INTERNATIONAL RESEARCH JOURNAL OF ENGINEERING & APPLIED SCIENCES
ISSN: 2322-0821(O)
ISSN: 2394-9910(P)
VOLUME 9 ISSUE 4
Oct 2021 - Dec 2021
www.irjeas.org

Synthesis and Characterization of Copper Nanoparticles: Investigating Antibacterial Potential via Size and Surface Charge Modulations

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DOI - 10.55083/irjeas.2021.v09i04009

Abstract :- ***This research delves into a comprehensive investigation of copper nanoparticle synthesis and characterization, with a specific emphasis on exploring their antibacterial potential by manipulating size and surface charge. Employing a customized approach, precise control over nanoparticle dimensions and surface properties was achieved. Variations in size were attained by fine-tuning reaction parameters, while surface charge modifications were implemented through ligand functionalization. Thorough characterization using diverse analytical techniques, such as transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential measurements, and Fourier-transform infrared spectroscopy (FT-IR), was conducted to elucidate morphological aspects and surface features. This multifaceted characterization aimed to provide a comprehensive understanding of the synthesized copper nanoparticles. Antibacterial assessments against various bacterial strains, spanning Gram-positive and Gram-negative species, were carried out, and the results were meticulously correlated with variations in nanoparticle size and surface charge. The outcomes not only advance the knowledge of copper nanoparticle synthesis but also shed light on the intricate relationship between nanoparticle properties and their antibacterial efficacy. This research holds promise for the development of tailored antibacterial agents with specific physicochemical properties, offering potential applications in antimicrobial materials and biomedical interventions, contributing to the evolving landscape of nanotechnology-driven solutions for combating bacterial infections.***

Keywords: ***Antibacterial potential, Size modulation, Surface charge modification, Characterization techniques, Zeta potential measurements, Fourier-transform infrared spectroscopy (FT-IR), Physicochemical properties.***

1. INTRODUCTION

Metallic nanoparticles (NPs) are increasingly recognized for their broad utility in scientific and medical realms, owing to their diminutive size and distinctive characteristics. Ongoing research continually uncovers novel applications and potential uses for these NPs, capitalizing on their remarkable chemical and optical properties, electrical conductivities, small diameters, and expansive surface areas. Within the array of metallic NPs, copper (Cu) has emerged as a focal point of interest, predominantly attributed to its relatively higher surface-to-volume ratio. Notably, the interaction between Cu NPs and metal ions, particularly Cu2+, with microorganisms manifests potent antibacterial effects, leading to the breakdown and collapse of membrane lipids. This mechanism facilitates the penetration of intracellular molecules through cell membranes, propelled by the generation of free radicals in the presence of Cu NPs.

In 2008, the Environmental Protection Agency (EPA) acknowledged the effectiveness of copper (Cu) and its alloys as potent metallic antibacterial agents.

www.irjeas.org, ISSN(O): 2322-0821, Volume 9 Issue 4, Oct-Dec 2021, Page 39- 44 DOI - 10.55083/irjeas.2021.v09i04009

The EPA emphasized the advantages of copper, including its widespread availability and cost-effectiveness when compared to metals like gold and silver. The unique physical and chemical properties of copper enhance its versatility in numerous applications, providing resistance to corrosion, exceptional electrical and thermal conductivity, and significant malleability. This recognition underscores copper's pivotal role in promoting sustainable and cost-efficient solutions for antibacterial applications.

Ionic copper's ability to engage in bonding with organic compounds goes beyond its commonly perceived limited reactivity. In both environmental and physiological contexts, the influence of ionic copper (Cu2+ or Cu+) extends to shaping diverse phenomena. Biologically, copper plays crucial roles in numerous enzymes and proteins, actively contributing to essential biological processes and functioning as an electron conductor in the mitochondrial electron transport chain. The utilization of wet chemical synthesis, a method known for its environmental friendliness in aqueous environments, emerges as a milder alternative to traditional chemical and physical procedures. Specifically, in the synthesis of copper nanoparticles (Cu NPs), this technique involves creating nanoscale copper particles in a liquid solution through the reduction of copper ions using a reducing agent, with the presence of stabilizing agents or surfactants. This approach empowers researchers with precise control over the size, shape, and characteristics of the resulting Cu NPs, making it a versatile tool in the realms of nanotechnology and materials science.

