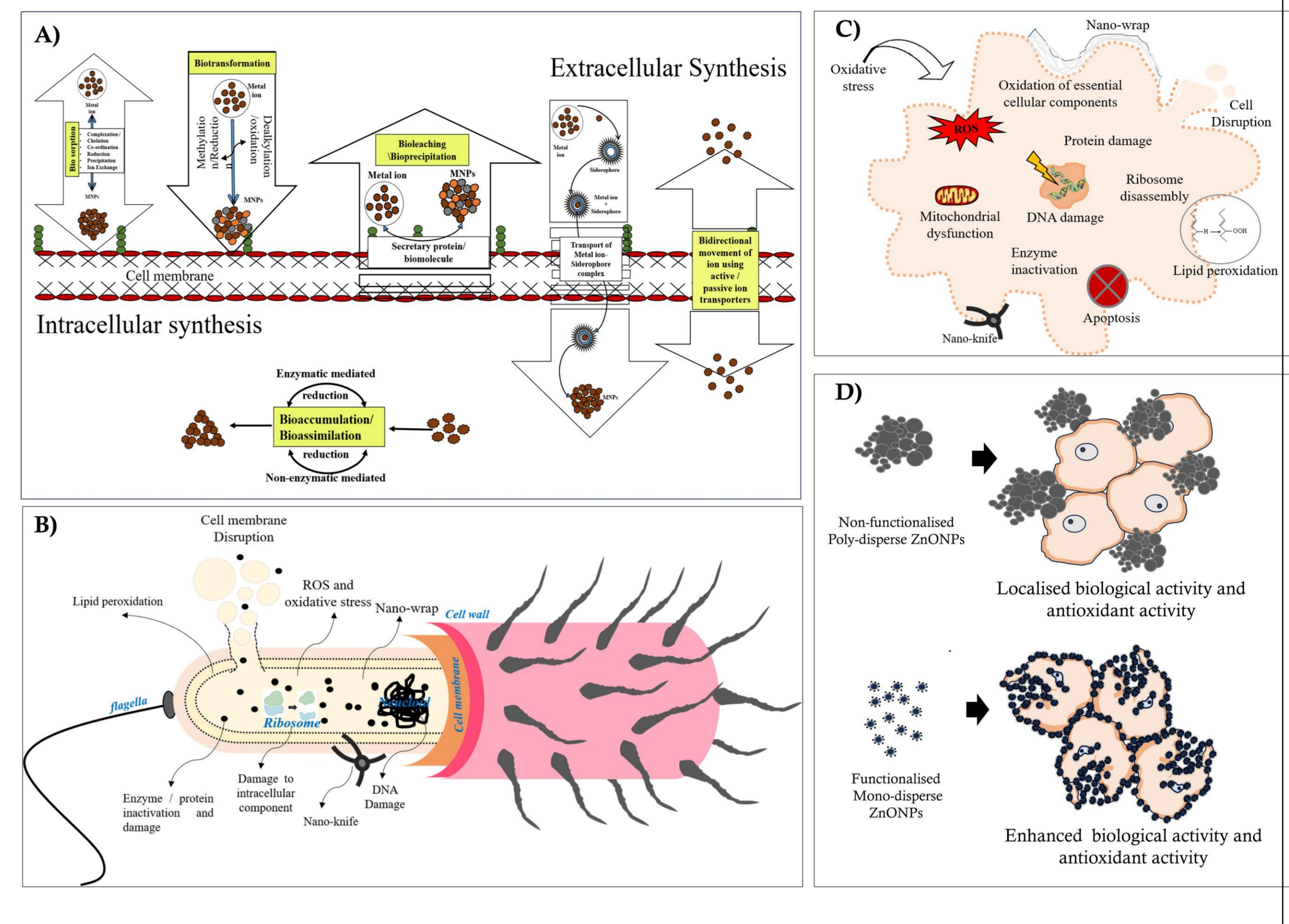
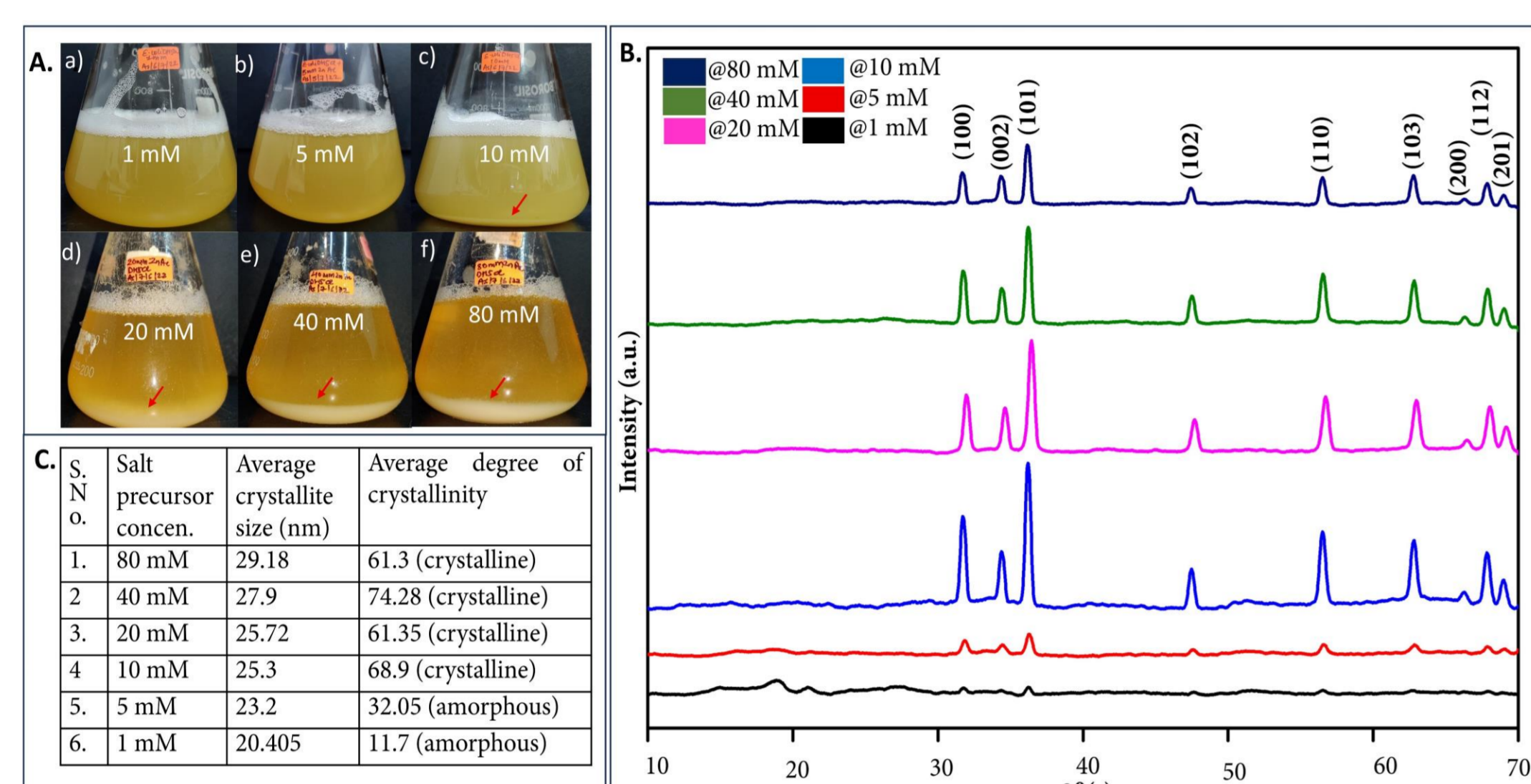


