

Development and Antibacterial Assessment of Meropenem Metallic Nanoparticles Against Multidrug Resistant Microorganisms

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ABSTRACT

Multidrug-resistant (MDR) bacterial infections have become a growing public health concern, significantly restricting the clinical use of traditional antibiotics such as meropenem, a broad-spectrum carbapenem antibiotic that is considered as last resort of drug while resistance has been reported in some bacterial strains. The study synthesized and comparatively analyzed meropenem silver (Mero-Ag), copper (Mero-Cu) and zinc (Mero-Zn) nanoparticles (NPs) as promising antimicrobial agents against MDR pathogens. The nanoparticles were synthesized by probe sonication method and characterized for organoleptic evaluation, UV-Vis spectroscopy, FTIR, XRD, FESEM and EDX to confirm the nanoparticles formation, morphology, elemental composition, and stability. The particle size, polydispersity index (PDI) and zeta potential were measured by DLS, and the colloidal stability was tracked over a six-month period under ICH Q1A(R2) conditions. The antibacterial activity was assessed against *E. coli*, *K. pneumoniae*, *S. aureus*, and *A. baumannii* including normal and resistant strains, using the agar well diffusion and resazurin microdilution MIC methods. In addition to having the largest zones of inhibition and lowest MIC values against both normal and resistant strains when compared to the copper and zinc-based counterparts, Mero-Ag NPs showed the smallest particle size, highest negative zeta potential and best colloidal stability among the three NPs. FTIR analysis confirmed that there was coordinate bond formation between drug and metal which resulted in lower crystallinity after the formation of NPs and EDX confirmed the successful incorporation of the metal. The results presented here indicate the great enhancement in the antibacterial activity and stability of meropenem in the presence of silver NPs, which makes Mero-Ag NPs a promising nanotherapeutic approach to combat antimicrobial resistance in Gram-positive and Gram-negative pathogens.

Keywords: Meropenem; Silver NPs; Multidrug-resistant bacteria; Antimicrobial resistance; NPs characterization; Zeta potential

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INTRODUCTION

Antimicrobial Resistance occurs when the microorganisms including bacteria, viruses, fungi and parasites develop such mechanisms that decrease the potency and efficacy of an antimicrobial agents which leads to continual infections, increased rate of transmission and high morbidity and mortality [1,2]. The growing prevalence of MDR infections poses a major threat to global health. Estimates from World Health Organization (WHO) indicate that, without effective interventions to restrain antimicrobial resistance, MDR infections may be associated with as many as 10 million deaths each year by 2050, emphasizing the need for timely and inventive approaches to combat this escalating challenge [3].

The spread of carbapenem resistance is a major global health problem, mainly attributed to the production of carbapenems but also to other mechanisms that include loss of porins and

overexpression of efflux pumps. These resistance mechanisms are very common in Gram-negative organisms, especially *Enterobacteriaceae*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*), and resistance is expected to lead to poor therapeutic choices, higher morbidity and mortality [4].

Meropenem an antimicrobial agent belonging to the carbapenem group that is used parenterally and has a wide spectrum of activity against Gram-positive and Gram-negative bacteria [5]. Chemically, it is (4R, 5S, 6S)-3-[(3S, 5S)-5-

(dimethylcarbamoyl)pyrrolidin-3-yl)sulfanyl 6-[(1R)-1-hydroxyethyl]-4-methyl Oxoazabicyclo [3.2.0] hept-2-ene-2-carboxylic acid and is resistant to hydrolysis by many β -lactamases. Meropenem is given for severe bacterial infections due to its broad spectrum of activity against bacteria [6]. Meropenem, like some other traditional antimicrobial agents, is a time- based drug and has a bactericidal action that suppresses bacterial cell wall synthesis [7]. The main issue with this drug is low bioavailability, as the β -lactam ring undergoes degradation at body temperature, not being stable for more than 2–3 h in the body. Continuous infusions to the patient is necessary to maintain drug concentrations above the therapeutic level throughout treatment. Therefore, nanotechnology offers a promising alternative to these problems through the use of nanocarriers such as nanoparticles (NPs) [8].

Nanoscale systems that can improve the pharmacokinetic and pharmacodynamic profiles of therapeutic agents can now be developed [9]. Nanocarriers can protect labile compounds against being prematurely degraded, increase drug solubility, prolong systemic drug circulation, and promote controlled or site-specific drug release, thereby enhancing therapeutic efficacy while minimizing adverse effects. These advantages have established NPs-based drug delivery systems as one of the major pillars of modern nanomedicine [10].

