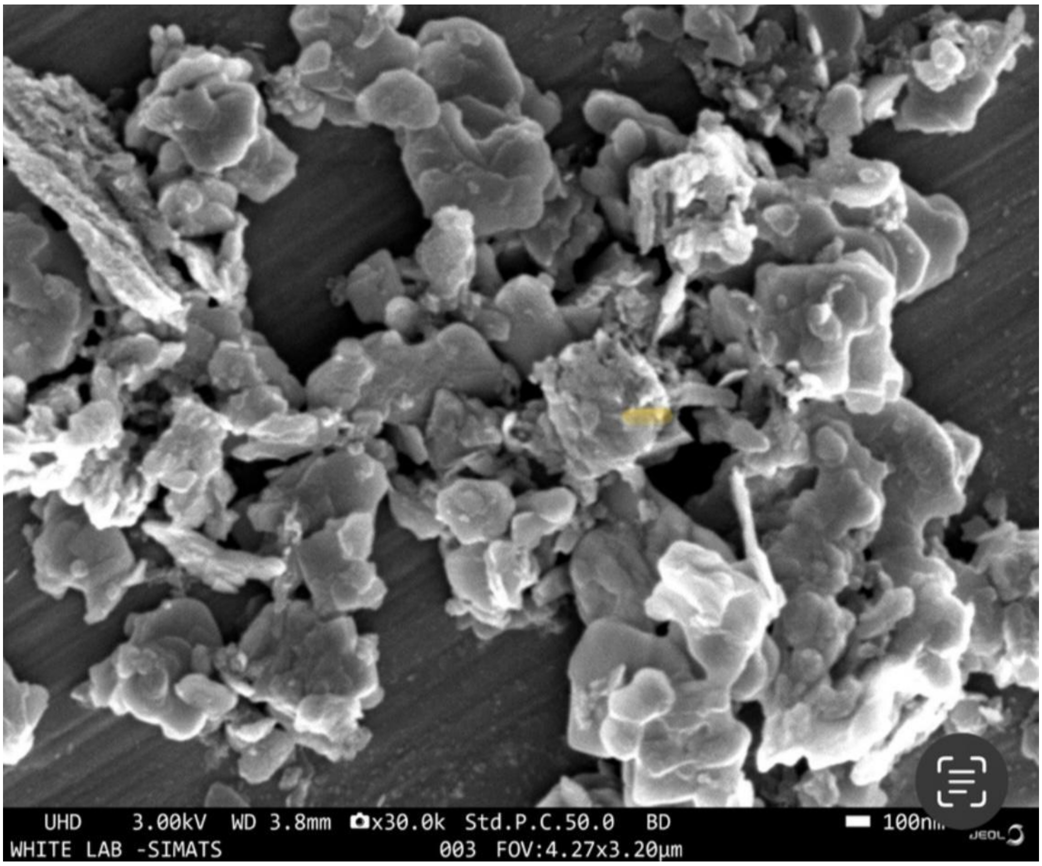

FIGURE 5 : SEM IMAGE

Figure shows the SEM image of $Ag@Cu_3(PO_4)_2–gC_3N_4$

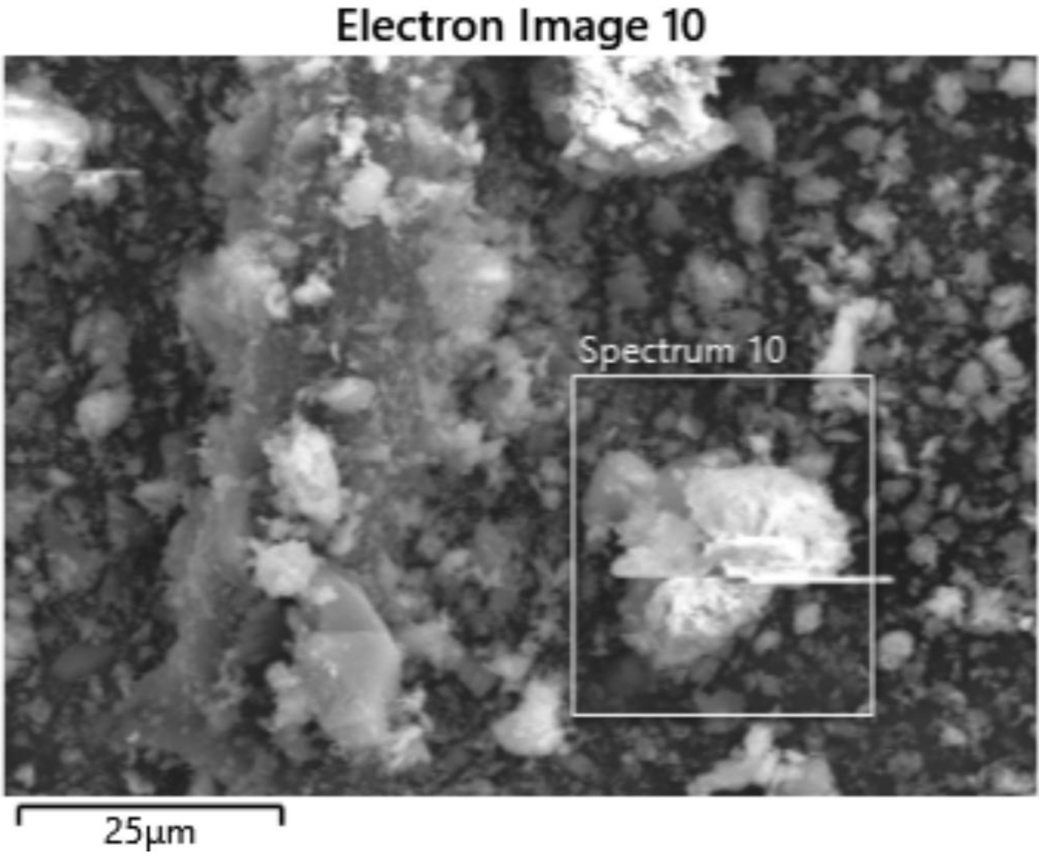

FIGURE 6 : ELECTRON MICROSCOPY IMAGE

The electron microscopy image shows surface morphology of $Ag@Cu_3(PO_4)_2–gC_3N_4$

