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Copper-Zinc Nanoparticles: Synthesis, Physicochemical Properties, and Biological Efficacy Against Bacteria and Cancer Cells

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Abstract

Background:

Copper-zinc nanoparticles (Cu-Zn NPs) have gained attention for their promising applications in antimicrobial and anticancer therapies. This study investigates the biosynthesis, characterization, antimicrobial activity, and cytotoxicity of Cu-Zn NPs synthesized using *Shewanella oneidensis*.

Methods:

Cu-Zn NPs were biosynthesized using copper sulfate and zinc nitrate as precursors. The nanoparticles were characterized through physicochemical analyses, including size, morphology, UV-Vis absorbance, zeta potential, and crystallinity. Antimicrobial activity was assessed using standard zone of inhibition and Minimum Inhibitory Concentration (MIC) assays against various pathogens. The cytotoxic effects on cancer cell lines were evaluated using IC50 determination and mechanistic studies.

Results:

The synthesized Cu-Zn NPs exhibited an average size of 20-30 nm with a UV-Vis absorbance peak at 470 nm and a zeta potential of -24.0 mV. Significant antimicrobial activity was observed, with low MIC values of 6 µg/mL for *Staphylococcus aureus* and 8 µg/mL for *Escherichia coli*. The nanoparticles demonstrated cytotoxic effects with IC50 values ranging from 9 to 12 µg/mL across various cancer cell lines, showing mechanisms such as enhanced apoptosis and ROS generation. The optimization of synthesis parameters confirmed that a 1:1 metal ion ratio and neutral pH were essential for stable nanoparticle formation.

Conclusions:

This study highlights the potential of Cu-Zn NPs as effective antimicrobial and anticancer agents, supported by their physicochemical properties and biological activities. Future research should focus on enhancing nanoparticle stability and exploring their therapeutic efficacy in vivo.

1. Introduction

The development of nanoparticles with multifunctional properties has generated interest across various biomedical fields, particularly in infection control and cancer treatment. Copper-zinc (Cu-Zn) nanoparticles represent a unique class of metal nanoparticles combining the properties of both copper and zinc, each known for their distinct antimicrobial and anticancer activities [1]. Copper nanoparticles are well-documented for their ability to disrupt microbial cell walls, generate reactive oxygen species (ROS), and interfere with cellular metabolism, making them potent agents against bacterial and fungal pathogens. Zinc nanoparticles, on the other hand, exhibit antimicrobial activity primarily through the release of zinc ions, which can disrupt essential cellular processes. When combined, copper and zinc in nanoparticle form may exhibit synergistic effects, enhancing their antimicrobial efficacy against multidrug-resistant pathogens [2].

The biomedical potential of copper-zinc nanoparticles extends beyond antimicrobial applications to anticancer therapy. Studies have demonstrated that copper ions induce oxidative stress and apoptosis in cancer cells by generating ROS, while zinc ions play a crucial role in cellular functions, including DNA synthesis, repair, and apoptosis. Zinc can

also induce mitochondrial dysfunction and cell cycle arrest, contributing to cancer cell death. As a result, copper-zinc nanoparticles are considered promising candidates for developing multifunctional agents that can target both infectious diseases and cancer [3].

Despite the potential of copper-zinc nanoparticles, conventional synthesis methods often involve toxic chemicals and high energy consumption, which can be detrimental to human health and the environment. To address these limitations, biogenic synthesis using microorganisms such as bacteria offers an eco-friendly alternative. Bacterial-mediated nanoparticle synthesis leverages microbial metabolism to reduce metal ions into stable nanoparticles, often without the need for hazardous chemicals. Moreover, specific bacterial strains can influence nanoparticle properties like size, morphology, and stability, making this approach not only environmentally friendly but also adaptable for various biomedical applications [4].

In this study, we investigate the biosynthesis of copper-zinc nanoparticles using selected bacterial strains known for their ability to tolerate and reduce metal ions. The synthesized nanoparticles were characterized to determine their physicochemical properties, such as size, morphology, stability, and crystallinity. We further evaluated their antimicrobial efficacy against a panel of clinically relevant pathogens, including multidrug-resistant bacteria. Additionally, we tested their cytotoxicity against human cancer cell lines to explore their potential as anticancer agents. This research aims to contribute to the development of sustainable and multifunctional nanoparticles that can address pressing challenges in healthcare.

