



Antimicrobial Effect of Biologically Synthesized Copper Sulfide Nanoparticles



Chaparro-Barrera, Eduardo A¹, Oquendo-Cruz, Abdiel J², Latorre-Esteves, Magda³

¹Department of Chemistry, University of Puerto Rico-Mayaguez Campus

²Bioengineering Graduate Program, University of Puerto Rico-Mayaguez Campus

³Department of Chemical Engineering, University of Puerto Rico-Mayaguez Campus

Introduction

- The growing number of drug-resistant bacteria is one of the major concerns in medical healthcare.
- Bacteria have developed multiple protection mechanisms of resistance to antibiotics such as enzyme inactivation, decreased cell permeability, altered antibiotic target site.¹
- Copper sulfide (CuS) nanoparticles (NPs) have shown excellent antibacterial activity by creating Reactive Oxygen Species (ROS) and disturbing the bacterial membrane by oxidative stress.²
- Bacterial biomolecules may reduce and stabilize nanoparticle synthesis; aiding nanoparticle growth and dispersion.
- Biologically synthesized nanoparticles are fabricated by green synthesis and do not produce toxic byproducts; making it more attractive for biomedical applications.³
- This novel biological synthesis will also reduce high energy waste usually made by traditional chemical synthesis.³

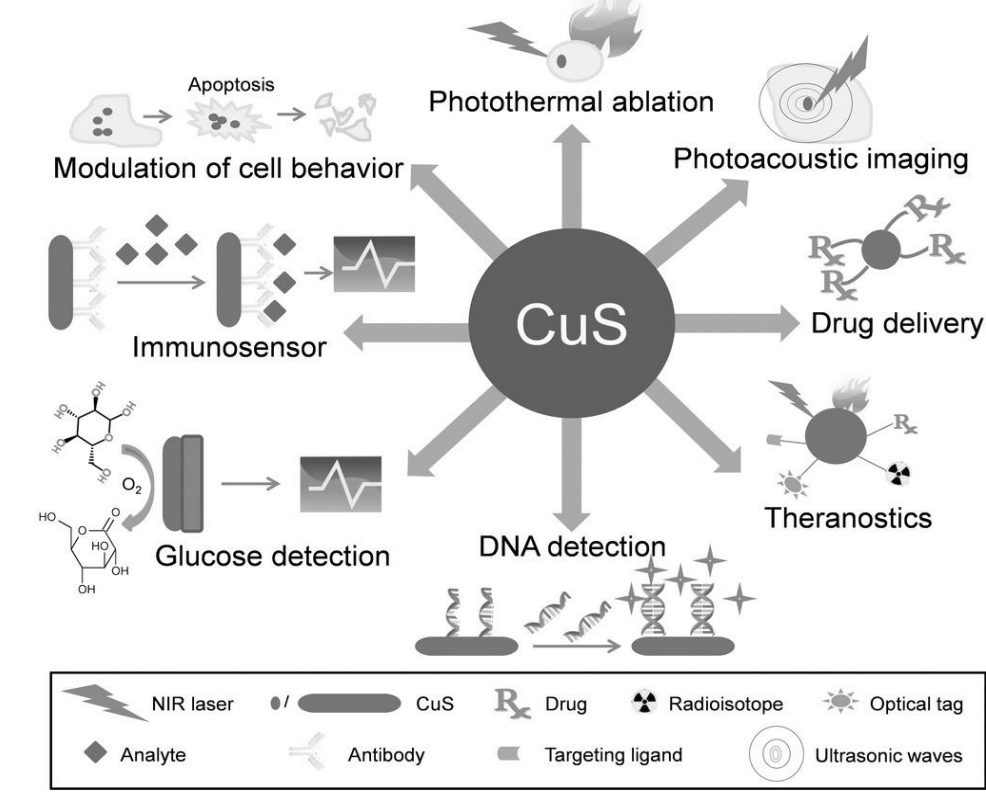


Fig 1. Different biomedical applications of chemically synthesized copper sulfide nanoparticles.⁴



Fig 2. Demonstrates a disk diffusion test treated with antibiotics; a concrete example of the mechanisms developed by bacteria.²