Metallic NPs have emerged as promising antimicrobial agents owing to their unique physicochemical properties, including a high surface-area-to-volume ratio, nanoscale dimensions, and enhanced interactions with microbial cells [11]. Unlike conventional antibiotics that typically target a single cellular process, metallic NPs exhibit broad-spectrum antimicrobial activity through multiple mechanisms, including disruption of the microbial cell membrane, generation of reactive oxygen species (ROS), release of metal ions, and interaction with intracellular biomolecules such as DNA and proteins. These multifaceted mechanisms enhance antibacterial efficacy while reducing the likelihood of resistance development, making metallic NPs attractive candidates for combination with conventional antibiotics to combat MDR pathogens [12]. Silver nanoparticles (AgNPs) have emerged as a promising alternative in combating MDR due to their unique antimicrobial properties. AgNPs possess multiple mechanisms of action that make them effective against a broad spectrum of pathogens, including those resistant to conventional antibiotics. They disrupt bacterial cell membranes, generate ROS that cause oxidative stress, and interfere with essential cellular functions such as DNA replication and protein synthesis. These properties enable AgNPs to target MDR pathogens effectively, offering a new avenue for treatment

Furthermore, AgNPs have demonstrated potential in enhancing the efficacy of existing antibiotics through synergistic effects, which could be pivotal in overcoming resistance [13]. Similarly, zinc nanoparticles (ZnNPs) exhibit potent antimicrobial activity through ROS generation, Zn^{2+} ion release, membrane disruption, and inhibition of biofilm formation and quorum sensing, making them effective against a wide range of pathogenic microorganisms [14]. Copper nanoparticles (CuNPs) have also shown significant antimicrobial potential against both Gram-positive and Gram-negative bacteria, as well as fungal pathogens, further highlighting the potential of metallic NPs as alternative or adjunct antimicrobial agents for combating antimicrobial resistance [15].

The combination of antibiotics with NPs has been shown to enhance antibacterial efficacy compared with conventional antibiotic therapy alone. Such combinations can reduce the development of bacterial resistance, lower the required antibiotic dose, and shorten the duration of treatment [16,17]. Although several studies have investigated antibiotic-metal NPs systems, comparative studies on meropenem-loaded AgNPs, CuNPs, and ZnNPs remain limited. Therefore, the present study aimed to synthesize, characterize, and evaluate meropenem-loaded silver (Ag), copper (Cu), and zinc (Zn) NPs and to investigate their antimicrobial activity against selected Gram-positive and Gram-negative bacterial strains.

Materials and Method

Materials

Meropenem trihydrate procured from Apitoria Pharma Pvt Ltd. Silver nitrate, Copper chloride, and Zinc acetate purchased from Loba Chemie Pvt Ltd. All solvents and reagents used were of analytical grade. The antibacterial activity was evaluated against four normal bacterial strains *E. coli* (NCIM 5662), *K. pneumoniae* (NCIM 5215), *S. aureus* (NCIM 5719), and *A. baumannii* (NCIM 5663) obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India. The resistant bacterial strains used in this study included *E. coli* (ATCC BAA 2469), *K. pneumoniae* (ATCC BAA-2146), *S. aureus* (ATCC BAA-44), and *A. baumannii* (NCTC 13304) were purchased from HiMedia Laboratories, India. All nutrient media, resazurin sodium salt and other reagents were procured from HiMedia Laboratories and were maintained under appropriate laboratory conditions.

Method

Synthesis of Meropenem metallic Nanoparticles

Meropenem-Ag NPs were synthesized by dissolving 1 mmol of AgNO_3 (42.45 mg) and 67.5 mg of polyvinyl alcohol (PVA) in 250 mL of distilled water in a clean beaker. In parallel, 2 mmol of meropenem (218.74 mg) was dissolved in an equal volume of distilled water (250 mL) in a separate

beaker. After complete dissolution of PVA in the AgNO_3 solution, the two solutions were combined under constant stirring. The resulting mixture subjected to probe sonication at 50% power for 20 minutes. Formation of Mero-AgNPs was confirmed by monitoring the characteristic colour change of the reaction mixture. The product was subsequently dried at room temperature, washed with distilled water to remove unreacted residues, and further air-dried to obtain the final solid nanoparticle preparation. Similarly, the synthesis of Mero-CuNPs and Mero-ZnNPs, using copper chloride (CuCl_2 , 33.61 mg) and zinc acetate dihydrate [$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, 54.87 mg], respectively, in place of AgNO_3 [18].

Characterization of Meropenem metallic Nanoparticles

Organoleptic characterization

The colour of the reaction mixture before and after NPs synthesis was observed visually against a clarity testing background. A distinct colour change served as a preliminary indicator of NPs formation. Similarly, pH of the drug solution and its corresponding NPs suspension was measured using a pH meter. The electrode was immersed in the sample contained in a small beaker and held in position until a stable pH reading was obtained [19].

UV Spectroscopic analysis:

A calibration curve for meropenem was prepared in distilled water at concentrations of 10, 20, 30, 40, and 50 $\mu\text{g/mL}$. For analysis, meropenem and its metal ion NPs (100 $\mu\text{g/mL}$) were dissolved in distilled water and scanned using a Shimadzu UV-1900 spectrophotometer in the wavelength range of 200–400 nm. The spectral data was used to confirm NPs formation and evaluate optical properties [19].