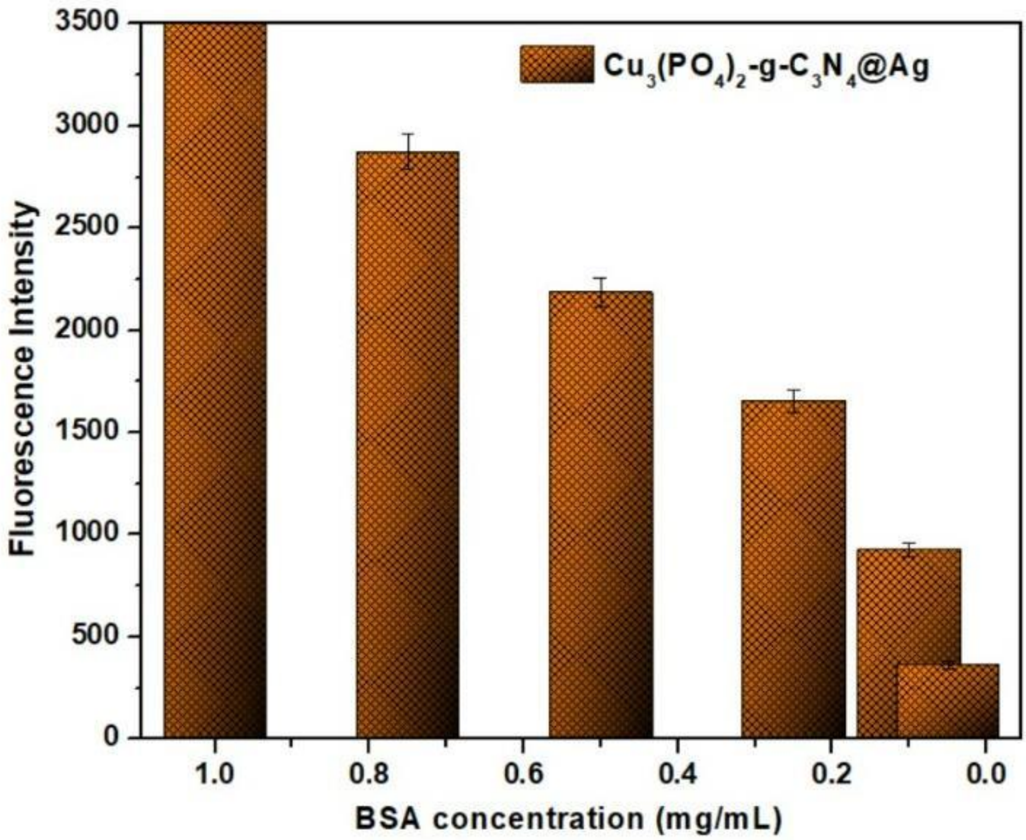

FIGURE 7 : BSA CONCENTRATION

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

DISCUSSION

C3N4 XRD pattern typically exhibits characteristic peaks that correspond to the arrangement of atoms within the crystal lattice. XRD pattern typically exhibits characteristic peaks that correspond to the arrangement of atoms within the crystal lattice. The specific positions and intensities of these peaks can provide valuable information about the crystal structure of C_3N_4 . $Cu_3(PO_4)_2$ crystallizes in a monoclinic crystal system, and its XRD pattern usually exhibits A strong peak around 2θ = 16° - 18°,Several medium to strong peaks in the range of 2θ = 22° - 32°,Additional peaks with lower intensities at higher 2θ values, typically in the range of 2θ = 35° - 60°(9).These peaks correspond to the crystallographic planes within the material and can be used to determine the crystal structure. Silver (Ag) often displays a specific pattern of diffraction peaks according to its crystal structure in its X-ray diffraction (XRD) pattern. Face cantered cubic structure, millet indices, and high intensity are the general descriptions of the silver XRD pattern(10).

EDS analysis shows that $Ag@Cu_3(PO_4)_2–gC_3N_4$ consists a composition(37.62 % - carbon, 23.03% - nitrogen, 31.94% - oxygen, 1.93% - phosphorous and 5.48% - copper)of various components, which confirms the proper chemical formation of $Ag@Cu_3(PO_4)_2–gC_3N_4$. The composite is likely to exhibit a layered or stratified structure(11). This layered arrangement can result from the combination of Ag nanoparticles, Cu3(PO4)2, and g-C3N4 sheets. The surface may contain Ag nanoparticles that appear as small, discrete, and often spherical or irregularly shaped entities. These nanoparticles may be dispersed or clustered on the surface of the composite. Depending on the synthesis process, Cu3(PO4)2 crystals may be present on the surface. They can exhibit distinct crystal facets and may appear as larger, well-defined structures compared to the nanoparticles. The C_3N_4 matrix is found to have many voids and silver particles according to SEM investigation, which expands the scope of the biosensing application(12).

Chitosan, ionic liquid, and graphene nanocomposites worked together synergistically to increase the sensor's sensitivity and electrochemical responsiveness. To characterize the sensor and look into its electrochemical response, various techniques including scanning electron microscopy (SEM), cyclic voltammetry (CV), electrochemical impedance spectrum (EIS), and differential pulse voltammetry (DPV) were applied(13). With a detection limit of 2 1011 g/L, the prepared MIPs/CS/IL-GR/GCE demonstrated a linear connection between the changes in current response and the logarithms of BSA concentrations in the range of 1.0 1010 to 1.0 104 g/L (R = 0.996). Additionally, the manufactured sensor had high selectivity, outstanding stability, good reproducibility, and acceptable recovery, indicating possible use in the clinical field.The Fluorescence intensity shows the degree of quantification of Bovine serum albumin by the electrochemical biosensor(14). At a BSA concentration of (1.0, 0.8, 0.6, 0.4 and 0.2) the corresponding fluorescence intensity values are (3500nm, 2900nm, 2250nm, 1750nm and 800nm)(15).

CONCLUSION

In our study, Ag compound is highly sensitive highly compatible high in strength and also highly selective. Because of using less amount 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 $Ag@Cu_3(PO_4)_2–gC_3N_4$ biosensor 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.

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CONFLICT OF INTEREST
The author declares that there were no conflicts of interests in the present study.

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

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