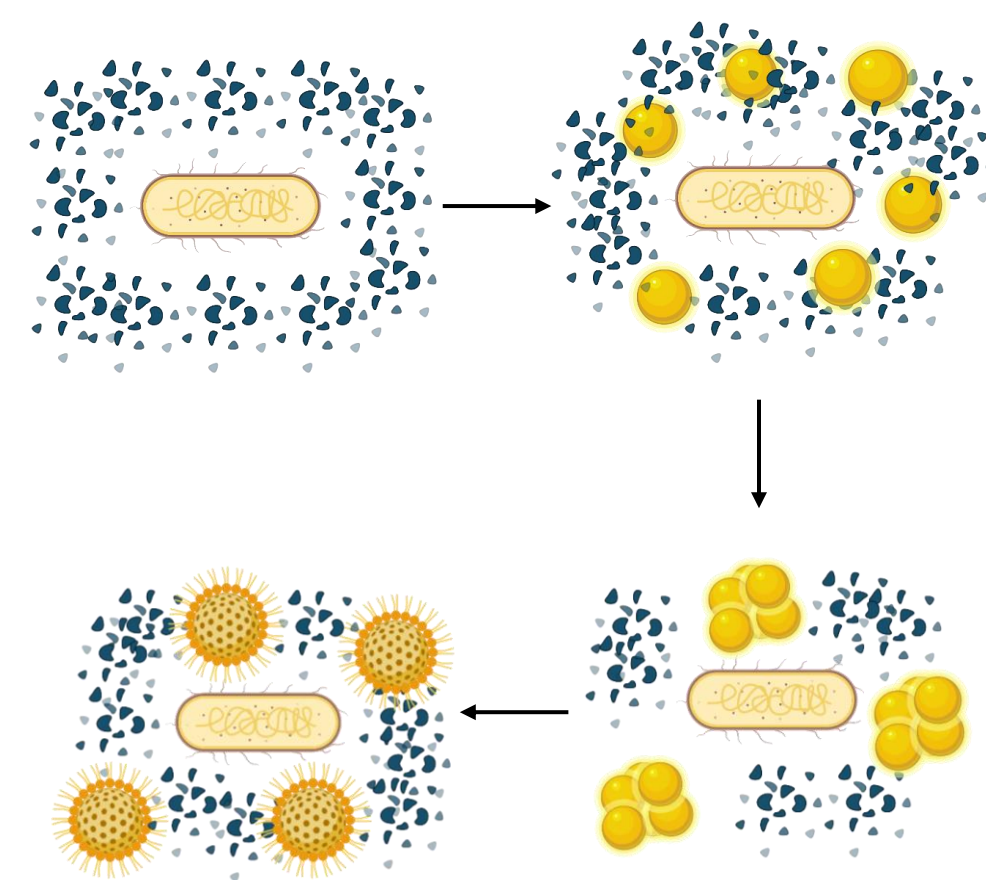
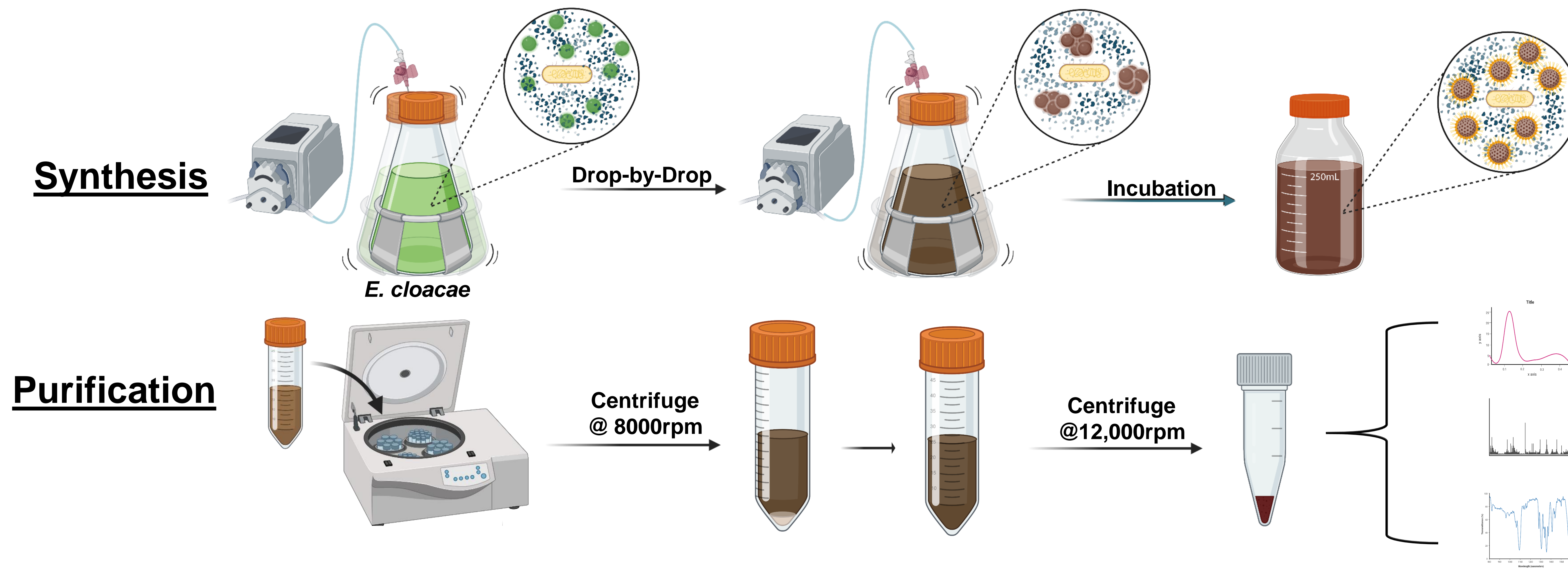