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify functional groups involved in NPs stabilization and to confirm drug-metal interactions. Dried samples of pure meropenem and its metallic NPs were taken as a sample. The spectra were recorded on a Bruker FTIR spectrometer in the range of $4000\text{--}400\text{ cm}^{-1}$. Shifts in characteristic absorption bands such as C=O, N-H, and O-H stretching vibrations were analyzed to determine the binding of meropenem to metal ions [6].

Field Emission Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

Field Emission Scanning Electron Microscopy (FESEM) employed to study the surface morphology of the NPs, while Energy Dispersive X-ray (EDX) spectroscopy used to confirm elemental composition. Dried NPs powder was measured on the JSM 7601F.

The sample was placed on the sample holder and the screw was screwed on slightly. After opening the door of the chamber for exchanging the specimens, the specimen holder was then inserted into the

specimen holder chuck device. The position of the arrow in the specimen chamber was noted. The EVAC button was depressed; chamber door was closed. The sample exchange rod was fully inserted with the rod in a horizontal position with the sample loaded and then fully pulled out of the rod for loading. It was confirmed that the indication bulb of the HLDR was lit in the specimen chamber. Finally, the specimen-exchange rod was completely pulled out until the end of the pipe was reached, and the sample was run. [20].

Particle Size, Polydispersity Index and Zeta Potential Determination

Dynamic Light Scattering (DLS) and zeta potential measurements were performed using a Horiba particle size analyzer to determine the hydrodynamic particle size distribution, Polydispersity Index (PDI) and colloidal stability of the synthesized NPs. Prior to measurement, the NPs were dispersed in distilled water and sonicated for 10 minutes to minimize aggregation. The average particle size and PDI values were recorded, as particle size in the nanometer range is associated with size-dependent physicochemical properties, including increased surface area and altered surface interactions, which can influence the behavior, reactivity, and performance of nanoparticles (NPs) in biomedical and pharmaceutical applications. Smaller particle sizes enhance surface area-to-volume ratio, which directly affects drug loading capacity, dissolution rate, and biological interactions. Zeta potential analysis was carried out to assess the stability of the colloidal suspension based on surface charge. A large positive or negative zeta potential value reflects strong electrostatic repulsion between particles, thereby preventing aggregation and indicating high stability. Together, particle size and zeta potential characterization provided essential insights into the physicochemical behavior of meropenem-metallic NPs, ensuring their suitability for further biological evaluation and therapeutic applications [21].

X-ray Powder Diffraction

X-ray Powder Diffraction (XRD) analysis was conducted to determine the crystalline structure of the NPs. Dried powders were mounted on glass slides and scanned using a diffractometer with $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406\text{ \AA}$) over a 2θ range of $10^\circ\text{--}80^\circ$. Sharp diffraction peaks confirmed crystalline nature, and average crystallite size was calculated using the Debye Scherrer equation. Theoretical interpretation emphasized that crystallinity influences nanoparticle stability, dissolution, and drug release kinetics, making XRD a critical tool for confirming structural integrity [22].

Antibacterial activity

Agar well diffusion (cup plate) method

The antibacterial activity of the synthesized meropenem-metallic NPs was evaluated using the

agar well diffusion (cup plate) method. Nutrient agar medium was prepared by dissolving 28 g of nutrient agar in 1000 mL of purified water, brought to a boil to ensure complete dissolution, and sterilized in an autoclave at 121 °C under 15 lbs/in² pressure for 15 minutes. The bacterial test organisms were cultured in nutrient broth overnight to achieve an appropriate colony forming unit (CFU) density. Sterilized nutrient agar was poured into sterile petri plates and allowed to solidify, after which the microbial strains uniformly spread across the surface using a sterile spreader. Wells of 6 mm diameter were then bored into the agar using a cork borer, and solutions of pure meropenem and meropenem-metallic NPs prepared in dimethyl sulfoxide (DMSO) were introduced into the wells. The plates were incubated at 37 °C for 24 hours, and antibacterial activity was assessed by measuring the diameter of the zones of inhibition (in mm) surrounding each well. The growth inhibition was compared against a positive control to determine relative efficacy. The theoretical basis of this assay lies in the diffusion of NPs through the agar medium, where larger zones of inhibition reflect stronger antibacterial activity. The method thus provided a preliminary yet reliable evaluation of the enhanced antimicrobial potential of meropenem-metallic NPs compared to pure meropenem [23].

Minimum Inhibitory Concentration Determination

The minimum inhibitory concentration (MIC) determined using resazurin dye microdilution method where the resazurin indicator interacted with an enzyme dehydrogenase in bacterial cells. The pink colour formation indicates live cells, whereas the blue colour indicates dead cells. The resazurin solution was prepared by dissolving 0.015g dye in 40 mL of sterile distilled water. Further the vortex mixer was employed to confirm the solubility and homogeneous solution formation.