## 1. Abstract

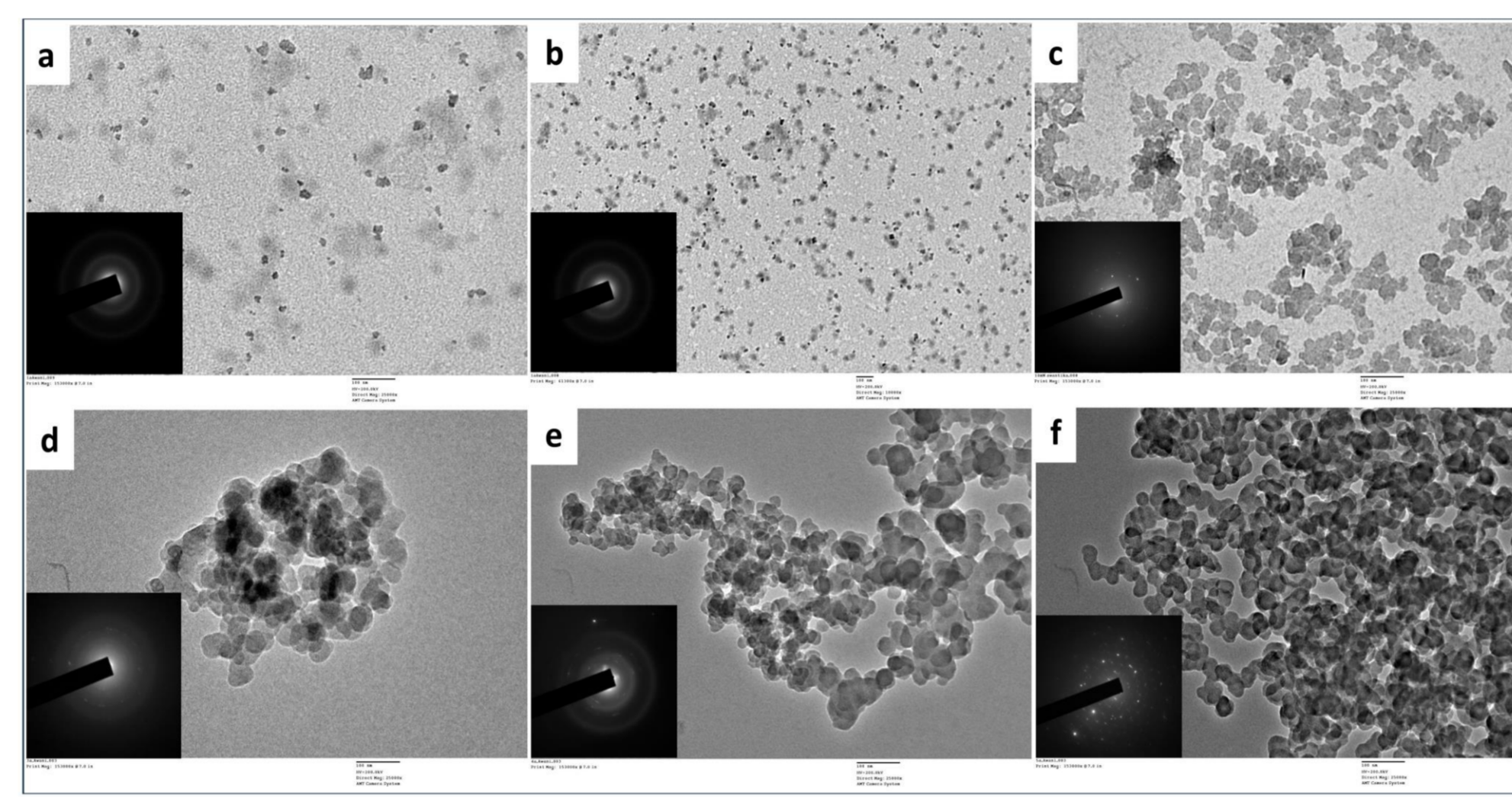
In the world of emerging alternative of conventional antibacterial agents, the nanoparticles synthesized via bacterial nanofactories have been shown an immense potential as they are cost effective, eco-friendly simple, less hazardous approaches. The bacterial nanofactories used in past research work are mostly pathogenic or difficult to culture, therefore, this study focuses on non-pathogenic *Escherichia coli* as a microbial nanofactory for the biosynthesis of zinc oxide nanoparticles (ZnONPs) with enhanced biomedical applications. Additionally, the microbial engineering of this bacterial nanofactories were carried out to fine tune the physicochemical properties of the synthesized nanoparticles. After that, the synthesized nanoparticles were characterised via various analytical technique and their biomedical application were evaluated for antibacterial, anticancer and antioxidant properties. The X-Ray diffractometer (XRD) spectrum suggests that pure, crystalline, hexagonal, wurtzite-type ZnONPs were formed when treated with zinc salt precursor concentrations (40 and 80 mM). The High-resolution transmission electron microscopy (HR-TEM) images showed the synthesis of spherical nanoparticles (26-39 nm range) were formed. The SEAD pattern also confirms the formation of polycrystalline ZnONPs at higher concentrations, validating XRD results. Biological entities present inside the bacterial cell and present at the surface plays significant role as reducing and stabilizing agents in nanoparticle formation and its antioxidant properties which can be clearly observed. The antibacterial activity of as-synthesized ZnONPs is noteworthy against gram-positive and gram-negative pathogenic bacteria, even at low concentrations of 50 µg/mL. Similarly, the significant anticancer activity against lung cancer A549 cells at similar doses of 50 µg/mL of as-synthesized ZnONPs was observed. This research highlights the potential of *E. coli*-based engineering for the sustainable and self-regulating synthesis of advanced biomaterials for enhanced biomedical applications.