Fig 3. Proposed enzyme-salts interaction during biogenic synthesis; where extracellular enzyme interactions could be the determinants in CuS biogenic synthesis.⁵

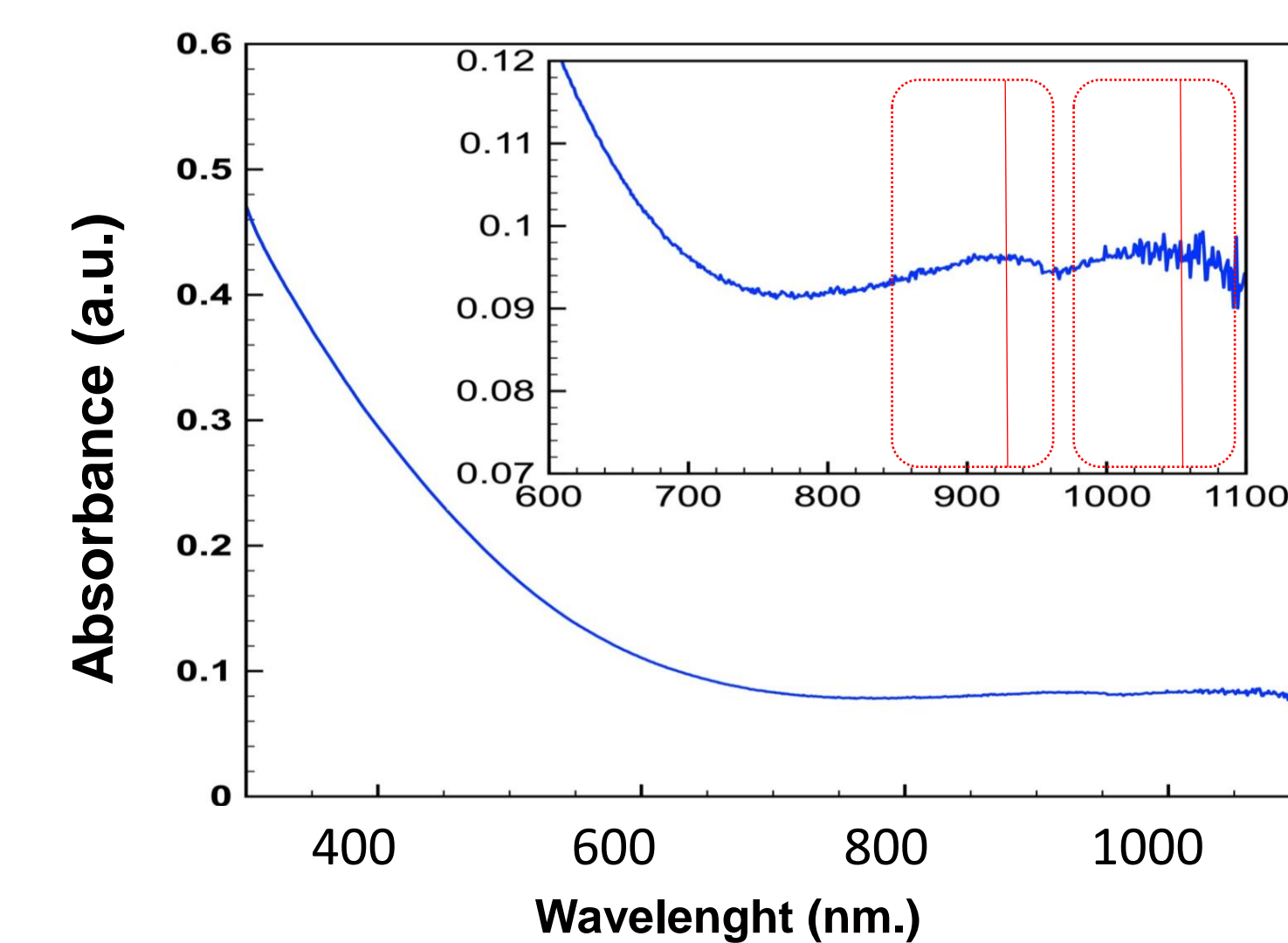
Objectives

- Develop a novel biogenic synthesis protocol.
- Evidence nanoparticle production with microbial cultures
- Corroborate the presence of a Protein Corona in NP's surface.
- Evaluate CuS NP's antimicrobial potential and minimum NP toxic concentrations for multicellular organisms.