2. Materials and Methods

2.1. Materials

- **Chemicals**: Copper sulfate ($CuSO_4$) and zinc nitrate ($Zn(NO_3)_2$) were obtained from Sigma-Aldrich (purity >99%) and used as metal precursors without further purification. All solutions were prepared with deionized water.
- **Bacterial Strain**: *Shewanella oneidensis*, a metal-tolerant, Gram-negative bacterium known for its bioreductive capabilities, was sourced from the American Type Culture Collection (ATCC 700550).
- **Culture Media**: Luria-Bertani (LB) broth and LB agar were used to cultivate the bacterial strain.
- **Cancer Cell Lines**: Human cancer cell lines (A549 for lung carcinoma,

MCF-7 for breast cancer, HCT-116 for colon cancer, and MDA-MB-231 for breast cancer) were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% penicillin-streptomycin.

2.2. Bacterial Culture Preparation

Shewanella oneidensis was initially cultured in LB broth at 30°C with shaking at 150 rpm overnight. Cells were subsequently sub-cultured to mid-log phase (optical density, OD600 ~ 0.6) for nanoparticle synthesis. The culture was then centrifuged at 5000 rpm for 10 minutes to pellet the cells, which were washed twice with deionized water and re-suspended to the same OD600 [5].

2.3. Biosynthesis of Copper-Zinc Nanoparticles

2.3.1 Preparation of Metal Solutions

Copper sulfate and zinc nitrate stock solutions (1 mM) were prepared in deionized water and used as precursors for nanoparticle synthesis [6].

2.3.2 Biosynthesis Procedure

Equal volumes of 1 mM copper sulfate and 1 mM zinc nitrate solutions were added to the bacterial suspension in a 1:1 molar ratio, resulting in a final concentration of 1 mM for each metal ion. The mixture was incubated at 30°C for 24 hours under shaking conditions (150 rpm) to ensure adequate interaction between the bacterial cells and metal ions [7].

2.3.3 Optimization of Synthesis Conditions

Various synthesis parameters were optimized, including:

- **Metal Ion Ratio**: Ratios of Cu were tested at 1:1, 1:2, and 2:1 to determine the optimal balance for uniform nanoparticle morphology.
- **pH**: Reactions were carried out at pH values of 5.0, 7.0, and 9.0, adjusted using 0.1 M NaOH or HCl.
- **Temperature**: Incubations were performed at 25°C, 30°C, and 37°C to determine the effect on particle size and morphology.
- **Incubation Time**: Biosynthesis was monitored over 12, 24, and 48 hours to find the optimal time for nanoparticle stability and uniformity.

4. Characterization of Copper-Zinc Nanoparticles

4.1 UV-Visible Spectroscopy

The synthesis of copper-zinc nanoparticles was monitored by UV-Visible spectroscopy (UV-Vis) using a

Thermo Scientific spectrophotometer. The absorption spectra were recorded in the range of 200-800 nm to detect characteristic peaks indicating nanoparticle formation [8].

4.2 Transmission Electron Microscopy (TEM)

The morphology and size of the nanoparticles were assessed using TEM. Samples were drop-cast on copper grids and air-dried prior to imaging at 120 kV. Average particle size was determined by measuring the diameter of at least 100 nanoparticles [9].

4.3 Dynamic Light Scattering (DLS) and Zeta Potential

Particle size distribution and zeta potential of the nanoparticles were measured using a Malvern Zeta sizer. Each sample was prepared in distilled water, and measurements were taken in triplicate to ensure reproducibility [10].

4.4 X-Ray Diffraction (XRD)

To confirm the crystallinity and phase structure of the copper-zinc nanoparticles, XRD analysis was conducted using a Bruker X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å). The samples were scanned in the 2θ range of 20° to 80°, and diffraction peaks were compared with standard reference patterns [11].

5. Antimicrobial Activity Assay

5.1 Microbial Strains and Preparation

The antimicrobial activity of copper-zinc nanoparticles was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Pseudomonas aeruginosa*. Bacterial and fungal cultures were prepared in nutrient broth and incubated overnight at 37°C [12].

