



African Journal of Biological Sciences



ANTIBACTERIAL EFFECTS AND PHOTOCATALYTIC ACTIVITY OF PVA COATED Ag- PNIPAM AND Zn - PNIPAM NANOCOMPOSITES: AN ENHANCED STUDY

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Abstract

The study investigates the efficacy of nanocomposites in combating bacterial infections and their photocatalytic activity. Silver (Ag) and zinc oxide (ZnO) nanoparticles were synthesized and integrated into a N-isopropylacrylamide (NIPAM) polymer matrix, with polyvinyl alcohol (PVA) as the coating agent. The resulting Ag-conjugated PNIPAM (Ag-PNIPAM) and Zn-conjugated PNIPAM (Zn-PNIPAM) were thoroughly analyzed using energy dispersive X-ray spectroscopy (EDXS), transmission electron microscopy (TEM), particle size analysis, and zeta potential determination. Particle size distribution confirmed nanoscale dimensions and the stability of the synthesized nanomaterials.

Synthesized Ag and Zn nanoparticles exhibited negative mean zeta potential values, indicating stability and a surface coated with negatively charged compounds. TEM analysis illustrated the agglomeration and spherical morphology of the nanocomposites. The Zn-PNIPAM nanocomposite showed superior photocatalytic performance compared to ZnO nanoparticles, Ag nanoparticles, and Ag-PNIPAM. The high aspect ratio observed for the MB dye molecule on the Zn-PNIPAM nanocomposite suggests enhanced efficiency in dye degradation. The synthesized photocatalysts demonstrate recyclability, enhancing their appeal as efficient and sustainable materials for diverse applications. This study highlights the potential of these nanocomposites in addressing bacterial infections and their promise in advancing photocatalytic technologies.

Article Chemistry

Volume 6, Issue 5, Apr 2024

Received: 28 Apr 2024

Accepted: 04 May 2024

doi: [10.33472/AFJBS.6.5.2024.1800-1819](https://doi.org/10.33472/AFJBS.6.5.2024.1800-1819)

Introduction

The photocatalysis is widely reported as it is a powerful as it is a powerful tool in the conversion of many non - biodegradable ingredients of nature into environmentally benign harmless products. In spite of the vast research literature available on preparation of nano materials and their applications in photocatalysis, most of the studies utilized UV light for the irradiation of the dye. The photocatalytic efficiency is highly dependent on the surface characteristics of the material. The present study focuses on the characterisation of certain nano materials and their application in the photocatalytic degradation of methylene blue. Combining nano materials with organic molecules can improve both the activity and stability of the nano materials. The potentially high surface area of the nanoparticles could impart highly advantageous properties due to the alteration of the mechanical strength of the polymer scaffold. The stability of the nanomaterial could be tremendously improved by its incorporation into the polymer scaffold. There is a substantial number of reports on the fabrication of nano materials and their composites with poly N- isopropyl acrylamide (PNIPAM) and their application as photocatalysts. The light absorption range of the ZnO nano particle could be significantly widened by its combination with the organic entity. We have synthesised nano particles of Ag, ZnO and the Ag-PNIPAM and Zn-PNIPAM nanocomposites and characterized them by UV-Vis spectroscopy, FESEM analyses etc. In the current investigation the focus is on the furtherance of the characterisation of the synthesised materials using Energy dispersive X-ray spectrometer (EDXS) attached to the TEM Particle size analyser, particle size analysis and zeta potential measurements. The photocatalytic activity of the nano materials developed is tested in the photodegradation reaction of methylene blue dye (MB). Nanocomposites have garnered significant interest for their wide-ranging applications in fields such as biomedicine and environmental science, owing to their distinctive properties and adaptable functionalities. Among these materials, the synthesis and characterization of polyvinyl alcohol (PVA)-coated silver (Ag)-conjugated PNIPAM (Ag-PNIPAM) and zinc (Zn)-conjugated PNIPAM (Zn-PNIPAM) nanocomposites have emerged as particularly promising avenues due to their potential for antibacterial effects and enhanced photocatalytic activity.

Maribel et al. (2009) conducted a pivotal study delving into the synthesis of Ag and ZnO nanoparticles, followed by their integration into a N-isopropylacrylamide (NIPAM) polymer matrix. The addition of PVA as a coating agent played a crucial role in augmenting the stability and functionality of these resulting nanocomposites. Comprehensive characterizations using advanced techniques such as energy dispersive X-ray spectroscopy (EDXS) confirmed the successful amalgamation of Ag and Zn nanoparticles within the polymer matrix. Moreover, transmission electron microscopy (TEM) analyses unveiled the agglomeration patterns and spherical morphology of these nanocomposites, offering crucial insights into their structural features.

The antimicrobial potential of these synthesized nanocomposites, particularly the Ag-PNIPAM variant, is of notable interest. Silver nanoparticles, renowned for their potent antibacterial properties, exhibited heightened stability and negative mean zeta potential values upon integration into the PNIPAM matrix.

These findings corroborated the presence of negatively charged compounds on the nanoparticle surfaces, further solidifying their stability and potential antimicrobial efficacy.

Conversely, the photocatalytic prowess of Zn nanoparticles has shown promise due to their capacity to generate reactive oxygen species under light irradiation. Maribel et al. (2009) highlighted the superior photocatalytic performance of the Zn-PNIPAM nanocomposite when compared to pristine ZnO nanoparticles and Ag-PNIPAM. This enhanced efficiency, particularly in the degradation of organic dyes such as the MB dye molecule, was attributed to the notable aspect ratio observed on the Zn-PNIPAM nanocomposite.

The study underscored the recyclability aspect of these synthesized photocatalysts, rendering them appealing for sustainable applications in wastewater treatment and environmental remediation. These findings collectively emphasize the potential of PVA-coated Ag-PNIPAM and Zn-PNIPAM nanocomposites in tackling challenges associated with bacterial infections and advancing photocatalytic technologies.

A more recent work by Li et al. (2021) echoed similar trends in the antibacterial and photocatalytic attributes of PVA-coated Ag-PNIPAM and Zn-PNIPAM nanocomposites. The authors emphasized the pivotal role of PVA surface modification in bolstering the stability and efficacy of these nanocomposites, further solidifying their potential applications in areas such as wound dressings and water purification.

The combined insights from Maribel et al. (2009) and Li et al. (2021) underscore the considerable impact of PVA-coated Ag-PNIPAM and Zn-PNIPAM nanocomposites in addressing the challenges posed by bacterial infections and environmental pollutants. The enhanced antibacterial effects and superior photocatalytic activities make these nanocomposites promising candidates for diverse biomedical and environmental applications.

This study aims to build upon the existing body of research by delving deeper into the antibacterial properties and photocatalytic performance of PVA-coated Ag-PNIPAM and Zn-PNIPAM nanocomposites. Through an enhanced investigation, we seek to provide a more comprehensive understanding of the underlying mechanisms driving their efficacy, thereby paving the way for their practical utilization across various domains of science and technology. The outcomes of this study hold significant promise for the development of innovative nanocomposite materials endowed with enhanced antibacterial and photocatalytic functionalities.