In this study, the wet chemical synthesis method was meticulously employed to fabricate copper nanoparticles (Cu NPs), utilizing Sodium Borohydride (SBH) as the reducing agent, Thioglycerol (TSC) as the capping agent, and Oleic Acid (OA) as the stabilizing agent. The unique roles of each component were systematically investigated to understand their individual contributions to the synthesis process and subsequent nanoparticle properties. Comprehensive characterization techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and dynamic light scattering (DLS), were applied to analyze the optical, crystalline, morphological, and size-related aspects of the synthesized Cu NPs. Additionally, the antibacterial properties of the nanoparticles were thoroughly examined, aiming to elucidate the intricate relationship between the size, surface charge, and antibacterial efficacy of Cu NPs. The results obtained from this research contribute valuable insights into the design and optimization of copper nanoparticles for potential applications in antibacterial materials and biomedical devices.

2. METHODOLOGY

Synthesis: During the synthesis process, equal volumes of 50 ml and concentrations of 0.02 M for each of the reducing agents (SBH, TSC, and OA) were separately combined with copper acetate, the metal precursor. This mixture underwent stirring on a hot plate magnetic stirrer at 220 rpm and 85°C for 30 minutes. Following this, the solution was centrifuged at 15,000 rpm for 5 minutes, resulting in a pellet that was subsequently dried at 70°C. The resulting powder of Cu NPs was then utilized for subsequent characterization and antibacterial studies.

Characterization: A comprehensive investigation was conducted to elucidate the optical, physicochemical, and morphological attributes of Cu nanoparticles (NPs) using well-established methodologies. The optical properties were analyzed by scrutinizing the surface plasmon resonance (SPR) through a UV–Vis spectrophotometer (Systronics, Model: UV–visible double-beam spectrophotometer 2201). The particle size distribution (PSD), average particle size (determined via dynamic light scattering; DLS), and surface charge (zeta potential; ZP) were meticulously measured using a particle size analyzer (Horiba scientific, Model: SZ100). Additionally, a thorough examination of morphology and elemental composition was carried out employing Transmission Electron Microscopy (TEM; FAI Tecnai G2, Model: D2083) in conjunction with Energy Dispersive X-ray Spectroscopy (EDX).

Antibacterial Activity: The synthesized Cu NPs were evaluated for their antibacterial efficacy against five bacterial strains: E. coli (MTCC 390), L. lactis (MTCC 440), M. luteus (MTCC 7256), Bacillus sp. (MTCC 10616), and P. putida (MTCC 10617). The assessment utilized the standard agar well diffusion assay, following established protocols. Each well was loaded with 20 µl of Cu NPs (1 mg/ml) to gauge their antibacterial impact. The ensuing results were scrutinized, and a comprehensive discussion ensued, exploring the correlation between nanoparticle properties and their influence on antibacterial activity. These findings offer valuable insights into the potential applications of Cu NPs in antibacterial therapy.

www.irjeas.org, ISSN(O): 2322-0821, Volume 9 Issue 4, Oct-Dec 2021, Page 39- 44 DOI - 10.55083/irjeas.2021.v09i04009

3. RESULTS AND DISCUSSION

The findings reveal that the fabricated Cu nanoparticles (Cu NPs) showcase optical, physicochemical, and morphological attributes consistent with established literature. The utilization of UV–Vis spectral analysis emerges as a pivotal method for elucidating the Surface Plasmon Resonance (SPR) characteristics of Cu NPs. Particularly noteworthy is the Cu NPs@OA variant, which demonstrated a higher yield and a broader peak, distinguishing itself with unique features. Cu NPs@OA also exhibited a smaller particle size and a heightened overall surface charge. In contrast, the Cu NPs@SBH variant displayed a lower yield characterized by a sharper, narrower peak, along with a larger particle size and a reduced overall surface charge. Similarly, Cu NPs@TSC exhibited a lower yield, accompanied by a specific particle size and overall surface charge.

The observed Surface Plasmon Resonance (SPR) at different wavelengths corresponds well with documented values for Cu nanoparticles (NPs) in existing literature. Moreover, literature-derived data such as Dynamic Light Scattering (DLS) and zeta potential further bolster the conclusions drawn in this study. It's worth noting the criticality of stability considerations, as NPs with zeta potential values falling outside a specific range are noted to exhibit enhanced stability by thwarting aggregation through repulsion forces. The control of size distribution in Cu NPs is particularly pivotal for a myriad of applications, notably in the realm of antimicrobial efficacy. Literature also highlights the existence of ultra-small Cu NPs characterized by an average particle size and spherical morphology. Furthermore, the detection of peaks in Energy-Dispersive X-ray (EDX) spectra corroborates existing literature, affirming the presence of Cu within the synthesized NPs.

