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Synthesis of ZnO Nanoparticles and Interaction with Protein and Surfactants

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Abstract

Zinc oxide nanoparticles (ZnO NPs) have gained significant attention in nanotechnology due to their exceptional physical and chemical properties, including a high surface area, unique optical characteristics, photosensitivity, and biocompatibility. These properties make ZnO NPs highly valuable for various biomedical and technological applications. This study focuses on the synthesis of ZnO NPs using a chemical synthesis approach, ensuring controlled particle formation. The synthesized nanoparticles were then characterized using UV-Vis spectroscopy to analyze their optical properties and confirm their formation. Furthermore, the interaction of ZnO NPs with bovine serum albumin (BSA), a model protein, was investigated through spectroscopic techniques. The results demonstrated substantial protein binding, leading to alterations in the structural properties of BSA. These findings indicate the strong affinity of ZnO NPs for biomolecules, highlighting their potential in drug delivery systems and biosensor applications. The study emphasizes the significance of ZnO NPs in biomedical research, showcasing their ability to interact with biological macromolecules. The observed protein-nanoparticle interactions suggest promising applications in targeted drug delivery, diagnostics, and biosensing. Overall, this research contributes to the growing interest in ZnO NPs and their role in developing advanced nanomaterials for future biomedical innovations. The interaction of Zn NPs with surfactants is also studied in this paper. It was found that with the increase in concentration, absorbance value increases.

Keywords: Bovine serum albumin, Drug delivery, Protein interaction, Biocompatibility, Surfactant, Zinc oxide nanoparticles

Introduction

Nanotechnology has revolutionized various scientific disciplines by enabling the manipulation of materials at the molecular and atomic levels. Among nanomaterials, zinc oxide nanoparticles (ZnO NPs) (Wang, 2004; Laurenti *et al*., 2015; Selvan *et al*., 2007) have received significant attention due to their unique optical, catalytic, photosensitivity, and antibacterial properties (Pal *et al*., 2011; Roy *et al*., 2012; Verma *et al*., 2014; Jha *et al*., 2016). ZnO NPs are widely employed in drug delivery systems, biosensors, cosmetics, and environmental remediation. The synthesis of ZnO NPs can be accomplished through physical, and chemical methods. While chemical methods provide high control over particle size and morphology. There are many chemical methods to prepare ZnO NPs, like the sol-gel method, the hydrothermal method, the co-precipitation method, and the green method (Parashar *et al*., 2008; Jain *et al*., 2009; Raghunandan *et al*., 2011; Hasan, 2015; Hong *et al*., 2017; Sharma *et al*., 2018; Nasir *et al*., 2020). The sol-gel method is more convenient, easy to set up, and gives a higher yield compared to other methods. Understanding the interaction between ZnO NPs and proteins is essential, as proteins are fundamental biomolecules involved in cellular functions. Protein-nanoparticle interactions (Chen *et al*., 2010; Yang *et al*., 2010; Waghmare *et al*., 2011; Yadav *et al*., 2013; Gao *et al*., 2015; Singh *et al*., 2021) may affect protein structure and function, influencing their biocompatibility and efficacy in medical applications. Bovine serum albumin (BSA), a widely studied model protein, offers insights into the binding mechanism of nanoparticles with biomolecules. Many researchers have studied the interaction between ZnNPs and surfactants (Moghadam & Azizian, 2014; Youn & Choi, 2022). This study aims to synthesize and characterize ZnO NPs, followed by an evaluation of their interaction with BSA to elucidate potential biomedical applications and also the interaction of Zn NPs with surfactants.

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Materials and Methods

Materials

For the preparation of zinc oxide nano-material, we used $(Zn(OAc)_{2.2H2O}$, NaOH, ethanol, and de-ionized water. Zn salt is used as a precursor, and NaOH is basically an additive and also maintains the pH of the medium. Ethanol helps with crystallization purposes. Bovine serum albumin (BSA), and $CuSO_45H_2O$ are also used for the protein and nanoparticle interactions. All the chemicals have high purity and these were purchased from Sigma Aldrich.

Methods for the Preparation of ZnO Nanoparticles

Our desirable material zinc oxide can be prepared with the help of the sol-gel process, which is one of the most well-known methods. For the preparation of this, we used 2g $Zn(OAc)_2.2H_2O$, 8g NaOH, and 100 ml ethanol. At first, we prepare an aqueous solution of precursor Zn $(OAc)_2$. $2H_2O$ in 15 mL deionized water with constant stirring by a magnetic stirrer and also an aqueous solution of NaOH by taking 10 mL deionized water separately. Then add NaOH solution dropwise to the clear solution of precursor with constant stirring. The clear solution becomes hazy with the addition of the aqueous solution of additive NaOH. Finally, we added 100 mL of ethanol dropwise from the burette with constant stirring and kept the whole mixture by covering the mouth of the beaker with parafilm for evaporation. An egg-like white crystal of

ZnO was obtained with good yield after 2 days. Using these various characterizations performed.

Experimental Set-Up for Protein-Nano Particle Interaction

ZnO nanomaterial is used extensively in the medical industry because of its special properties, which include non-toxicity, biocompatibility, antibacterial and antimicrobial activity, etc. Consequently, we have used our recently created ZnO material in our lab to conduct a protein-nanoparticle interaction. As a phosphate buffer, also known as a phosphate-buffered saline (PBS) solution, it is necessary for biological research and applications because it helps to maintain a steady pH, which is vital for protein stability and avoiding denaturation. For this experiment, we make a 1% BSA protein solution using PBS. Additionally, we used PBS solution to create a 1% solution of $CuSO_4$ $5H_2O$ and freshly made ZnO nanomaterial. To ensure adequate interaction, we create two sets for the experiment and incubate them for 30 minutes. BSA solution $CuSO_4$ $5H_2O$ solution and ZnO nanomaterial solution are mixed in one set, and BSA solution and $CuSO_4$ $5H_2O$ solution are mixed in another. Lastly, we independently measured and noted each set of the solution's UV-VIS absorbance **(Figure 2)**.