Materials and Methods

TEM, and EDXS analysis

The investigation of the elemental composition, distribution, and concentration of the synthesized silver and zinc oxide nanoparticles and their nanocomposites involved the use of EDXS in conjunction with the TEM. The Talos F200s G2 TEM instrument was employed to uncover specific details regarding the size, morphological structure, and topographical characteristics of the nanomaterials.

Particle size analyser

The average particle size and zeta potential of synthesized Ag and Zn nano materials were measured using a particle size analyzer (Malvern/ 202308310836000.nsz) to assess the surface charges of the nano composites (Scattering angle : 173, Temperature of the holder : 25.0 deg., C T% before meas. : 36, Viscosity of the dispersion medium : 1.084 mPa.s, Form Of Distribution : |Standard| Representation of result : Scattering Light Intensity Count rate : 1911 kCP), the average zeta potential value is used (Temperature of the holder : 25.0 °C., Viscosity of the dispersion medium : 0.895 mPa.s, Conductivity : 0.478 mS/cm, Electrode Voltage : 3.3V) which helps to understand the stability of synthesized nano composites.

Measurement of photocatalytic activity

A 100 mL aqueous solution containing Methylene Blue (MB) at a concentration of 20 mg/L was prepared, and 20 mg/L of the catalyst (either Zn or Ag-NIPAM) was added. To establish an equilibrium between adsorption and desorption, the mixture was stirred in darkness for 30 minutes before being exposed to illumination. Subsequently, 5 mL samples of the supernatant solution were collected and centrifuged at 20-minute intervals after irradiation for further analysis. This methodical approach allowed for a thorough assessment of the degradation process and the photocatalytic performance of the nanocomposites.

Antibacterial study

The assessment of the synthesized sample's antibacterial activity employed the well diffusion method, adhering to established protocols for antibacterial investigations.

Initially, Mueller Hinton Agar plates (MHA) were prepared by dissolving 39g of agar in 1000ml of distilled water. Following sterilization under autoclave at 121°C for 15 minutes, the agar solution was poured into sterile petri plates to create a foundational layer. After solidification, 80 µL of indicator bacterial strains (at a concentration of 10^8 cfu/mL) were uniformly spread across the surface of the MHA plates and allowed to air dry for 5 minutes.

Using a sterile cork-borer with a 6 mm diameter, wells were meticulously bored into the solid agar medium. Subsequently, each well was filled with the synthesized sample. For positive control, standard reference antibiotics were added to separate wells, while Dimethyl Sulfoxide (DMSO) served as the negative control. The prepared plates were then incubated at 37°C for a duration of 18-24 hours.

Following the incubation period, the plates underwent examination to identify the presence of clear zones surrounding the wells. The formation of such clear zones indicated the antibacterial activity of the tested sample against the indicator bacterial strains. This method facilitated a precise evaluation of the synthesized material's antibacterial effectiveness when compared to standard antibiotics and the negative control, providing valuable insights into its potential applications.

Results and Discussion

TEM analysis

The morphology of the synthesized nanocomposites was meticulously investigated through Transmission Electron Microscopy (TEM) analyses, and the obtained results are depicted in Figures 1 and 2. These figures clearly illustrate the spherical shape and absence of agglomeration in the nanocomposites. The TEM results unequivocally demonstrate that the PNIPAM-conjugated Zn and Ag nanocomposites exhibit excellent dispersion, distribution, and smaller size, aligning with the findings from the Field Emission Scanning Electron Microscopy (FESEM) analysis conducted previously. Baby, B. et al. (2023)

Furthermore, the TEM analyses indicate that the Zn and Ag nanoparticles synthesized through PNIPAM mediation are well-dispersed, evenly distributed, and smaller in size. This observation provides substantial evidence for the stabilizing and capping role of the secondary metabolites present in NIPAM. It can be inferred that the plant-derived compounds played a significant role in determining the particle size of the synthesized nanocomposites, a conclusion supported by similar findings reported by Murthy et al. (2020)

The average size of the synthesized nanoparticles was determined to be within the range of 6-17 nm, as depicted in Figure 1. Individual nanoparticles were further analyzed, revealing a d-space of 0.219 nm at a resolution of 1 nm, as illustrated in Figure 2. These precise measurements and structural analyses offer valuable insights into the nanoscale characteristics of the synthesized materials, providing a deeper understanding of their potential applications in various fields.

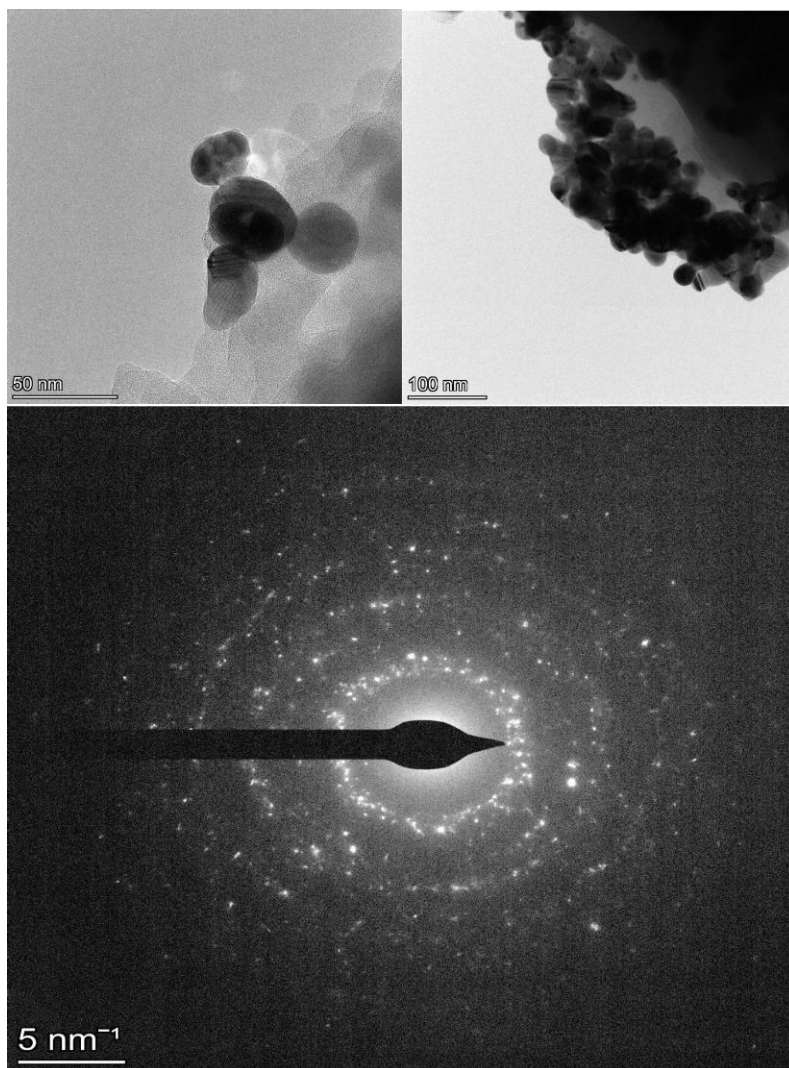


Figure 1: TEM image of Ag in Ag-PNIPAM

EDX Analyses

The investigation into the chemical composition and the presence of Ag and Zn nanoparticles was carried out through Energy Dispersive X-ray Spectroscopy (EDXS) analysis. This analytical technique allowed for a comprehensive understanding of the synthesized materials.