A 96-well sterile plate was labelled and prepared in aseptic conditions. The test substances (Meropenem), synthesized NPs with Ag, DMSO and silver NPs pipetted into the first four rows of the plate in a volume of 50 µL with different concentrations (0.125-32) µg/ml for Meropenem; 0.03-32 µg/ml for Mero-Ag NPs; 16-64 µg/ml for Silver NPs for normal strain and 32-256 µg/ml for Meropenem; 0.25-256 µg/ml for Mero-Ag NPs; 16-128 µg/ml for Silver NPs), 50 µL of muller hinton broth were introduced to all wells and 50uL of each bacterial strain suspension (1×10^6 CFU ml⁻¹), were added separately except in negative control in the last row of the plate. The plates were made in triplicate and left in a 37 °C incubator for 18 to 24 hours. Thereafter, resazurin indicator solution (40 µL) was added to each well after incubation. The plate was further incubated for 30 min at 37 °C. Any colour changes from blue to pink or absence of colour were seen in live cells. The lowest dilution without colour change will be indicated as MIC. The MIC for the Meropenem and its synthesized metallic NPs against bacterial strains was computed as the average of three results [24].

Stability Studies

The stability studies of meropenem metallic NPs were accomplished by keeping the NPs for 6 months in two stability conditions as per ICH Q1A(R2) Guideline: Room Temperature (25±3°C) and Accelerated Stability Condition (40°C & 75%RH). The samples were continuously monitored during the storage. After the duration of 6 months, their colour, physical state, particle size, PDI, zeta potential, UV and FTIR analysis was performed [25].

Result and Discussion

Physicochemical Characterization Organoleptic Properties

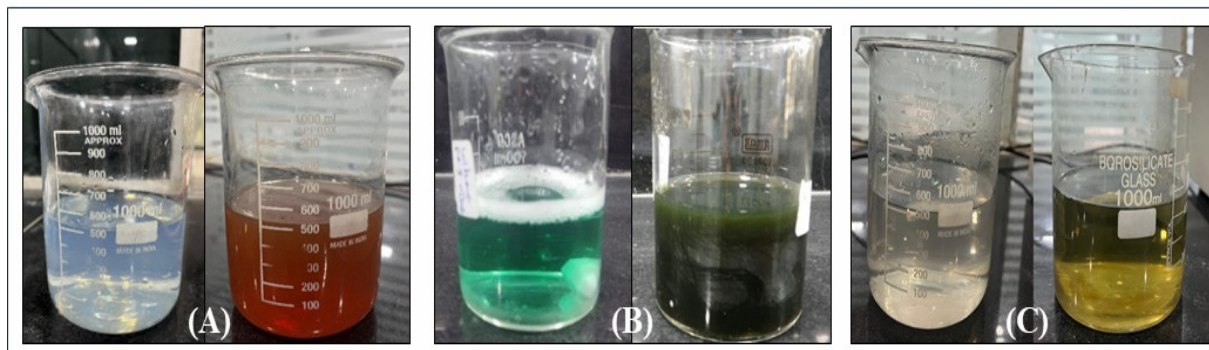
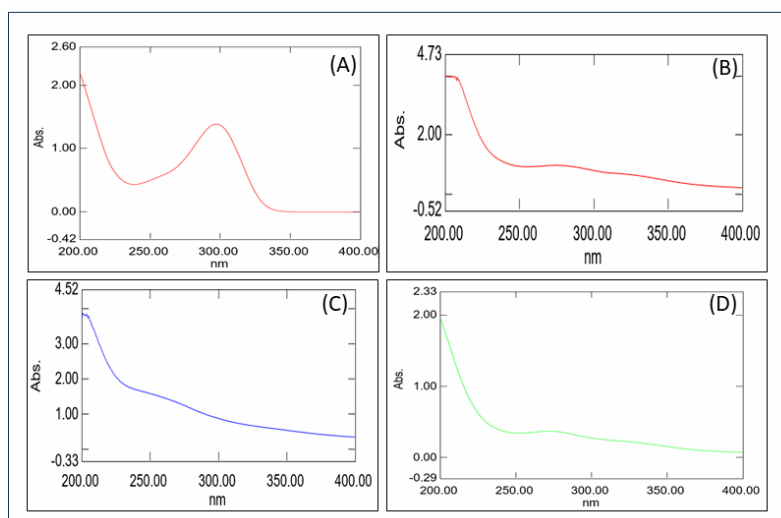


Figure 1. Colour changes before and after sonication in (A) Mero-Ag (B) Mero-Cu, and (C) Mero-Zn NPs with the pH increasing from 6.43 before synthesis to 9.75 after synthesis, and it showed a percentage yield of 72%. The Mero-Cu NPs appeared blue (Figure 1B), with the pH increasing from 5.17 to 9.37 after synthesis, and

The samples were prepared and evaluated based on their colour, pH, and percentage yield. Meropenem appeared white in colour and exhibited a pH of 7.02 before synthesis. The Mero-Ag NPs was brown in colour (Figure 1A),



it achieved the highest percentage yield of 78%. The Mero-Zn NPs was yellow in colour (Figure 1C), with the pH changing from 5.73 before synthesis to 9.36 after synthesis, and it showed a percentage yield of 62%. Change in colour,

shifting of pH towards alkaline pH indicated primary formation of meropenem metallic nanoparticles.