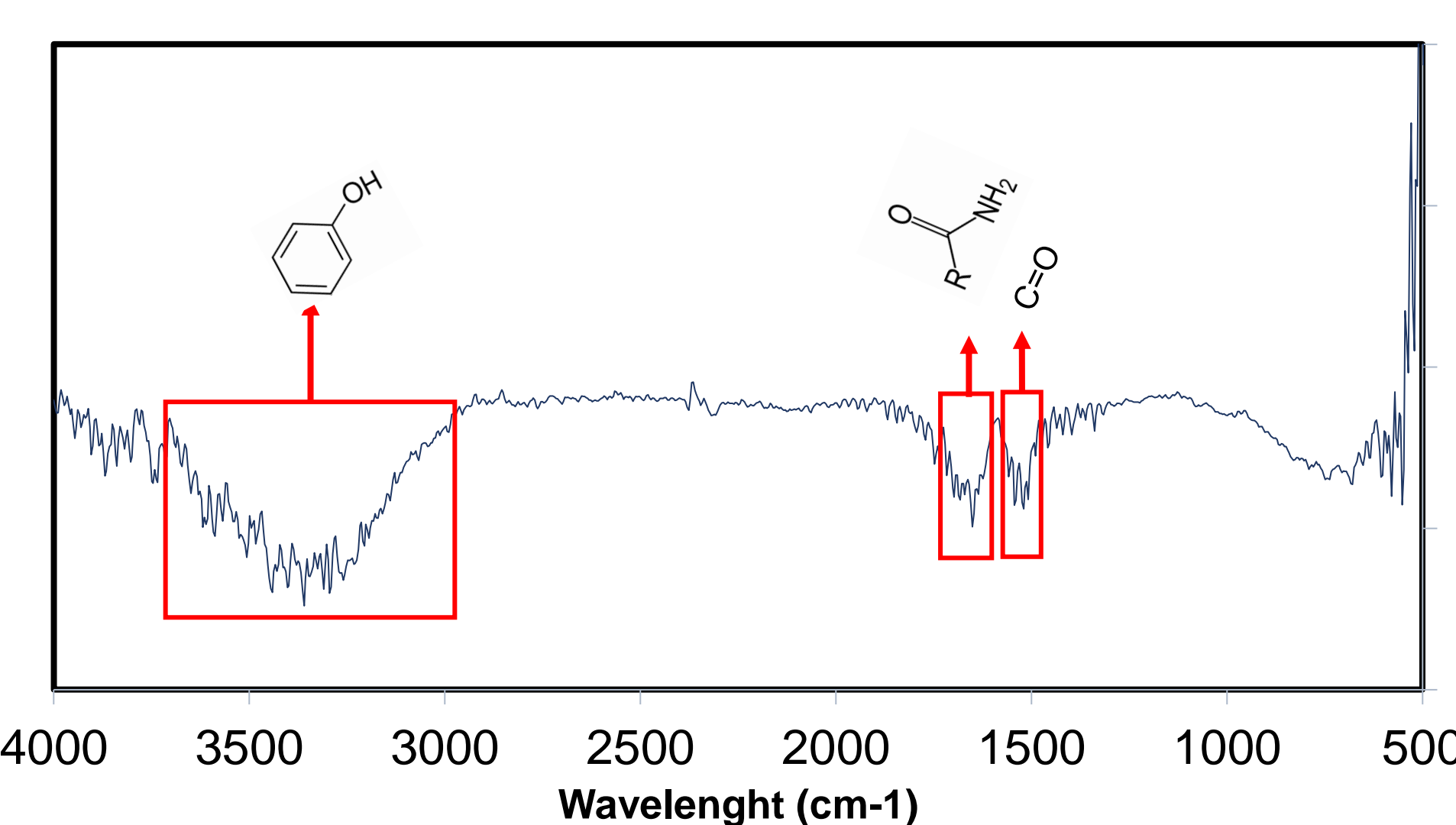
Methodology



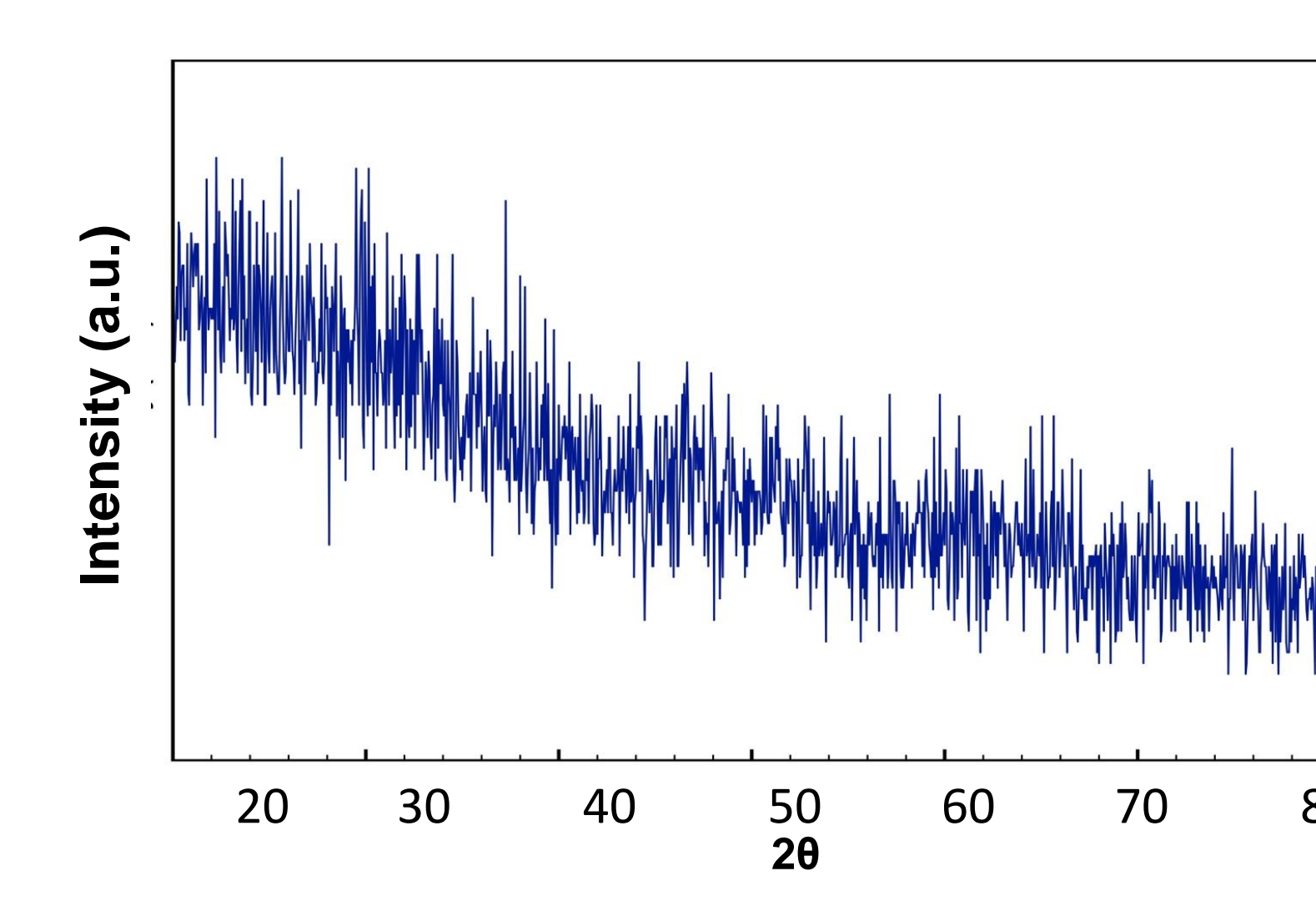
Results



Graph 1. UV-Vis of CuS NPs biosynthesized with *E. cloacae*.