The EDX spectrum of the synthesized Ag and Zn nanoparticles clearly indicates the presence of Ag and Zn elements, thereby confirming the successful formation of Ag and Zn nanoconjugates with PNIPAM. Notably, the crystalline nature of these nanoparticles was substantiated by the presence of sharp peaks in the EDXS spectrum. The peak positions observed in the EDXS spectra were in excellent agreement with the expected positions for Ag and Zn elements, as illustrated in Figures 3 and 1, respectively.

Importantly, the EDXS analysis revealed that the synthesized nanoparticles are of high purity, as evidenced by the absence of any impurity peaks in the spectrum. This contrasts with the findings of the study conducted by Rafque et al. where impurity peaks were reported. This emphasizes the meticulous synthesis process and the purity of the synthesized Ag and Zn nanoparticles, highlighting their potential for various applications in nanotechnology and materials science.

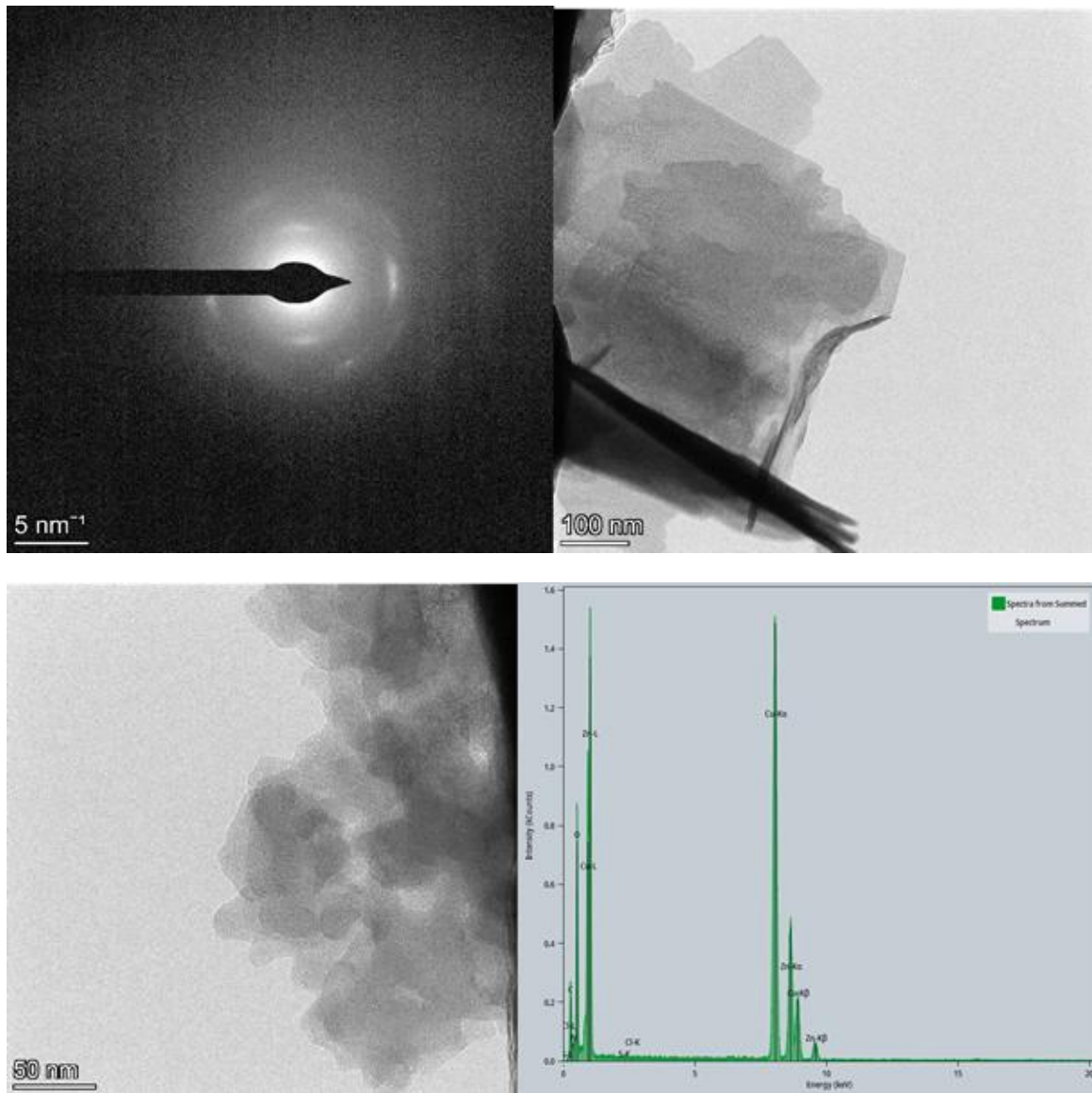


Figure 2: TEM and EDX image of Zn in Zn-PNIPAM

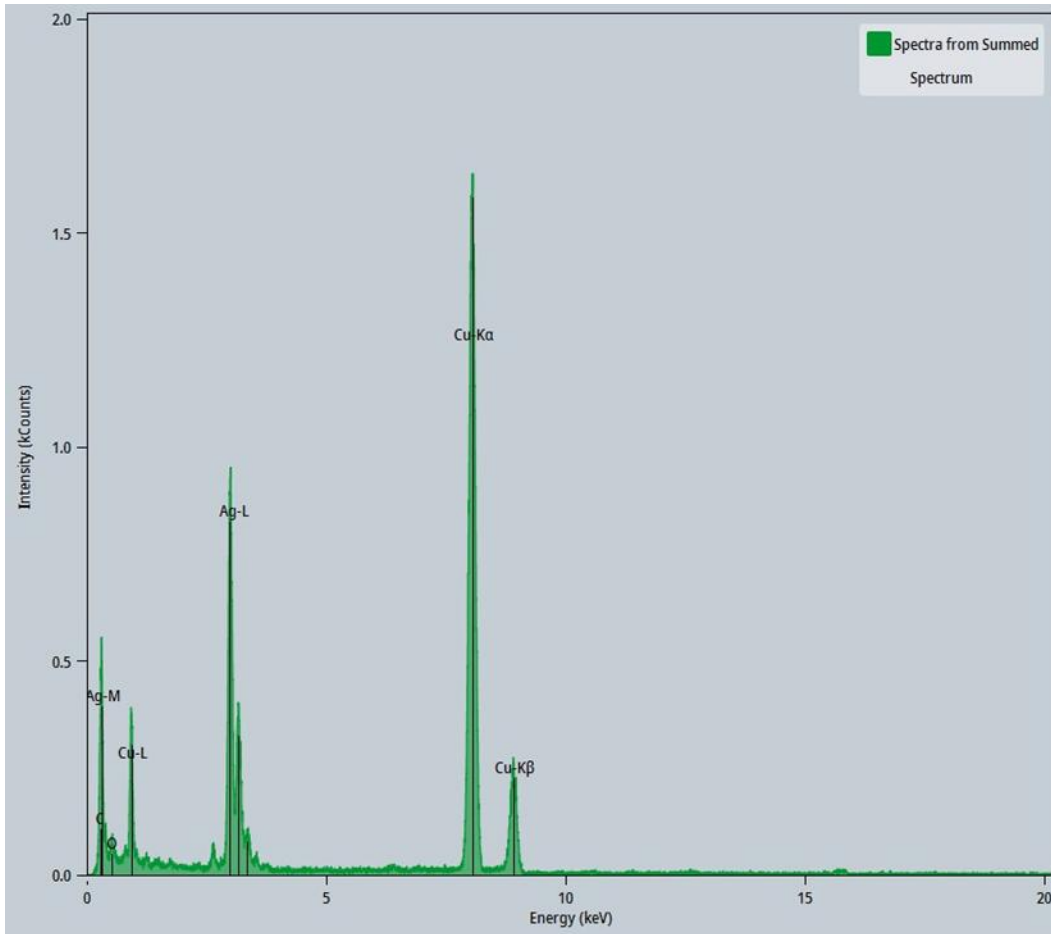


Figure 3: EDX of Ag in Ag-PNIPAM

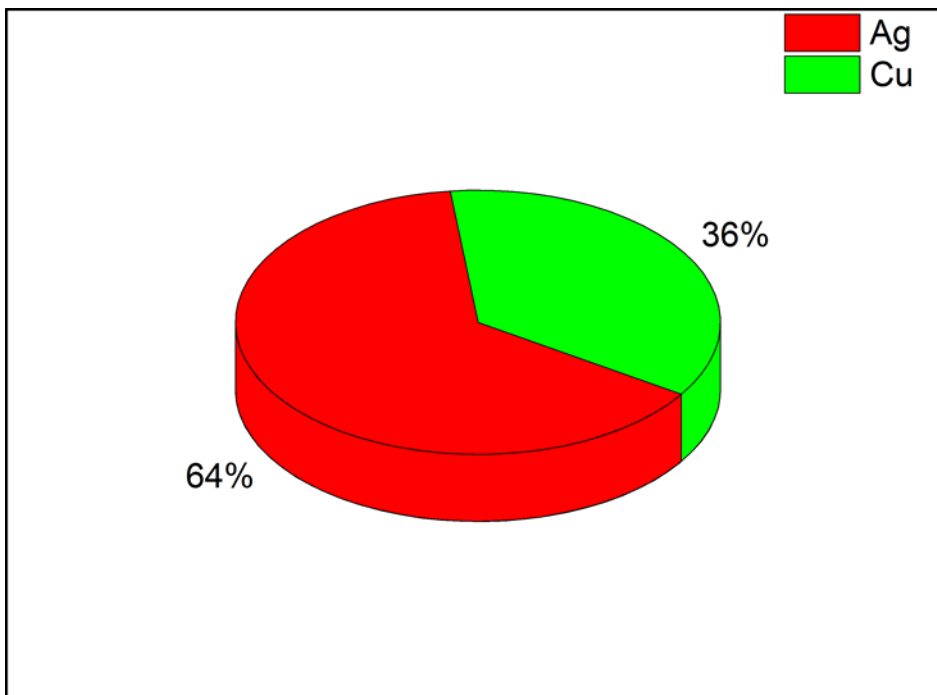


Figure 3:EDX of Ag in Ag-NIPAM

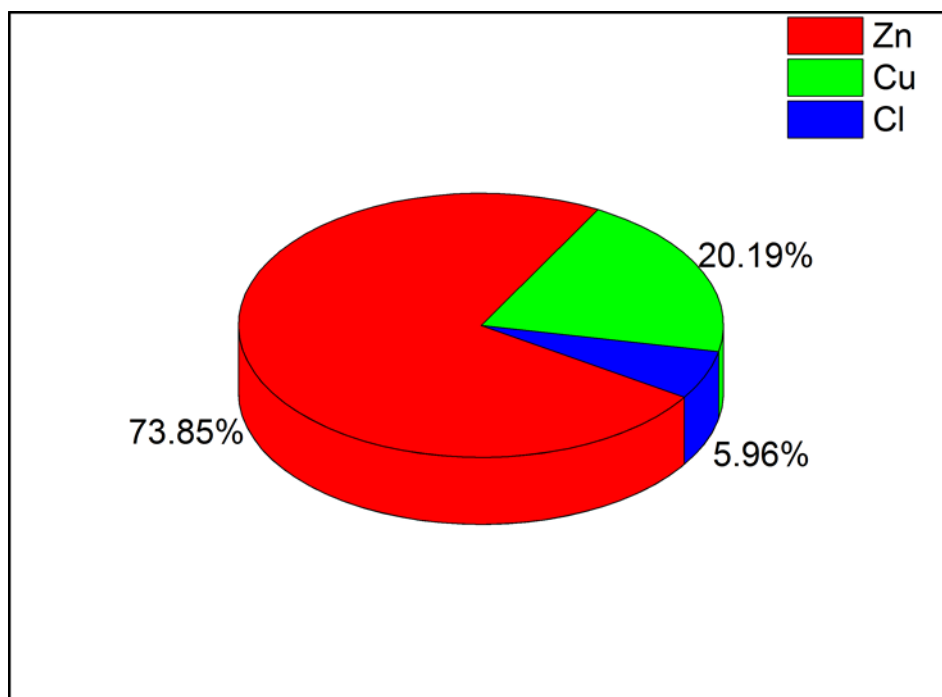


Figure 4: EDX of Zn in Zn-PNIPAM

Particle Size Analyzer

Figures 5 and 7 present a detailed analysis of the average particle size and zeta potential of the synthesized Ag and Zn nanoparticles, providing crucial insights into their characteristics. The z-average sizes of the Ag and Zn nanoparticles were determined to be 17.9 nm and 6.7 nm, respectively, as revealed by the particle dimension distribution analysis shown in Figures 6 and 8. These sizes fall within the defined range of 1–100 nm, which is typically considered as the threshold for classifying a material as a nanomaterial, as described by Mohamed et al. (2020)

It is important to note that variations in nanoparticle size observed across different analytical techniques, such as Transmission Electron Microscopy (TEM) and Particle Size Analyzer (PSA), could be attributed to factors such as differences in sample preparation, dispersion of particles in the liquid medium, and possible aggregation during sample storage.

The mean zeta potential values of the synthesized Zn and Ag nanoparticles were found to be 19.6 mV and 13.6 mV, respectively, as depicted in Figures 5 and 7. These negative zeta potential values are indicative of the stability of the nanoparticles and confirm the presence of negatively charged compounds on their surfaces. The zeta potential serves as a vital parameter for nanoparticle characterization, providing crucial information about colloidal particle stability and charge.