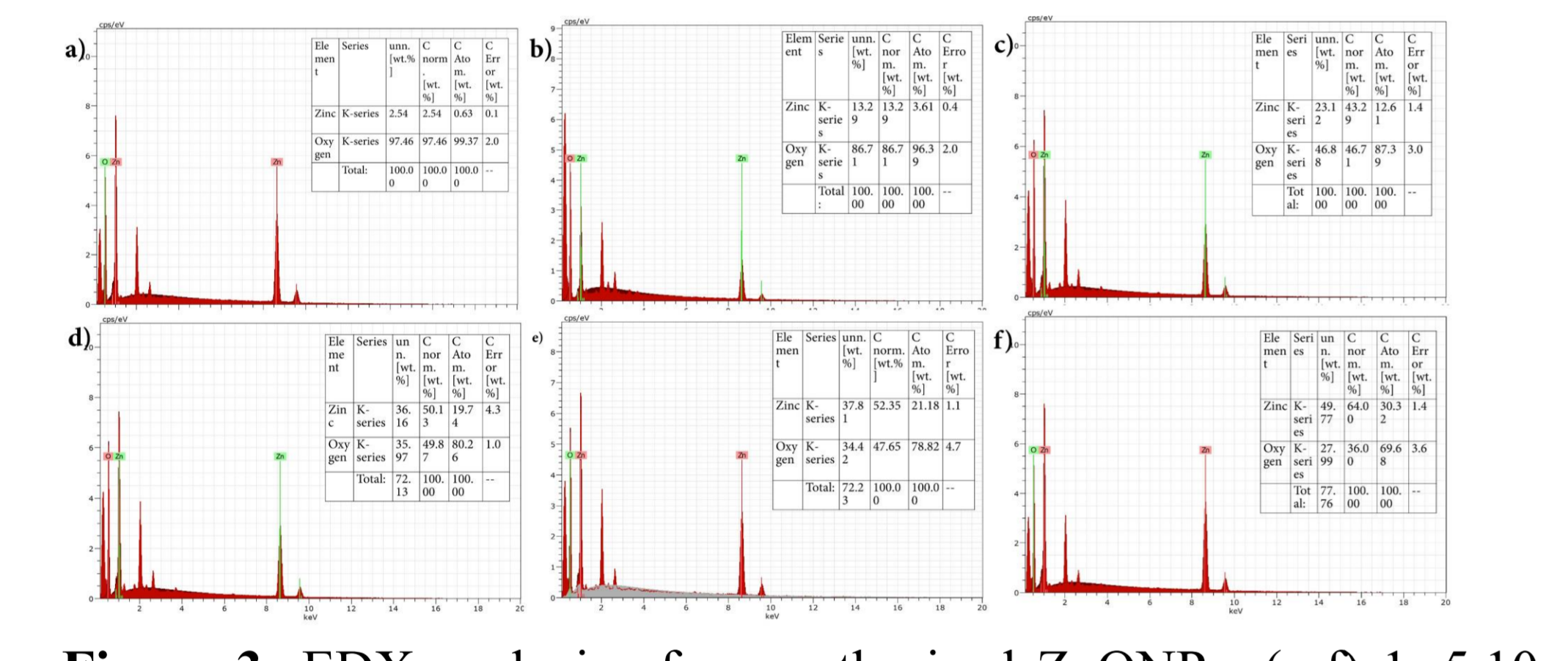
## 2. Synthesis of ZnO NPs and its characterizations studies