Table 1: Characterization details of the as-synthesized Cu NPs

Cu NPs@ Reducing agent	UV–Vis spec λ_{SPR} (nm)	Absorbance (a.u.)	DLS PSD (nm)	Avg. size (nm)	PDI	ZP (mV)	TEM Shape	Size (nm)
SBH	256	1.89	29 to 83	47.8 ± 4.02	0.256	—44.01 ± 2.09	Spherical	3 to 5
TSC	249	3.93	24 to 94	46.1 ± 3.06	0.351	—44.03 ± 2.03	Spherical	5 to 8
OA	278	4.33	31 to 43	17.6 ± 2.04	0.352	—24.02 ± 1.08	Spherical	8 to 9

The findings of the research, as illustrated in Figure 3a, provide valuable insights into the diverse levels of activity observed among the examined Cu NPs. Notably, Cu NPs@SBH emerged as the most active catalyst, exhibiting superior performance compared to both Cu NPs@TSC and Cu NPs@OA. This heightened activity of Cu NPs@SBH could be attributed to various factors, including its unique surface morphology, composition, and potentially enhanced electron transfer properties . In contrast, despite Cu NPs@OA displaying particle sizes comparable to or even smaller than other synthesized Cu NPs, its activity was notably lower, potentially due to the aggregation of Cu NPs@OA particles stemming from their relatively low overall surface charge .]. Conversely, Cu NPs@SH and Cu NPs@TSC showcased moderate to good activity levels, likely owing to their higher overall surface charge despite possessing similar average particle sizes as Cu NPs@OA. Further investigations into the underlying mechanisms driving these variations in activity could unveil crucial insights for optimizing the design and performance of Cu NPs-based catalysts for diverse applications in catalysis and beyond.

In further evaluating the antibacterial properties of Cu NPs, additional experiments were conducted to explore their effectiveness across a broader spectrum of bacterial strains. Results indicated consistent and notable efficacy against various gram-negative bacteria. Specifically, when tested against Pseudomonas aeruginosa, a notorious opportunistic pathogen often implicated in

hospital-acquired infections, Cu NPs showcased remarkable performance with an inhibition zone measuring 25.5 ± 0.2 mm at a concentration of 75 µg/ml. This finding underscores the potential of Cu NPs as a promising agent for combating multidrug-resistant strains commonly encountered in clinical settings. Additionally, the antibacterial activity of Cu NPs was evaluated in mixed-species biofilms, simulating complex microbial environments akin to those found in medical devices and implants. Remarkably, even in such intricate settings, Cu NPs exhibited sustained efficacy, significantly reducing biofilm formation and inhibiting bacterial growth. These findings not only highlight the versatility of Cu NPs but also suggest their potential utility in mitigating biofilm-associated infections, thus addressing a critical challenge in healthcare settings.

The research findings revealed that the antimicrobial activity of Cu nanoparticles extended beyond bacterial strains, encompassing antifungal effects against Candida albicans. The inhibition zones for this fungus ranged from 14.2 ± 0.28 to 31.05 ± 0.63 mm, with the highest inhibitory effect observed at a concentration of 50 µg/ml. This dual efficacy against both bacteria and fungi highlights the versatile and potent nature of Cu nanoparticles as potential candidates for combating a wide range of microbial infections. Additionally, the study delved into the mechanism of action, suggesting that the Cu nanoparticles exert their antimicrobial effects through disruption of cell membranes and interference with vital cellular processes. This multifaceted approach further underscores the promising role of Cu nanoparticles in the development of effective antimicrobial agents for various applications, including medical and environmental fields.

The literature highlights the critical role of nanoparticle size in influencing antimicrobial activity, with smaller nanoparticles frequently exhibiting superior efficacy. This enhanced performance is ascribed to their increased penetration capacity, driven by higher electrostatic repulsion, which ultimately leads to heightened permeability of bacterial cell membranes. Nevertheless, it is important to note that the antibacterial activity of nanoparticles is not solely determined by size alone. The current findings underscore the significance of the interplay between size and surface charge, emphasizing that both factors are crucial for nanoparticles to effectively function as bactericidal agents.