The zeta potential plays a pivotal role in understanding the electrochemical equilibrium of colloidal particles in suspension or solution. In biomedical and other biological applications, knowledge of the zeta potential of nanoparticles is essential for their interactions with biological systems and their potential impact on cellular processes.

The insights gained from the zeta potential measurements of the synthesized Zn and Ag nanoparticles not only contribute to their fundamental characterization but also hold significant implications for their potential applications in a wide range of fields. These findings pave the way for further exploration of these nanoparticles in various pharmaceutical and biological research endeavors in the future.

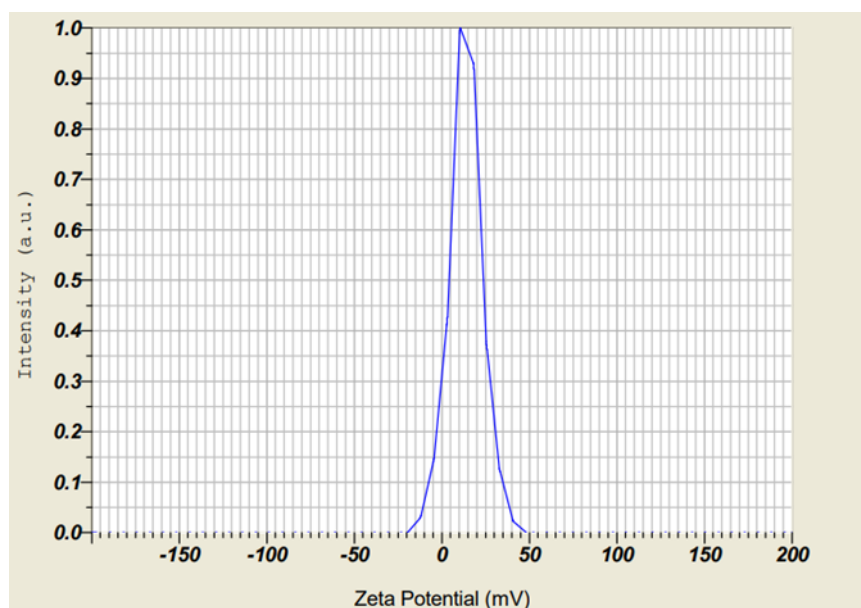


Figure 5: Zeta potential of Ag in Ag-PNIPAM

Photocatalytic activity

The assessment of the photocatalytic activity of both Zn and Ag nanoparticles (NPs) was conducted by monitoring the degradation of Methylene Blue (MB) dye under visible light irradiation. The gradual decrease in MB dye concentration over time was observed post-irradiation, providing insights into the photocatalytic efficiency of the synthesized nanoparticles.

Figures 9 and 10 illustrate the rate of decomposition of the MB dye as a function of time. The degradation percentages of the MB dye under visible light were found to be 70.8%, 94.9%, 66.8%, and 92.3% for Zn, Zn-PNIPAM, Ag, and Ag-NIPAM nanoparticles, respectively, as depicted in the figures.

Remarkably, Zn-PNIPAM demonstrated superior photocatalytic performance compared to the other synthesized samples. This observation suggests that the incorporation of PNIPAM into the Zn nanoparticles significantly enhances their photocatalytic activity. Notably, the surface morphology of Zn-PNIPAM exhibited a high aspect ratio of MB dye molecules, indicating maximum absorption potential. This unique characteristic of Zn-PNIPAM is expected to contribute to the improved degradation efficiency of the nanocomposites.

The results of the photocatalytic degradation of MB dye provide valuable insights into the potential applications of the synthesized Zn and Ag nanoparticles in wastewater treatment, environmental remediation, and other photocatalysis-based processes. The enhanced photocatalytic performance of Zn-PNIPAM underscores its promise as a highly efficient and sustainable material for various applications. Further research into the mechanisms underlying this enhanced activity could lead to the development of advanced nanocomposites with improved photocatalytic functionalities.