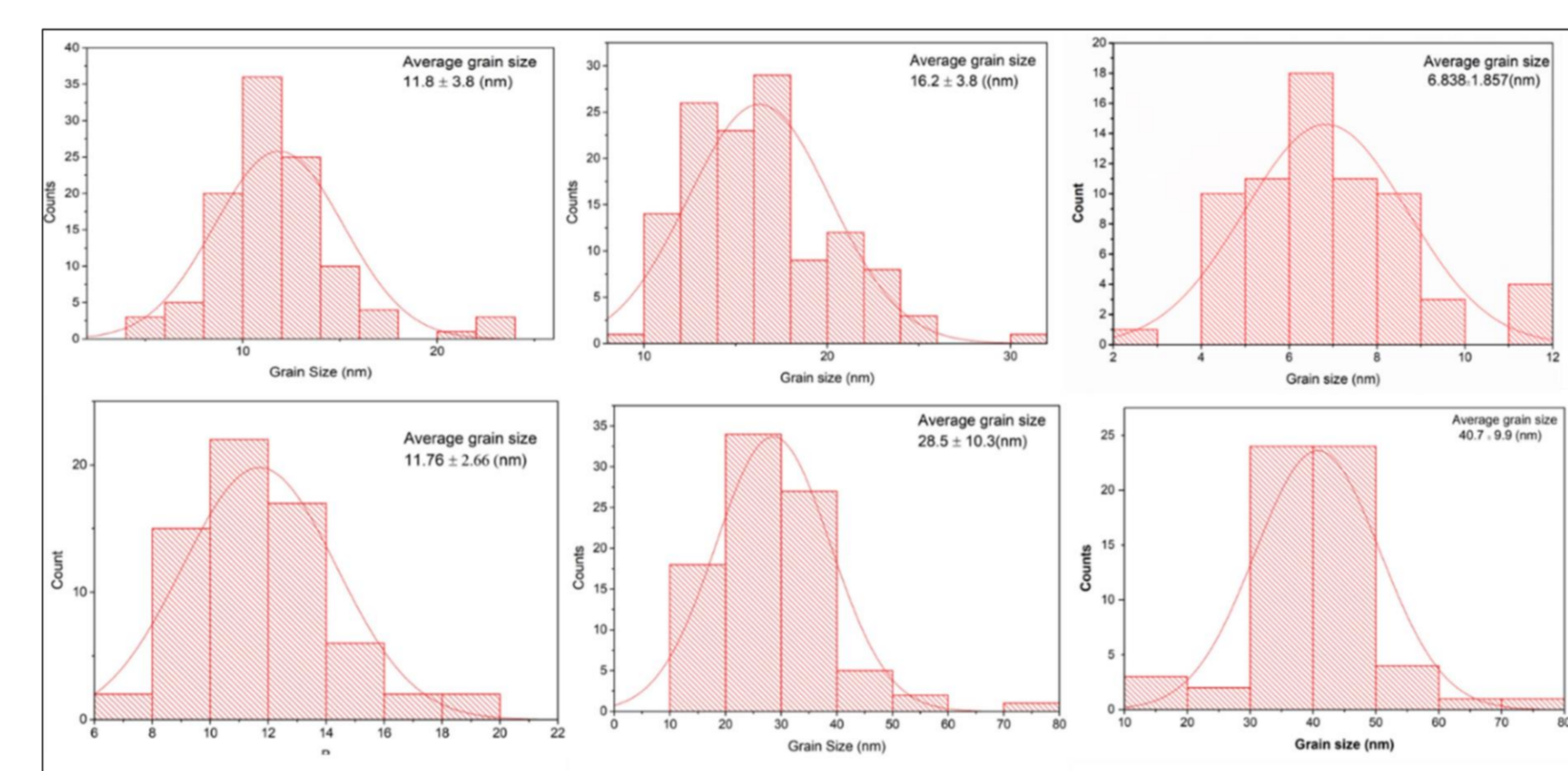
**Figure 1:** (A) Visual observation of white pellet of as-synthesized ZnONPs, (B) XRD pattern of as-synthesized ZnONPs, and (C) Average crystallite size of as-synthesized ZnONPs and Average degree of crystallinity.