Graph 2. FTIR of CuS NPs biosynthesized with *E. cloacae*.



Graph 3. Evaluation of crystallinity with XRD suggests an amorphous structure.

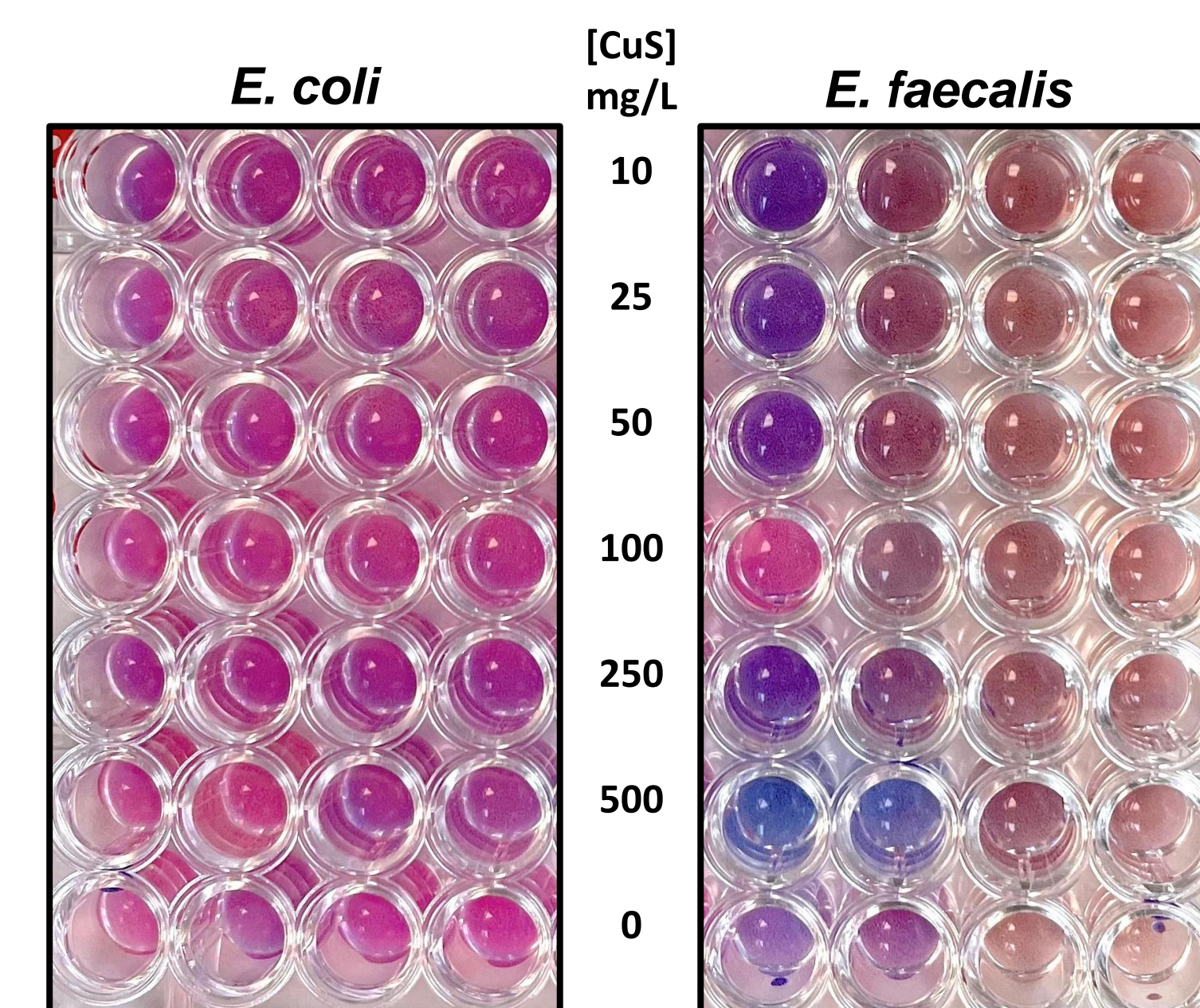


Fig. 4. Minimum Inhibitory Concentration was evaluated against Gram-negative *E. coli* (left) and Gram-positive *E. faecalis* (right).