UV-Spectrophotometric analysis

Figure 2. UV Spectrum of (A) Meropenem, (B) Mero-Ag, (C) Mero-Cu, and (D) Mero-Zn NPs formation.

The UV-visible spectra of the meropenem and metallic NPs were recorded between 200 to 400nm. The meropenem showed an absorption maximum of 299nm as shown in Figure 2 which resembles to reference spectrum and indicate the characteristic electronic absorption property of drug. The synthesized Mero-Ag NPs, Mero-Cu NPs and Mero-Zn NPs exhibited a broad absorption peak at 299nm and decrease in absorbance in comparison of plain meropenem. The alterations in absorption intensity and spectral profile after introducing metallic components were indicated the interactions between meropenem and these metal ions upon

FTIR Spectroscopy

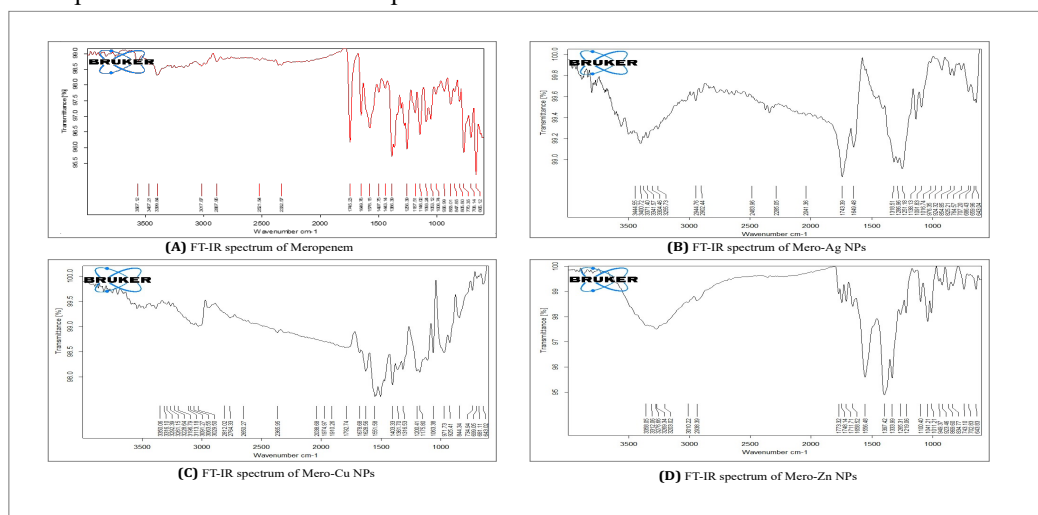


Figure 3. FTIR Spectrum of (A) Meropenem (B) Mero-Ag (C) Mero-Cu and (D) Mero-Zn NPs

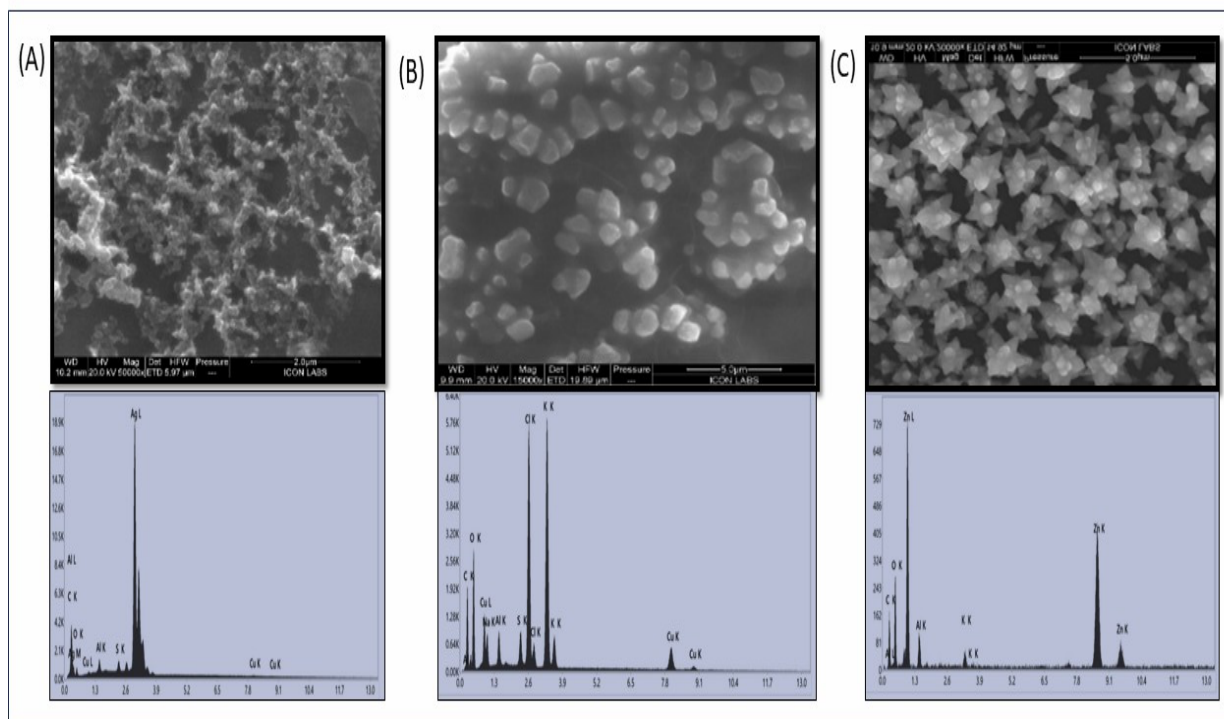
The IR spectrum of Meropenem and its metallic