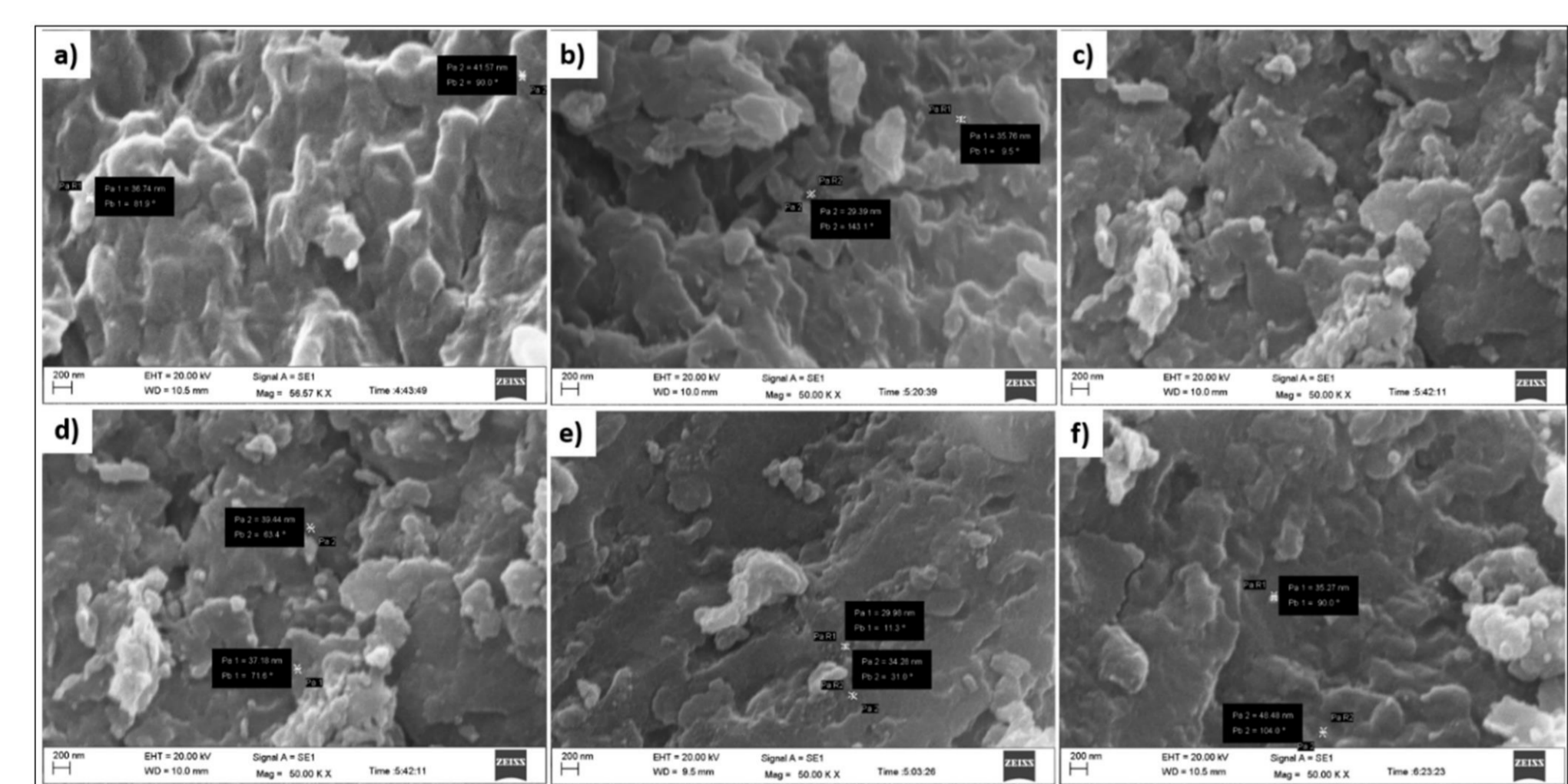
**Figure 2:** HR-TEM micrographs (a-f) as-synthesized ZnONPs at 1,5,10,20,40, and 80 mM salt precursor concentration. Inset: SAED pattern showing (a-b) amorphous structure, and (c-f) polycrystalline structure



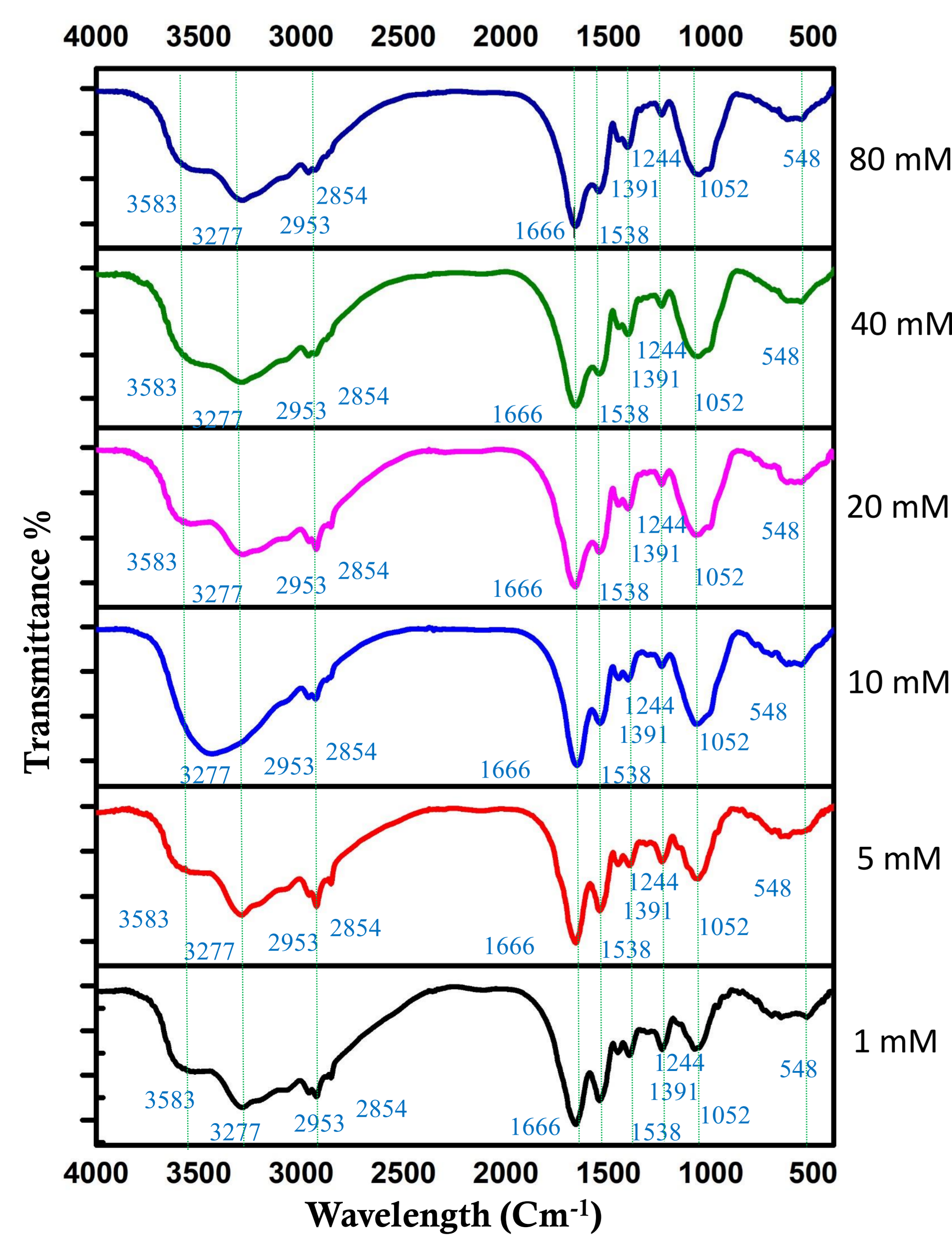
**Figure 3:** EDX analysis of as-synthesized ZnONPs: (a-f) 1, 5, 10, 20, 40, and 80 mM zinc acetate salt precursor concentration.