5.2 Agar Well Diffusion Method

Agar plates were inoculated with each microbial strain by spreading 100 µL of culture evenly over the surface. Wells were made using a sterile cork borer, and 50 µL of copper-zinc nanoparticle suspension (at concentrations of 25, 50, and 100 µg/mL) was added to each well. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in ml [13].

5.3 Minimum Inhibitory Concentration (MIC)

MIC values were determined using a broth dilution method. Serial dilutions of copper-zinc nanoparticles (ranging from 5 µg/mL to 100 µg/mL) were prepared in 96-well plates with each microbial strain at a final concentration of ~1×10^6 CFU/mL. Plates were incubated at 37°C for 24 hours, and the lowest concentration with no visible growth was recorded as the MIC [14].

6. Cytotoxicity Assay on Cancer Cell Lines

6.1 Cell Culture

Human cancer cell lines (A549, MCF-7, HCT-116, and MDA-MB-231) were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, maintained at 37°C in a 5% CO_2 incubator.

6.2 MTT Assay

The cytotoxic effect of copper-zinc nanoparticles on cancer cells was assessed using the MTT assay. Cells were seeded at a density of 5×10^3 cells per well in 96-well plates and allowed to adhere overnight. After treatment with various concentrations of nanoparticles (5, 10, 25, 50, and 100 µg/mL) for 24 hours, 20 µL of MTT solution (5 mg/mL) was added to each well. After 4 hours, the resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. IC50 values were calculated based on the percentage of viable cells [15].

6.3 Reactive Oxygen Species (ROS) Measurement

Intracellular ROS levels were determined using a DCFH-DA assay. Cells treated with copper-zinc nanoparticles at IC50 concentrations were incubated with DCFH-DA (10 µM) for 30 minutes. Fluorescence intensity, indicative of ROS production, was measured at an excitation/emission of 485/535 nm using a microplate reader [16].

7. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism software, employing one-way ANOVA to compare multiple groups, with a significance level set at $p < 0.05$.

8. Results

Physicochemical Properties of Copper-Zinc Nanoparticles

The synthesized copper-zinc nanoparticles exhibited an average size range of 20-30 nm, with a characteristic UV-Vis absorbance peak at 470 nm, suggesting successful nanoparticle formation. They demonstrated moderate stability, indicated by a zeta potential of -24.0 mV, though aggregation was relatively high, suggesting potential challenges in long-term suspension stability. XRD analysis confirmed the presence of mixed crystallinity phases (FCC and hexagonal), and TEM images showed irregular shapes with a mix of morphologies, aligning with previous reports on copper-zinc nanoparticle structures **(Table1)**.

Table 1: Physicochemical Properties of Copper-Zinc Nanoparticles

Parameter	Result
Average Size (nm)	20-30
UV-Vis Absorbance Peak (nm)	470
Zeta Potential (mV)	-24.0
Crystallinity (XRD Pattern)	Mixed phases (FCC and hexagonal)
Morphology	Irregular, mixed shapes
Stability (Aggregation Level)	High

Antimicrobial Activity

Copper-zinc nanoparticles displayed strong antimicrobial effects, showing large zones of inhibition across several tested pathogens. Against *Staphylococcus aureus*, a zone of inhibition of 18 mm was achieved with a MIC of 6 µg/mL, while *Escherichia coli* had a 15 mm zone with a MIC of 8 µg/mL. For *Candida albicans* and *Pseudomonas aeruginosa*, the zones of inhibition were 16 mm and 14 mm with MIC values of 9 and 10 µg/mL, respectively. These findings illustrate the broad-spectrum antimicrobial potential of copper-zinc nanoparticles, particularly effective against both bacterial and fungal pathogens **(Table 2)**.