NPs were analyzed within the range of 4000-

400 cm^{-1} to investigate the structure of the drug. The IR Spectrum was shown in figure 3 confirmed the characteristic functional groups of meropenem, while the synthesized Mero-Ag, Mero-Cu and Mero-Zn NPs exhibited distinct shifts in their corresponding vibrational bands. The O-H/N-H stretching band shifted from 3399.84 cm^{-1} in standard meropenem to 3371.40, 3350.06 and 3276.66 cm^{-1} in Mero-Ag, Mero-Cu and Mero-Zn NPs, respectively. The β -lactam C=O band also showed changes from 1745.23 cm^{-1} to 1743.39, 1792.74 and

changes indicated alterations in the chemical environment of meropenem functional groups and suggested their interaction with the respective metal species during nanoparticle formation. The retention of the characteristic meropenem bands further suggested preservation of its principal structural features after metal incorporation. Overall, the FTIR findings supported the successful formation of the Mero-Ag, Mero-Cu and Mero-Zn nanoparticulate systems.

Field Emission Scanning Electron



1748.14 cm^{-1} , respectively. Additional shifts were observed in the amide C=O, C-H, C-N/C-O and ring-vibration regions. These spectral

Figure 4. FESEM and EDX Analysis of (A) Mero-Ag (B) Mero-Cu and (C) Mero-Zn NPs

FESEM followed by EDX analysis was performed to investigate the morphology and elemental composition of the meropenem metallic NPs, as shown in figure 4. In FESEM of Mero-Ag (Figure 4A), the surface morphology was a rough, irregular and highly agglomerating. In EDX spectrum, a distinctive peak of Ag was observed in relation to other elements and provides evidence of Ag content in NPs at an elemental weight percentage of around 45.7%. In FESEM of Mero-Cu (Figure 4B) showed predominantly a mixed irregular, rounded shape with a more discrete distribution and some clustering. The EDX spectrum presented characteristic peaks attributed to Cu, confirming the introduction of Cu with elemental weight percentage of around 6.2%. In FESEM of micrograph of Mero-Zn (Figure 4C),

Microscopy and Energy Dispersive X-ray Spectroscopy Analysis

showing irregular and angular particles with extensive aggregation and a surface densely dispersed in Zn particles. As evident from the corresponding EDX spectrum, there was a specific Zn peak as can be denoted which

confirms reinforcing of zinc with elemental weight percentage of around 49.5%.

Particle Size, Polydispersity Index and Zeta Potential Determination

The Mero-Ag NPs showed a mean particle size of 135.7 nm, and the PDI was 0.23 which means Mero-Ag NPs have more homogeneous size distribution. The zeta potential was -21.23 mV that indicated the moderately colloidal stability. The Mero-Cu NPs had a mean particle size of 123.7 nm, PDI 0.23 and zeta potential -22.23 mV indicating uniformity in particle size distribution with moderate colloidal stability

appeared to have been achieved. The mean particle size of Mero-Zn NPs showed particle size 148.7 nm, the PDI was 0.23, and zeta potential -26.23 mV indicated smaller size with

homogeneous distribution and moderately stable nanoparticles.

X-ray Diffraction Analysis

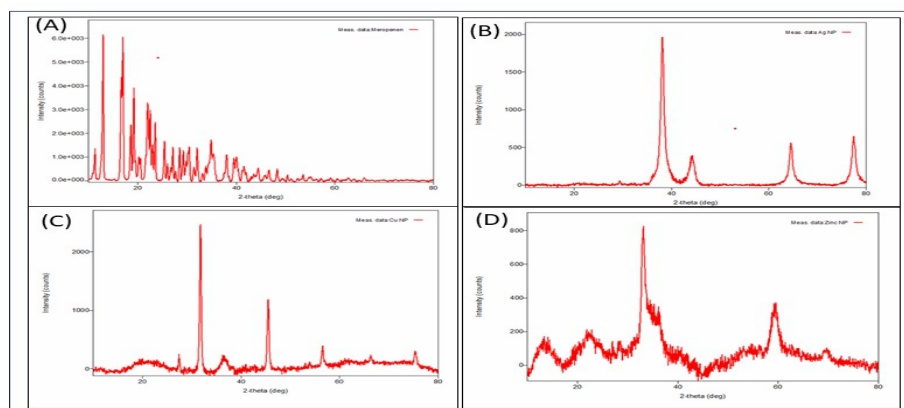


Figure 5. XRD Analysis of (A) Meropenem (B) Mero-Ag (C) Mero-Cu and (D) Mero-Zn NPs