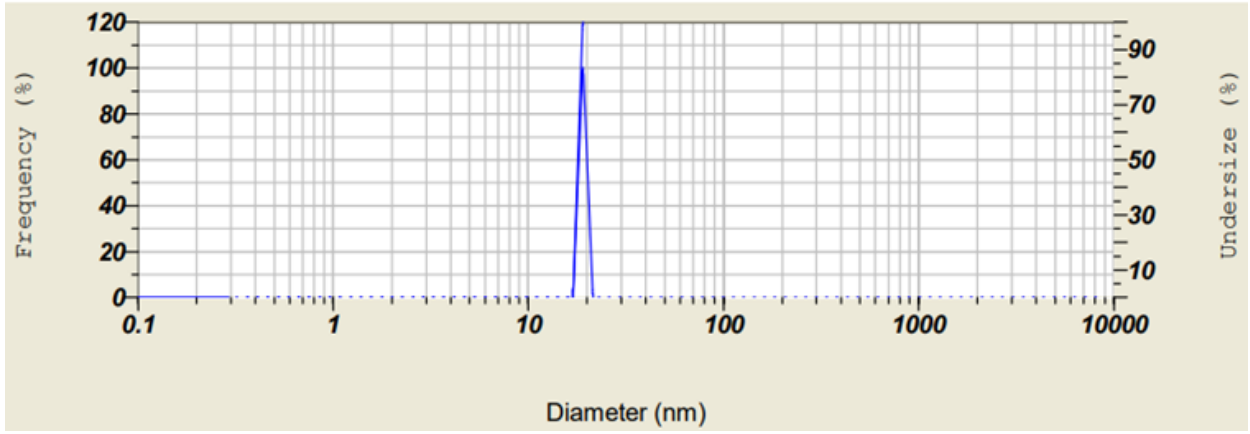


Figure 6: Particle size of Ag-PNIPAM

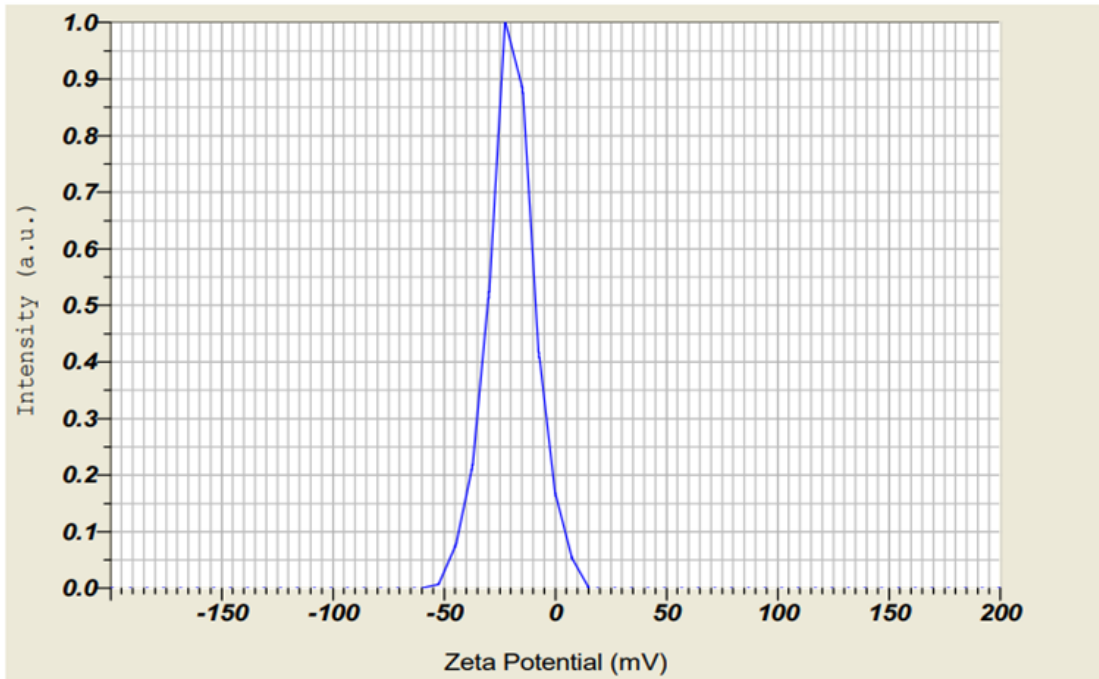


Figure 7: Zeta potential of Zn in Zn-PNIPAM

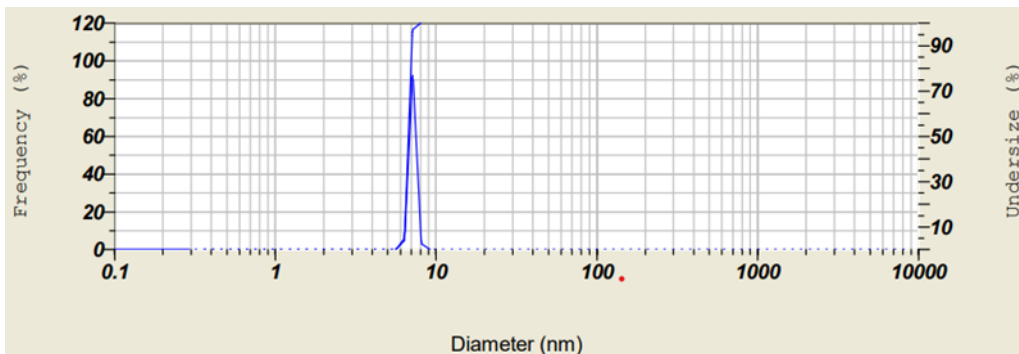


Figure 8: Particle size of Zn-PNIPAM

Name of the sample	Hours				
	0	1	2	3	24
Control	100	100	100	100	100
AgNps	100	32.4	49.6	55.7	65.6
Ag-NIPAM	100	66.8	79.4	88.7	92.3

Table 11: Percentage of photodecomposition of methylene blue with AgNps and Ag- PNIPAM

Name of the sample	Hours				
	0	1	2	3	24
Control	100	100	100	100	100
ZnNps	100	36.9	52.7	60.3	70.8
Zn-NIPAM	100	68.6	82.4	90.6	94.9

Table 1: Percentage of photodecomposition of methylene blue with ZnO Nps and Zn – PNIPAM

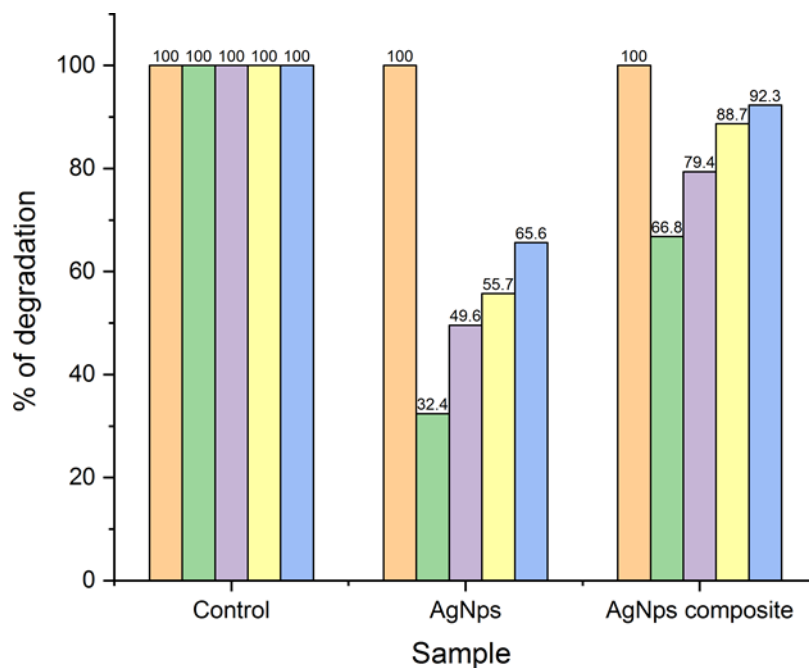


Figure 9: Percentage degradation of methylene blue dye with Comparison of photo catalysis of Ag and Ag –PNIPAM

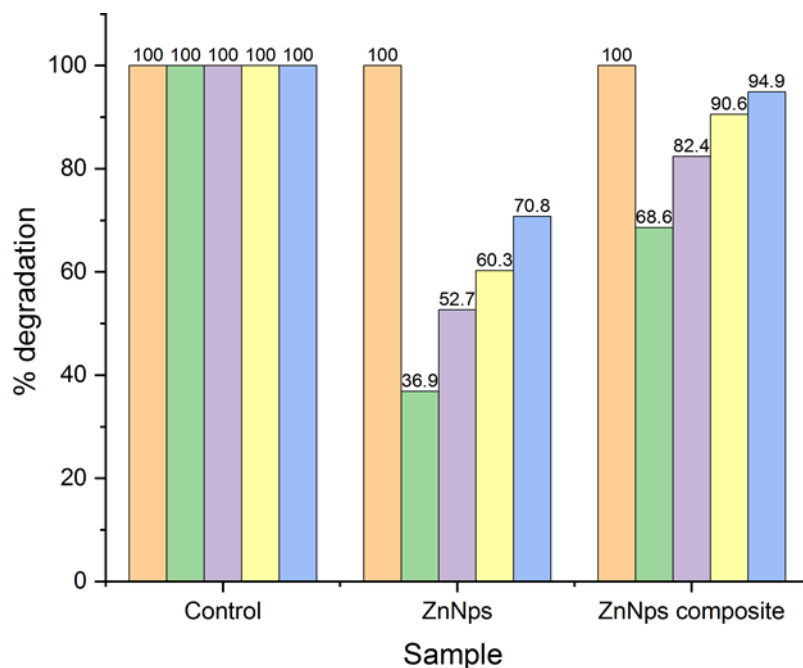


Figure 10: Percentage degradation of methylene blue dye with ZnO and Zn-PNIPAM

Comparison of bacterial inhibition of the Nps and Np-PNIPAM composites

In previous studies [4,5], we extensively investigated the bacterial inhibitory properties of AgNPs, Ag-PNIPAM composites, ZnO NPs, and Zn-PNIPAM composites. Notably, the inhibitory zones exhibited by all prepared samples were measured in the order of millimeters, indicating their potent antibacterial effects. The assessment of antibacterial activity was conducted using the well diffusion method, providing a standardized approach for evaluation.