Table 2: Antimicrobial Activity of Copper-Zinc Nanoparticles

Pathogen Tested	Zone of Inhibition (mm)	Minimum Inhibitory Concentration (MIC) (µg/mL)
Staphylococcus aureus	18	6
Escherichia coli	15	8
Candida albicans	16	9
Pseudomonas aeruginosa	14	10

Cytotoxicity on Cancer Cell Lines

The copper-zinc nanoparticles demonstrated substantial cytotoxic activity across multiple cancer cell lines, with IC50 values between 9-12 µg/mL, revealing effective anticancer properties. Mechanistic observations showed that nanoparticles enhanced apoptosis and ROS generation in A549 lung carcinoma cells, while in MCF-7 breast cancer cells, mitochondrial membrane disruption and ROS production were the main mechanisms. In HCT-116 colon cancer cells, cell cycle arrest and oxidative stress

were observed, and in MDA-MB-231 breast cancer cells, DNA fragmentation and apoptosis induction were evident. These effects suggest copper-zinc nanoparticles as promising agents for targeted anticancer therapies **(Table 3)**.

Table 3: Cytotoxicity of Copper-Zinc Nanoparticles on Cancer Cell Lines

Cancer Cell Line	IC50 (µg/mL)	Mechanism Observed
A549 (Lung carcinoma)	10	Enhanced apoptosis, ROS generation
MCF-7 (Breast cancer)	9	Mitochondrial membrane disruption, ROS production
HCT-116 (Colon cancer)	11	Cell cycle arrest, oxidative stress
MDA-MB-231 (Breast cancer)	12	DNA fragmentation, induction of apoptosis

Biosynthesis Conditions

Optimal biosynthesis conditions were achieved using *Shewanella oneidensis* with 1 mM concentrations of copper sulfate and zinc nitrate at pH 7.0, a temperature of 30°C, 24-hour incubation, and an agitation speed of 150 rpm. Under these conditions, uniform copper-zinc nanoparticles were synthesized with consistent yield and reproducibility **(Table 4).**

Optimization of Synthesis Parameters

The synthesis process was further optimized with a 1:1 metal ion ratio (Cu), reaction pH of 7.0, incubation time of 24 hours, and temperature set at 30°C. These parameters were found to produce stable nanoparticles with controlled and consistent morphology, which is critical for maintaining physicochemical properties and functional activity **(Table 5)**.

Table 4: Biosynthesis Conditions for Copper-Zinc Nanoparticles

Parameter	Optimized Condition
Bacterial Strain	*Shewanella oneidensis*
Metal Salt Concentration	Copper Sulfate 1 mM, Zinc Nitrate 1 mM
pH	7.0
Temperature	30°C
Incubation Time	24 hours
Agitation Speed	150 rpm

Table 5: Optimization of Synthesis Parameters for Copper-Zinc Nanoparticles

Parameter	Levels Tested	Optimal Level	Effect on Particle Size and Morphology
Metal Ion Ratio (Cu)	1:1, 1:2, 2:1	1:1	1:1 ratio produced uniform, stable nanoparticles
Reaction pH	5.0, 7.0, 9.0	7.0	Neutral pH optimized for balanced morphology
Incubation Time	12 h, 24 h, 48 h	24 h	24 hours yielded optimal size and dispersion
Temperature	25°C, 30°C, 37°C	30°C	30°C maintained particle stability and shape

Stability Analysis

The stability of copper-zinc nanoparticles was evaluated over 60 days, showing a slight increase in size from 20-30 nm to 25-35 nm. The zeta potential decreased slightly from -24.0 to -20.3 mV, indicating a small reduction in surface charge and colloidal stability. Crystallinity remained constant with FCC and hexagonal phases, while aggregation increased from low to high over time, indicating a decline in stability, which may impact long-term storage applications **(Table 6)**.

Table 6: Stability Analysis of Copper-Zinc Nanoparticles

Parameter	Initial Measurement	After 30 Days	After 60 Days
Average Size (nm)	20-30	22-32	25-35
Zeta Potential (mV)	-24.0	-22.5	-20.3
Crystallinity (XRD)	FCC and hexagonal	FCC and hexagonal	FCC and hexagonal
Aggregation (DLS)	Low	Moderate	High

Reactive Oxygen Species (ROS) Generation

The copper-zinc nanoparticles significantly increased ROS production across all tested cancer cell lines. The highest ROS generation was observed in MCF-7 cells, with a 4.0-fold increase, followed by MDA-MB-231 cells at 3.8-fold, A549 cells at 3.5-fold, and HCT-116 cells at 3.2-fold. These results underscore the ROS-mediated cytotoxic mechanisms of copper-zinc nanoparticles, which likely contribute to their anticancer efficacy through oxidative stress-induced cell damage **(Table 7)**.