The XRD analysis revealed distinct crystallinity patterns for meropenem and its metallic NPs (Figure 5). Pure meropenem exhibited characteristic diffraction peaks at 2θ values of 12.93° , 16.62° , 19.18° , 22.02° , 23.25° , and 35.65° , indicating its crystalline nature. Similarly, Mero-Ag NPs showed prominent peaks at 38.98° , 44.16° , 64.62° , and 77.37° , confirming that the AgNPs also possessed a crystalline structure. Mero-Cu NPs displayed diffraction peaks at 35.73° , 45.47° , 56.49° , 66.13° , and

75.45° , suggesting a crystalline nature. In contrast, Mero-Zn NPs exhibited peaks at 13.2° , 22.92° , 34.98° , 59.32° , and 63.5° , but the overall diffraction pattern indicated an amorphous structure. These findings demonstrate that the type of metal used in NPs synthesis significantly influences the crystalline properties of the resulting NPs.

Antibacterial Assessment

Agar well diffusion (cup plate) method

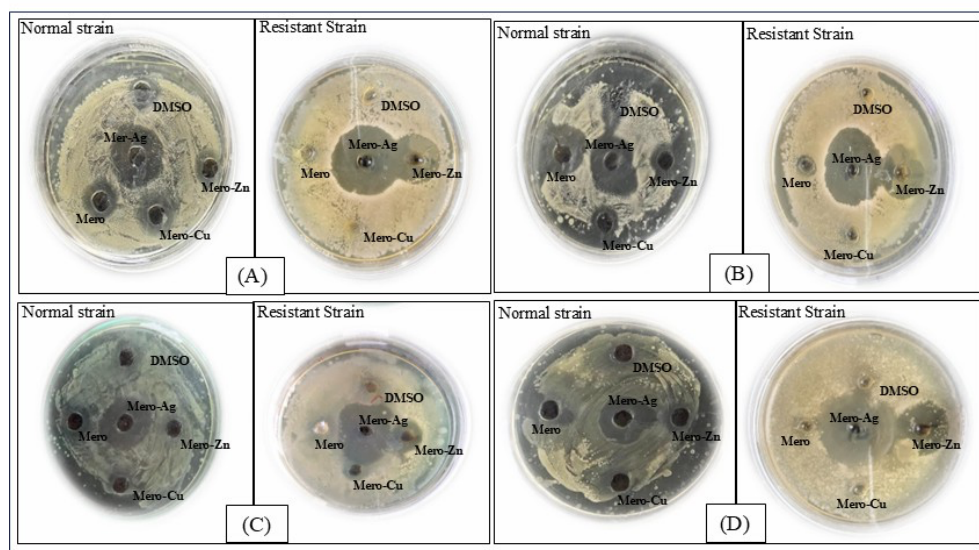


Figure 6. Zone of inhibition of Meropenem and Meropenem metallic nanoparticles against normal and resistant bacterial strains (A) *E. coli* (B) *K. pneumoniae* (C) *A. baumannii* and (D) *S. aureus*

Antibacterial activity of meropenem and metallic-NPs were determined against normal and resistant bacteria using the agar diffusion method. The respective zones of inhibition

shown to form around the NPs are shown in figure 6. The zone of inhibition of meropenem and its metallic NPs was evaluated against *E. coli*, *K. pneumoniae*, *A. baumannii*, and *S.*

aureus under normal and resistant conditions. Meropenem showed zones of inhibition of 10 and 6 mm against *E. coli*, 13 and 6 mm against *K. pneumoniae*, 10 and 6 mm against *A. baumannii*, and 10 and 6 mm against *S. aureus*, respectively. Mero-Ag exhibited higher zones of inhibition, ranging from 13–26 mm, with values of 16 and 22 mm against *E. coli*, 16 and 23 mm against *K. pneumoniae*, 18 and 24 mm against *A. baumannii*, and 13 and 26 mm against *S. aureus*. Mero-Zn showed zones of inhibition of 12 and 14 mm against *E. coli*, 10 and 16 mm against *K. pneumoniae*, 6 and 11 mm against *A. baumannii*, and 8 and 15 mm against *S. aureus*. Mero-Cu exhibited zones of inhibition of 10 and 6 mm against *E. coli*, 10 and 6 mm against *K. pneumoniae*, 8 and 8 mm against *A. baumannii*, and 6 and 6 mm against *S. aureus*. Overall, Mero-Ag demonstrated the highest antibacterial activity among the tested NPs, particularly against resistant bacterial strains. Mero-Ag NPs exhibited the highest antibacterial performance among all other NPs tested indicated by larger zones of inhibition against each of the bacterial strains, both normal and resistant. Compared to pure meropenem, Mero-Ag NPs displayed increased inhibition of Mero-Ag against normal strains. This result suggested that the NPs in the form of AgNPs was more effective for the antibacterial effect. Mero-Ag NPs were highly effective against the normal strains, while Mero-Zn and Mero-Cu NPs showed lower antibacterial activity.