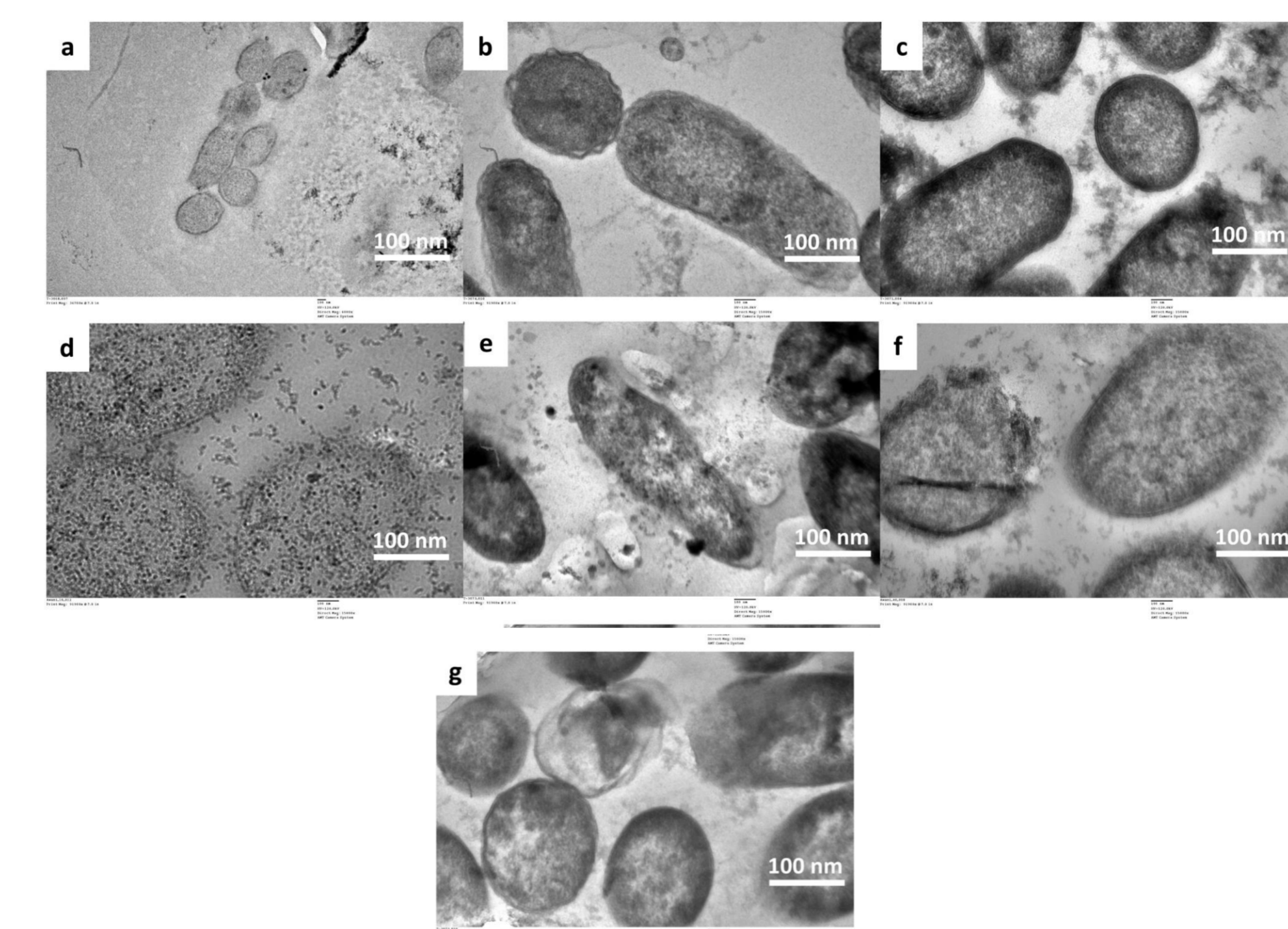
**Figure 4:** Histograms showing the average size range of (a-f) as-synthesized ZnONPs at 1, 5, 10, 20, 40, and 80 mM salt-precursor concentrations.