σ potential	-30.9 mV
Hydrodynamic size	779.9 nm

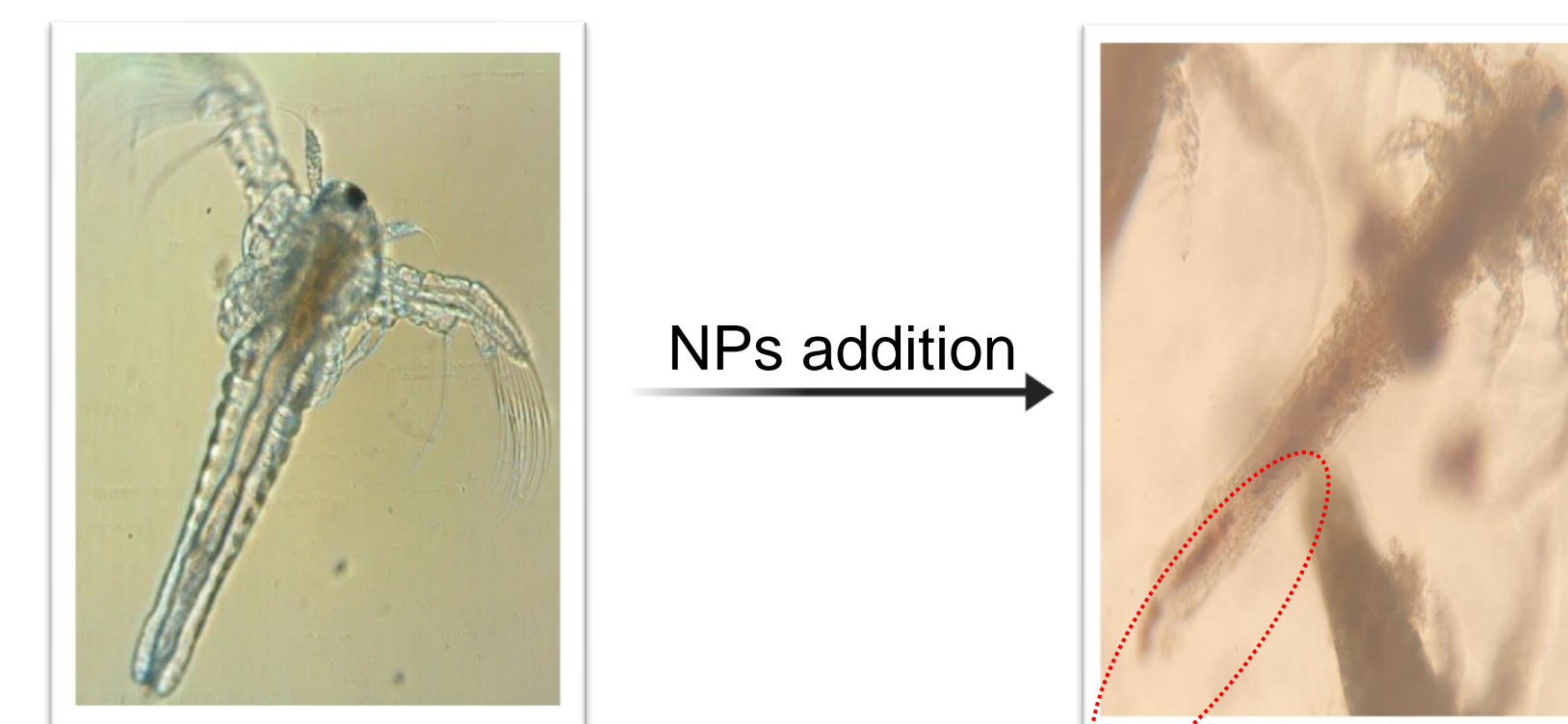
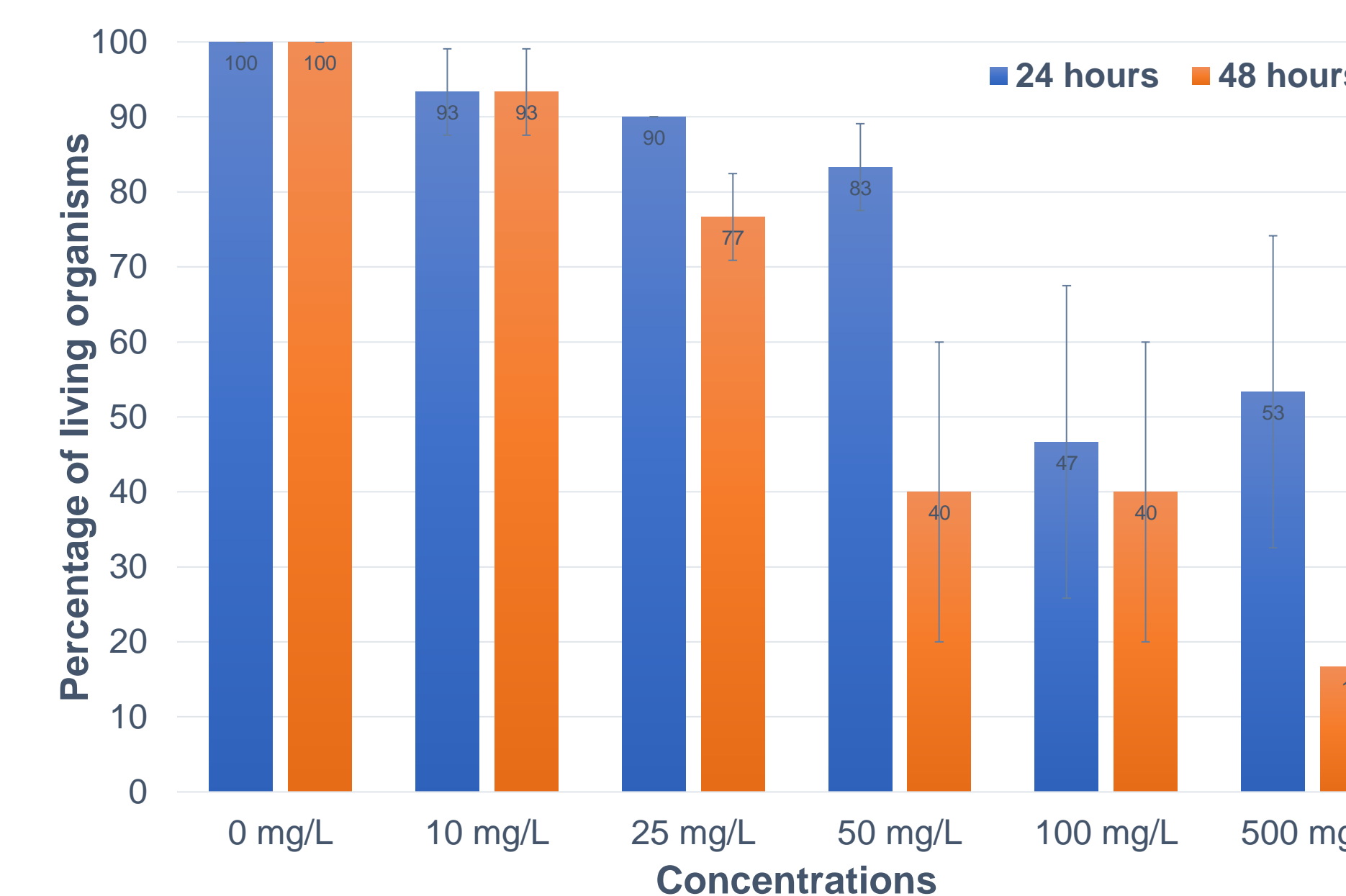


Fig. 6. Brine shrimp toxicological evaluation process.



Graph 4. Brine shrimp viability after 24 and 48 hours of exposure to biosynthesized CuS NPs at different concentrations (0-500 mg/L CuS NPs).

Conclusions

- The pellet obtained after 8000rpm centrifugation implies an extracellular biogenic synthesis.
- UV-Vis showed two smooth peaks suggesting possible difference in NPs size due to absorption.
- FTIR indicates the presence of biologic material on nanoparticle's surface.
- XRD suggests the formation of amorphous nanoparticles.
- Zetasizer characterization measured a total Hydrodynamic size of ~780nm, including Protein Corona on NP surface.
- CuS nanoparticles showed a minimum inhibitory concentration for *E. faecalis* at 500 mg/L whereas *E. coli* was showed resistance at this concentrations.
- The brine shrimp lethality test showed a decrease in viability starting at 50 mg/L after 24h.

Future Work

- Measure bacterial growth curves to verify the effects of the CuS salts when added to the bacteria culture medium.
- Characterize the possible Protein Corona in NP's surface through 2D Electrophoresis.
- Determine the NP's morphology by HRTEM and SEM characterizations.

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