Minimum Inhibitory Concentration Determination

The MIC study demonstrated a pronounced enhancement of the antibacterial activity of meropenem following its incorporation into silver nanoparticles (Mero-Ag NPs). In normal strains, the MIC of meropenem was 0.125, 0.25, 0.50 and 0.125 µg/mL against *E. coli*, *K. pneumoniae*, *A. baumannii* and *S. aureus*, respectively, which decreased to 0.06, 0.06, 0.06 and 0.03 µg/mL, respectively, with Mero-Ag NPs.

In resistant strains, meropenem exhibited substantially elevated MICs of 64 µg/mL (*E. coli*), 64 µg/mL (*K. pneumoniae*), 64 µg/mL (*A. baumannii*) and 32 µg/mL (*S. aureus*), whereas Mero-Ag NPs reduced these values to 0.50, 1, 1 and 0.25 µg/mL, respectively.

Overall, Mero-Ag NPs produced significant reduction in MIC against normal strains and resistant strains, demonstrating substantially enhanced in vitro antibacterial potency, particularly against meropenem-resistant pathogens. The improved efficacy may result from the complementary actions of meropenem-mediated inhibition of cell-wall synthesis and Ag NP-mediated membrane

disruption, oxidative stress, and interaction with essential cellular components. These findings support the potential of Mero-Ag NPs for potentiating meropenem activity against resistant bacterial pathogens.

Stability Study of Nanoparticles

The stability of the prepared metal NPs of meropenem was evaluated by comparing the characteristics of initial and after 6 months of storage under room temperature ($25 \pm 3^\circ\text{C}$) and accelerated stability conditions as per ICH Q1A(R2) guidelines. At accelerated condition, the size of Mero Ag NPs was reduced to 87 nm with a PDI of 0.28 under well-distributed conditions. The zeta potential was -18.22 mV, indicating a moderate colloidal stability. This suggests that the smaller particles formed at accelerated condition have lower colloidal stability, whereas the negative zeta potential values of room-temperature samples indicate a comparatively higher colloidal stability. The particle size of Mero-Cu NPs decreased to 64 nm with PDI and zeta potential values of 0.29 and -15.22 mV, respectively. Results showed that, under the accelerated condition, very small NPs were obtained whereas at room temperature which gave rise to larger intermediates, in part due to their more negative zeta potential leading up to greater colloidal stability. For Mero-Zn NPs, the particle size decreased to 87 nm, PDI was 0.28 and zeta potential -17.15 mV. Consequently, the accelerated condition sample yielded smaller NPs than room temperature which was positively assessed by its greater colloidal stability determined by zeta potential. No significant changes were noticed in the colour, physical state, UV-visible spectral characteristics and FTIR spectra of the NPs after 6 months when compared to their initial characteristics. The NPs retained their physical and physicochemical properties under the two storage conditions, showing good stability during the storage period.

Conclusion:

Antimicrobial resistance had reduced the effectiveness of last-resort antibiotics such as meropenem, which was limited by physiological instability, short half-life and hydrolysis of the β -lactam ring, less effective. The study was intended to overcome these drawbacks by the synthesis of meropenem loaded AgNPs, CuNPs and ZnNPs with the help of probe sonication technique employing PVA as stabilizer and focused upon their evaluation. The synthesis indicated color and pH changes after the synthesis. Interactions between meropenem and metal ions were confirmed by UV-Vis analysis and FTIR, and XRD analysis revealed that the crystallinity was reduced;

Mero-Zn had the highest degree of amorphicity. The presence of Ag, Cu and Zn was confirmed using EDX and there were observed differences in both morphology and aggregation. It was confirmed that nanometer-sized particles exist by DLS, and the highest zeta potential magnitude and colloidal stability was observed in Mero-Zn followed by Mero-Ag and Mero-Cu. Stability studies under room-temperature and accelerated conditions for 6 months also confirmed the stability of NPs. Mero-Ag NPs exhibited the highest level of antibacterial effects against the normal and resistant strains of *E. coli*, *K. pneumoniae*, *S. aureus* and *A. baumannii* with highest inhibition and lowest MIC. This increased activity might be due to a combined mechanism between AgNPs and meropenem which involves membrane disruption, generation of ROS, release of silver ions, inhibition of DNA and protein synthesis, and meropenem-mediated cell wall inhibition. In general, Mero-Ag NPs exhibited good physicochemical properties and better antibacterial activity against the bacteria than Mero-Cu and Mero-Zn. Thus study projects novel process for enhanced combination delivery of Mero-Ag, holding promise for antibacterial effect against MDR microorganisms.

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