**Figure 5:** Scanning Electron Microscope (SEM) image of bacterial synthesized ZnO nanoparticles



**Figure 7:** FT-IR spectrum of as-synthesized ZnONPs

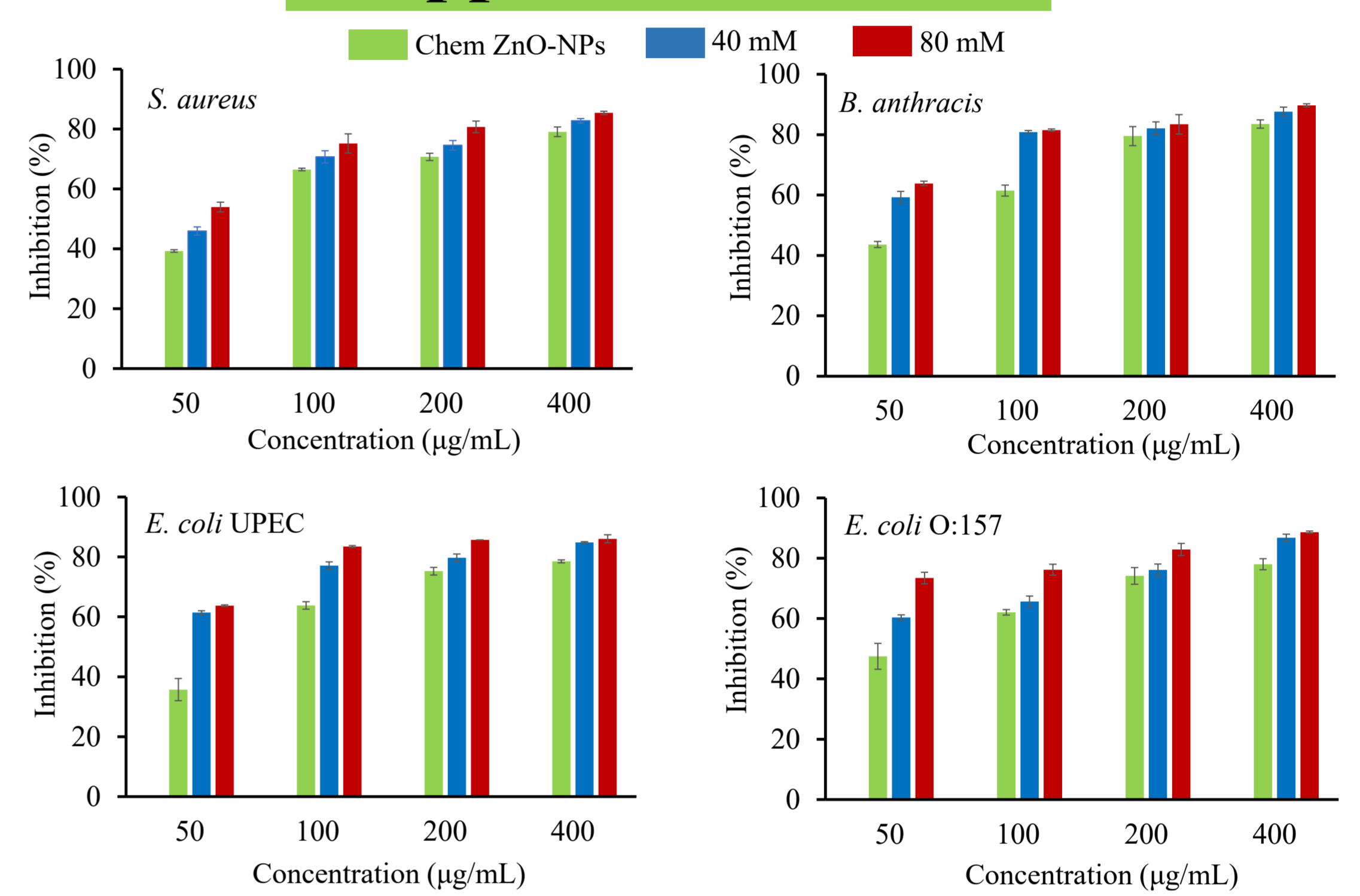


**Figure 6:** TEM Analysis of ultramicrotome of *E. coli* DH5α (a) wildtype, (b) treated with 1 mM, (c) treated with 5 mM, (d) treated with 10 mM, (e) treated with 20 mM, (f) treated with 40 mM, and (g) treated with 80 mM salt precursor conc. showing formation of ZnONPs in extracellular and intracellular vicinities.