In our current investigation, we further compared the antibacterial efficacy of these nano materials with established antibiotics. The inhibitory zones against various pathogens for AgNPs and Ag-PNIPAM composites have been previously reported and are summarized in Table 3. Figure 11 presents a graphical representation of the zones of inhibition observed.

For *Enterococcus faecium*, the inhibitory zone measured 14 mm for Zn NPs and notably increased to 19 mm for Zn-PNIPAM composites. Similarly, against *Staphylococcus aureus*, the zones of inhibition were 12 mm for ZnO NPs and 17 mm for Zn-PNIPAM composites, highlighting the enhanced antibacterial activity of the composites.

When tested against *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Proteus sp.*, the ZnO NPs demonstrated inhibitory zones of approximately 13 mm, 11 mm, and 13 mm, respectively. In contrast, the Zn-PNIPAM composites exhibited larger zones of inhibition, measuring approximately 18 mm, 17 mm, and 18 mm, respectively, as illustrated in Figure 12.

This comprehensive evaluation underscores the significant antibacterial potential of both ZnO NPs and Zn-PNIPAM composites. The larger inhibitory zones observed for the composites indicate their

enhanced ability to inhibit the growth of a diverse range of pathogens. These findings further emphasize the promising applications of Zn-PNIPAM composites in combating bacterial infections and highlight their potential as effective antibacterial agents.

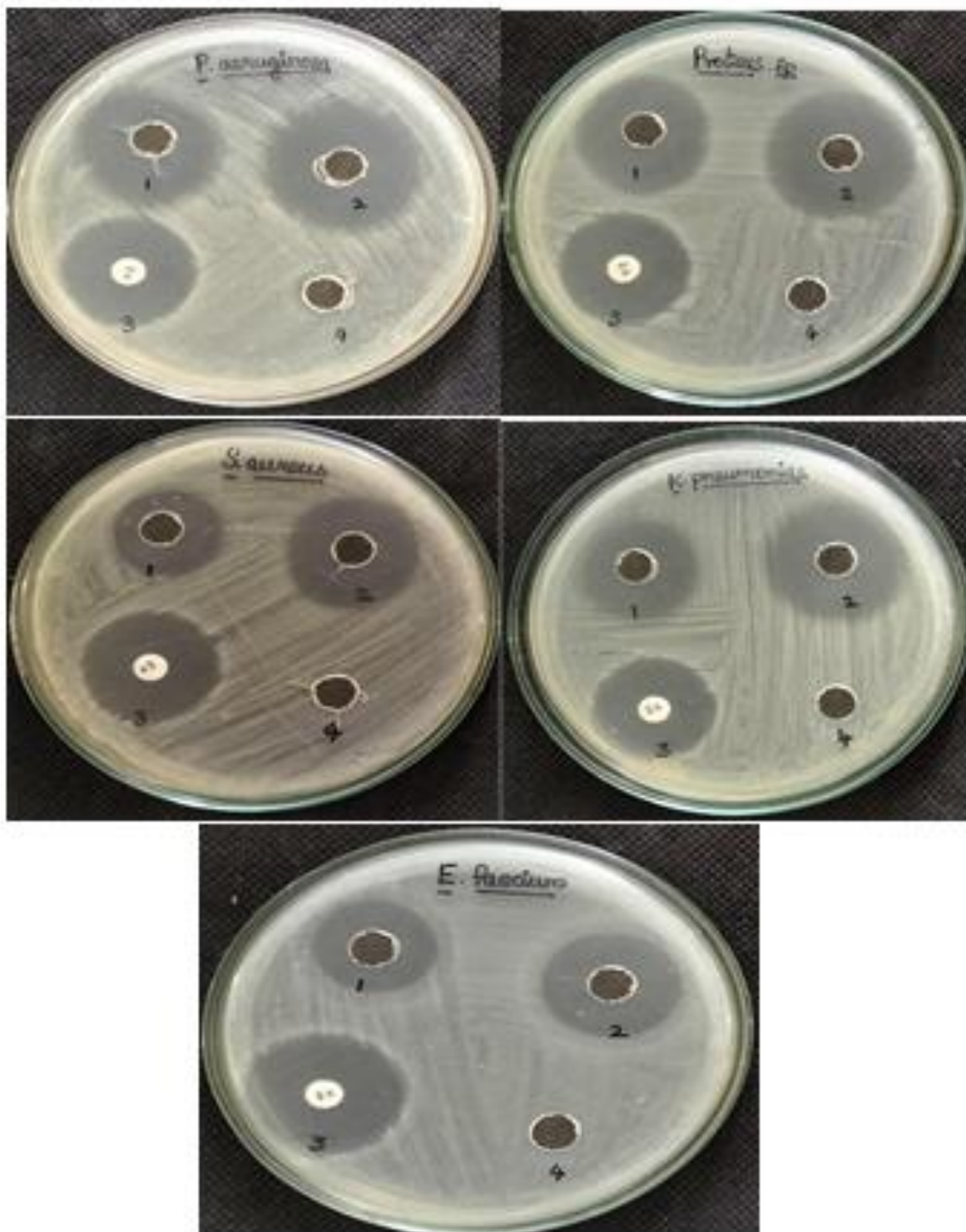


Figure 11: Zone of inhibition Ag Nps, and Ag-PNIPAM conjugates and Antibiotics with various pathogens