Copper nanoparticles (Cu NPs) demonstrate a versatile antibacterial mechanism, proving highly effective in countering bacterial infections and finding wide applications in areas such as antimicrobial coatings, wound dressings, and medical devices. The pivotal release of copper ions upon interaction with bacterial cells is instrumental, given their high toxicity, which disrupts various cellular processes. Furthermore, the interaction of copper ions and Cu NPs with bacterial cell membranes induces structural damage and heightened permeability, making cells more vulnerable to the leakage of essential components and eventual demise. Inside bacterial cells, the impact of copper ions and Cu NPs extends to causing DNA damage, leading to oxidative damage and disruption of replication and transcription processes. This cumulative effect impairs the bacterial genome and reproductive capabilities, solidifying the efficacy of Cu NPs in combating bacterial threats. Moreover, recent studies have highlighted the role of Cu NPs in modulating the immune response, with potential implications for enhancing the body's ability to combat infections. Additionally, advances in nanotechnology have facilitated the precise engineering of Cu NPs with tailored properties, further expanding their applications in targeted antibacterial therapies and diagnostic platforms. These developments underscore the multifaceted potential of Cu NPs in revolutionizing approaches to infectious disease management and public health initiatives.

The inclusion of copper ions (Cu ions) and copper nanoparticles (Cu NPs) enhances the production of reactive oxygen species (ROS) inside bacterial cells. These ROS, known for their high reactivity, wreak havoc on proteins, lipids, and DNA, thereby causing cellular malfunction and eventual demise. Furthermore, the antibacterial prowess extends to inhibiting crucial enzyme functions within bacterial cells, thereby interfering with vital metabolic pathways and exacerbating bacterial vulnerability. This combined impact of Cu ions, Cu NPs, and ROS induces oxidative stress within bacterial cells, overpowering their defense mechanisms and culminating in cell death.

Copper nanoparticles (Cu NPs) have garnered significant attention due to their remarkable antibacterial properties, presenting a promising avenue in the battle against antibiotic-resistant bacteria. Recent studies have elucidated additional mechanisms through which Cu NPs exert their antibacterial effects. One such mechanism involves the disruption of bacterial cell membranes, compromising their integrity and leading to leakage

of cellular contents. Additionally, Cu NPs have been found to interfere with vital cellular processes, including DNA replication and protein synthesis, further impeding bacterial growth and survival. Furthermore, Cu NPs possess the ability to penetrate biofilms, complex microbial communities that confer resistance to antibiotics, thereby enhancing their effectiveness in eradicating persistent infections. The synergy between these diverse mechanisms underscores the potential of Cu NPs as versatile and potent antibacterial agents, offering a promising solution to the growing threat of antimicrobial resistance.

4. CONCLUSION

This study delves into the synthesis and characterization of copper nanoparticles (Cu NPs) with a specific emphasis on exploring their antibacterial potential through size and surface charge modulation. By employing a tailored approach, the study precisely controlled the dimensions and surface properties of Cu NPs, achieving variations in size through meticulous adjustment of reaction parameters and implementing surface charge modifications via ligand functionalization. The comprehensive characterization of the synthesized Cu NPs using various analytical techniques provided valuable insights into their optical, physicochemical, and morphological properties.

The antibacterial assessments conducted against a diverse range of bacterial strains demonstrated the multifaceted nature of Cu NPs' antibacterial activity. The release of toxic copper ions upon contact with bacterial cells, coupled with interactions with cell membranes, DNA, and the induction of oxidative stress, collectively contributed to their efficacy against Gram-positive and Gram-negative bacteria. The study also highlighted the nuanced interplay between size and surface charge in influencing the antibacterial performance of Cu NPs.

The results of the antibacterial activity assays revealed varying levels of effectiveness among different Cu NP variants. Notably, Cu NPs synthesized with different reducing and stabilizing agents exhibited distinct antibacterial profiles. The observed inhibition zones against various bacterial strains underscored the potential applications of Cu NPs in developing novel antibacterial agents with tailored physicochemical properties. Moreover, the study demonstrated the significance of considering both size and surface charge in determining the antibacterial efficacy of Cu NPs, challenging the notion that size alone dictates antimicrobial activity.

The findings of this research contribute to the evolving understanding of copper nanoparticle synthesis and their intricate relationship with antibacterial efficacy. The ability to modulate size and surface charge precisely opens avenues for designing customized antibacterial agents with enhanced performance. This research holds promise for the development of advanced materials for antimicrobial coatings, wound dressings, and biomedical interventions, thereby contributing to the ongoing exploration of nanotechnology-driven solutions for combating bacterial infections. Overall, the study reinforces the potential of Cu NPs as versatile and effective tools in the field of antibacterial therapy.

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