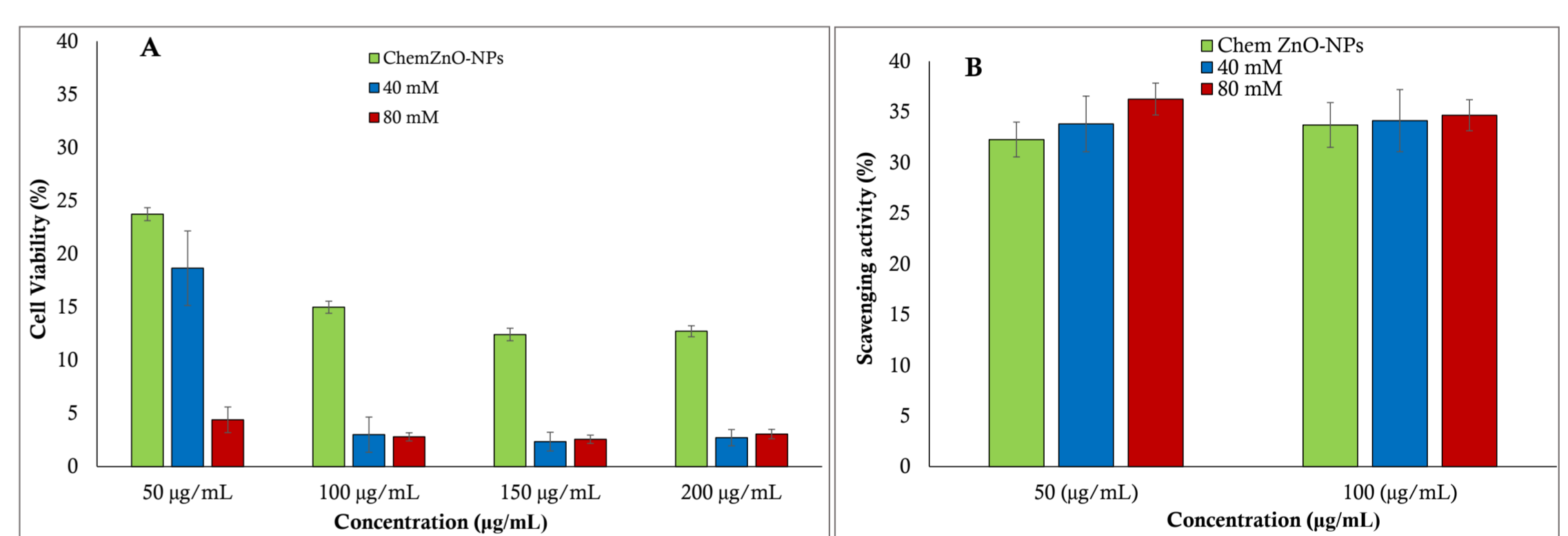
S.No.	Peak (cm <sup>-1</sup> )	Functional group	Present in biomolecules	Mechanism	Examples
1.	3583.7	-OH	polysaccharides, proteins, or enzymes	Acts as reducing agents, stabilizing agents	The abundant hydroxyl groups on sugars (like glucose, mannose), reductase enzymes, and liposaccharides reduce metal ions and initiate nanoparticle formation.
1.	3277	Hydroxy			
1.	2953.3	-C-H <sub>2</sub> Methyl	Amino acid side chains, phospholipids	Building block, prevent aggregation, spacer in surface ligands/ capping agents, steric stabilization and colloidal control, influence on hydrophobic interactions	A bridge between carbon and hydrogen — is a versatile building block, critical in designing nanoparticle interfaces and forming the structural framework of life in <i>E. coli</i> .
1.	2854.9	-C-H Aromatics	polysaccharides and peptidoglycan		
1.	1666	C=O Esters, aldehyde, ketones	Protein and polysaccharides, or small organic molecules	Reducing and stabilising agents/ capping agents	Carbonyls donate electrons to metal ions. Coordination via C=O or -COO <sup>-</sup> prevents aggregation. Carboxylate groups (charged) anchor onto nanoparticle surfaces
1.	1538.8	N-H			
1.	1391.5	-C-N/-C-O	Protein, peptidoglycan	Reducing and capping agents	nitrate reductase uses electron-rich functional groups like amine and hydroxyl to reduce metal salts.
1.	1244.3	O of ethers or alcohols/ -C-H bending within CH <sub>2</sub> CH <sub>2</sub>			
1.	1052.9	-ZnO	lipopolysaccharides,		
1.	548.9		Due to salt precursors	Nucleating molecules	ZnO NPs synthesis

**Figure 8:** functional group present on the surface of synthesized ZnONPs and their possible role

## 3. Application studies



**Figure 7:** Analysis of antibacterial assay of as-synthesized ZnONPs against Gram-positive (*S. aureus* and *B. anthracis*) and Gram-negative (*E. coli* UPEC, *E. coli* O:157) pathogenic bacteria



**Figure 8:** Dose-dependent MIC assay of the as-synthesized ZnONPs and compared with commercially available chemically synthesized ZnONPs against Gram-positive (*S. aureus* and *B. anthracis*) and Gram-negative (*E. coli* UPEC, *E. coli* O:157) bacteria.

## 4. conclusion

- The study establishes a novel strategy of using non-pathogenic engineered *E. coli* as a microbial nanofactory to synthesize biocompatible ZnO nanoparticles.
- Microbial engineering fine-tuned the physicochemical properties of ZnONPs, enhancing their crystalline index, size uniformity, and surface reactivity.
- The tailored physicochemical features directly improved biomedical efficacy, showing potent antibacterial, anticancer, and antioxidant activities.
- The antibacterial activity for the bacterial pathogenic species observed is in the order: *Staphylococcus aureus* > *E. coli* UPEC > *Bacillus anthracis* > *E. coli* O:157. Further, anti-cancer activity studies against A549 cell lines using MTT assay showed that the ZnO exhibited cytotoxicity against the A549 cancer cell line. Additionally, the synthesized ZnONPs displayed significant antioxidant capabilities compared to chemically synthesized zinc salt and ascorbic acid (standard antioxidants).
- Synthesized nanoparticles show promising results over other reported literature; however, achieving optimal material properties and scalability of the manufacturing process are essential for potential commercial applications.

## 5. Acknowledgment

- I would like to thank organising committee, my coworkers, my friends and my mentors Dr. Hemant R Kushwaha, DR. Anu Singh, Prof Rupesh Chaturvedi and Dr. Mohan Joshi.

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