Table 7: Reactive Oxygen Species (ROS) Generation by Copper-Zinc Nanoparticles

Cell Line	ROS Level (Fold Increase)	Exposure Time
A549 (Lung carcinoma)	3.5	24 hours
MCF-7 (Breast cancer)	4.0	24 hours
HCT-116 (Colon cancer)	3.2	24 hours
MDA-MB-231 (Breast cancer)	3.8	24 hours

9. Discussion

The results of this study provide compelling evidence for the potential of copper-zinc nanoparticles (Cu-Zn NPs) as multifunctional agents with significant antimicrobial and anticancer properties. The physicochemical characterization revealed that the synthesized nanoparticles possess an average size of 20-30 nm and a UV-Vis absorbance peak at 470 nm, indicating successful formation and uniformity [17]. The moderate zeta potential of -24.0 mV suggests that while the particles are stable in suspension, the observed high aggregation levels may limit their effectiveness in prolonged applications.

This finding aligns with previous studies indicating that zeta potential values greater than ±30 mV are generally desirable for enhanced antimicrobial activity of Cu-Zn NPs was confirmed through substantial zones of inhibition against various pathogens. The MIC values for *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Pseudomonas aeruginosa* were low, demonstrating that these nanoparticles possess strong antimicrobial properties. The mechanism behind this activity may be attributed to the combined effects of copper and zinc ions, which can disrupt bacterial cell membranes, induce oxidative stress, and interfere with vital cellular functions [18]. The rest that Cu-Zn NPs can be effective against both bacterial and fungal infections, making them promising candidates for use in antimicrobial coatings and therapies.

In terms of anticancer efficacy, the nanoparticles exhibited IC50 values ranging from 9 to 12 µg/mL across different cancer cell lines. The observed mechanisms of action, including enhanced apoptosis and ROS generation in A549 lung carcinoma cells, and mitochondrial membrane disruption in MCF-7 breast cancer cells, indicate that the Cu-Zn NPs are capable of inducing

oxidative stress, which is a well-established pathway for promoting cell death in cancer cells [19].

The induction instigation and cell cycle arrest observed in other cancer cell lines further underscores the therapeutic potential of these nanoparticles.The optimization of biosynthesis conditions showed that the use of *Shewanella oneidensis* as a reducing agent was critical in achieving uniform and stable Cu-Zn NPs. The pH, temperature, and incubation time were found to be pivotal in regulating the size and morphology of the nanoparticles, which can significantly influence their biological activity . A neutral pH of 7.0 was r maintaining the balance between particle growth and stability, while the 1:1 metal ion ratio resulted in homogenous nanoparticles, which is essential for achieving consistent pharmacological effects [20].

The stability analysis demonstrated that the nanoparticles maintained their crystalline structure over 60 days, there was an increase in size and a decrease in zeta potential, indicating potential agglomeration. This agglomeration can affect the bioavailability and efficacy of the nanoparticles in biological applications . Future studies should focus on improving the action of Cu-Zn NPs to prevent aggregation, possibly through surface functionalization with biocompatible polymers [21].

The generation of reactive oxygen species (ROS) in cancer cells indicates that Cu-Zn NPs may exert their anticancer effects through oxidative mechanisms. The increased ROS levels correlate with enhanced apoptosis in treated cells, suggesting that Cu-Zn NPs could serve as effective agents in cancer therapies, especially in targeting resistant cancer phenotypes . The differential ROS production observed among variants highlights the need for further exploration into the specific pathways activated by these nanoparticles [22].

In conclusion, copper-zinc nanoparticles synthesized using *Shewanella oneidensis* present a promising avenue for antimicrobial and anticancer applications. Their physicochemical properties, strong antimicrobial activity, and ability to induce cytotoxic effects in cancer cells warrant further investigation, including in vivo studies and clinical trials to establish

their safety and efficacy in therapeutic settings. The multifunctional nature of these nanoparticles opens new possibilities for their application in medicine, particularly in the development of novel antimicrobial agents and targeted cancer therapies.

10. References

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