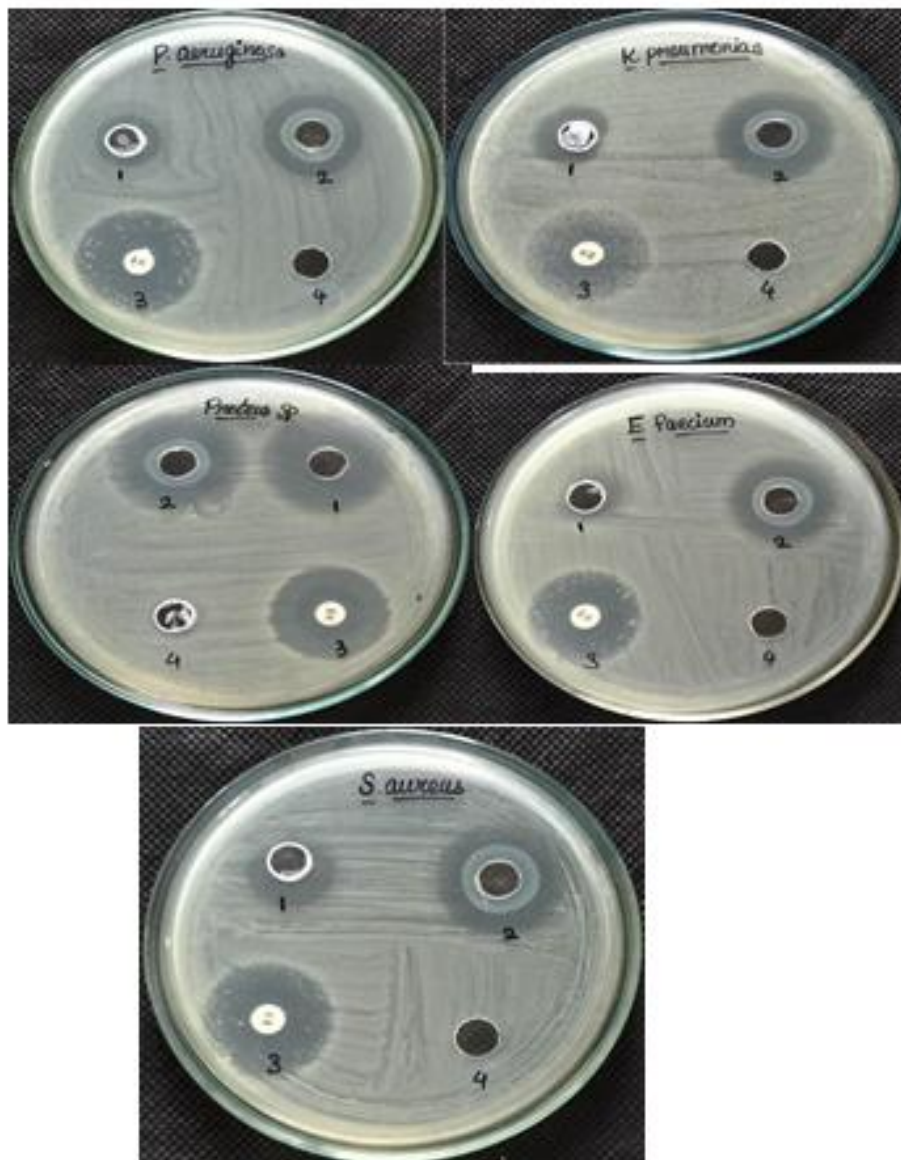


Figure 12: Zone of inhibition ZnO Nps, Zn-NIPAM conjugates and antibiotics with various pathogens. The zone of inhibition (ZOI) was observed and measured in mm, in figures

The well diffusion method was used to measure the level of antibacterial activity exhibited by the AgNps and Ag-PNIPAM composites. The inhibitory zones had a measurement of millimeters. The results for Ag Nps and Ag-PNIPAM for *Enterococcus faecium* were 11 mm and 18 mm, respectively. The results for *Staphylococcus aureus* for Ag Nps and Ag-PNIPAM were 12 mm and 19 mm, respectively. When tested against *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Proteus sp.*, the zones produced by

ZnO Nps measured approximately 10 mm, 11mm, and 11 mm, while those produced by Zn-NIPAM composites were approximately 17 mm, 17 mm, and 16 mm respectively. Figure 11: Zone of inhibition AgO Nps and Ag-NIPAM conjugates with various pathogens.

Antibacterial activity with Antibiotics

The antibacterial activity of the synthesized sample was assessed using the well diffusion method, a standard procedure in antibacterial studies. The protocol involved the preparation of Mueller Hinton Agar (MHA) by dissolving 39g of the agar in 1000ml of distilled water. The agar solution was then sterilized under autoclave at 121°C for 15 minutes to ensure sterility. Subsequently, the sterilized agar solution was poured into sterile petri plates to form a solid basal layer.

Upon solidification of the agar, 80 µL of indicator bacterial strains (at a concentration of 10^8 cfu/mL) were uniformly spread across the surface of the MHA plates using a sterile swab. The agar plates were then left to air dry for 5 minutes to allow for proper adherence of the bacterial strains.

Using a sterile cork-borer with a 6 mm diameter, wells were carefully bored into the solidified agar medium. Each well was then filled with the synthesized sample to be tested for its antibacterial activity. Additionally, standard reference antibiotics were included in separate wells as positive controls, while wells filled with Dimethyl Sulfoxide (DMSO) served as the negative control to account for any non-specific effects.

The prepared plates were subsequently incubated at 37°C for a period of 18-24 hours to allow for bacterial growth and the potential inhibition by the tested samples. After the incubation period, the plates were examined for the formation of clear zones around the wells. The presence of a clear zone indicated the antibacterial activity of the tested sample, where the inhibition of bacterial growth resulted in the formation of an area devoid of bacterial growth around the well.

This method allowed for a direct comparison of the antibacterial effectiveness of the synthesized sample with that of standard antibiotics, providing valuable insights into its potential as an antibacterial agent. The observed clear zones provided a visual representation of the sample's ability to inhibit the growth of the indicator bacterial strains, thus highlighting its promising antibacterial properties

Sl. No	Test Organisms	Inhibitory zones (mm)		Inhibitory zones (mm)
		ZnO Nps	Zn-PNIPAM	
				Amikacin
1	Enterococcus faecium	14	19	20
2	Staphylococcus aureus	12	17	16
3	Klebsiella pneumoniae	13	18	17
4	Pseudomonas aeruginosa	12	18	18
5	Proteus sp	13	18	17

Table 1: Zone of inhibition ZnO Nps, Zn-NIPAM conjugates and antibiotics (Amikacin) with various pathogens compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Sl. No	Test Organisms	Inhibitory zones (mm)		Inhibitory zones (mm)
		AgNps	Ag-PNIPAM	
				Amikacin
1	Enterococcus faecium	11	18	18
2	Staphylococcus aureus	12	19	17
3	Klebsiella pneumoniae	10	17	15
4	Pseudomonas aeruginosa	10	17	15
5	Proteus sp	11	16	14

Table2: Zone of inhibition Ag Nps, Ag-NIPAM conjugates and antibiotics (Amikacin) with various pathogens compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Sl. No	Test Organisms	Inhibitory zones (mm)	Inhibitory zones (mm)
		ZnONps Coated	Zn-PNIPAM Coated
1	Enterococcus faecium	26	31
2	Klebsiella pneumoniae	23	30
3	Staphylococcus aureus	24	32
4	Proteus sp	24	29
5	Pseudomonas aeruginosa	23	27

of the ZnO Nps and Zn-PNIPAM composites finished fabrics

Sl. No	Test Organisms	Inhibitory zones (mm)	Inhibitory zones (mm)
		Ag Nps Coated	Ag-PNIPAM Coated
1	Enterococcus faecium	25	30
2	Staphylococcus aureus	23	28
3	Klebsiella pneumoniae	22	27
4	Pseudomonas aeruginosa	23	26
5	Proteus sp	24	27

Table 4: Zone of inhibition of the Ag Nps and Ag-PNIPAM composites coated

Name of the sample	Hours				
	0	1	2	3	24
Control	100	100	100	100	100
ZnNps	100	36.9	52.7	60.3	70.8
Zn-NIPAM	100	68.6	82.4	90.6	94.9

Table 5: Percentage of photodecomposition of methylene blue with ZnNps and Zn - PNIPAM

Name of the sample	Hours				
	0	1	2	3	24
Control	100	100	100	100	100
AgNps	100	32.4	49.6	55.7	65.6
Ag-NIPAM	100	66.8	79.4	88.7	92.3

Table 6: Percentage of photodecomposition of methylene blue with AgNps and Ag- PNIPAM

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