Experimental Set-Up for Surfactant-Nano Particle Interaction

Preparation of Solutions

- *Sunlight (Marketable surfactant) Stock Solution*

Prepare a 1% (w/v) Surfactant solution by dissolving 1g of Sunlight in 100 mL of distilled water. Stir until completely dissolved.

- *ZnO Nanoparticles Suspension*

Prepare a series of AgO nanoparticle suspensions (e.g., 10 ppm, 20 ppm, 50 ppm, 100 ppm) by dispersing the nanoparticles in distilled water.

Use sonication for uniform dispersion if needed.

- *Experimental Steps*

  - *Sample Preparation*

In labeled beakers or test tubes, mix equal volumes of Sunlight solution with varying concentrations of ZnO nanoparticles (e.g., 5 mL SLS + 5 mL ZnO NPs suspension).

Prepare a control sample with Sunlight only (no nanoparticles).

  - *Incubation*

Allow the mixtures to react for a fixed period (e.g., 30 minutes to 1 hour) at room temperature.

Stir gently or use a shaker for uniform interaction.

  - *Colorimetric Measurement*

After incubation, transfer each sample to a clean cuvette.

Measure the absorbance using the colorimeter at a suitable wavelength (typically 370-400 nm). Using distilled water as the blank.

Results and Discussion

Evidence for the Formation of ZnO Nanoparticles

Zinc oxide (ZnO) nanoparticles are among the most extensively studied metal oxide nanomaterials due to their remarkable physicochemical and optical properties. Their wide band gap and high exciton binding energy make them ideal candidates for a variety of applications, including biosensing, photocatalysis, drug delivery, and optoelectronics. In the present study, ZnO nanoparticles were synthesized in the laboratory using a controlled process. To confirm the successful formation of ZnO nanoparticles, UV-visible (UV-Vis) spectroscopic analysis was carried out. UV-Vis spectroscopy is a fundamental and widely used technique for the preliminary characterization of metal oxide nanoparticles, as it provides information about their optical properties and electronic transitions. The ZnO nanoparticles synthesized in our lab were dispersed in ethanol (EtOH) and subjected to UV-Vis analysis. The absorbance spectrum, as shown in **Figure 1**, reveals a distinct and sharp absorption peak around 370 nm. This peak is widely recognized in the literature as the characteristic absorption edge for ZnO nanoparticles due to their intrinsic band gap transition. The presence of this absorption peak at 370 nm provides strong evidence of the formation of ZnO at the nanoscale. The sharpness and intensity of the peak also indicate that the nanoparticles are well-formed and possibly monodispersed to a significant extent. Furthermore, the absence of any major secondary peaks suggests high purity of the synthesized nanoparticles, without significant contamination or the presence of other metal oxides.

Overall, the UV-Vis spectral data supports the successful synthesis of ZnO nanoparticles. This confirmation lays a solid foundation for subsequent studies involving the interaction of ZnO NPs with biological molecules, evaluation of their stability, and exploration of their potential in various biomedical and technological applications (Duraimurugan *et al*., 2022; Anushree *et al*., 2023; Malinga & Laing, 2024; Zafeiraki *et al*., 2024).

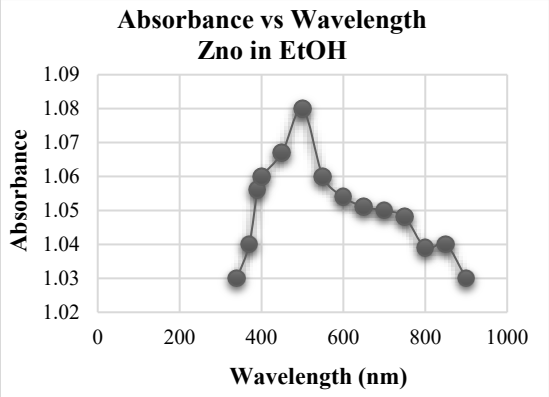

Figure 1. Plot of Absorbance Vs. The wavelength of ZnO nanoparticles

Biocompatibility of ZnO Nanomaterials

Zinc oxide (ZnO) nanoparticles are considered highly promising materials due to their unique physicochemical properties, including a wide band gap, excellent chemical stability, non-toxicity, and notable biocompatibility (Daivasigamani *et al*., 2022; Mohandas *et al*., 2022; Mubayrik *et al*., 2022; Alsubeie, 2023; Broers *et al*., 2023; Hackenberg *et al*., 2023; Dobrzynski *et al*., 2024; Makakova *et al*., 2024). These attributes make them suitable for various biomedical applications such as biosensing, drug delivery, and imaging. In our study, we specifically examined the interaction between our newly synthesized ZnO nanoparticles and bovine serum albumin (BSA), a model protein widely used to assess nanoparticle–protein interactions. To investigate this interaction, UV-visible spectroscopy was conducted on a mixture of ZnO nanoparticles and BSA protein. BSA, in its native form, exhibits a strong absorbance peak around 280 nm due to the presence of aromatic amino acid residues, such as tryptophan and tyrosine (Al-Khotani *et al*., 2022; Enwa *et al*., 2022; Liu *et al*., 2022; Zhang *et al*., 2022; İlaslan *et al*., 2023; Kulkarni *et al*., 2023; Makhoahle *et al*., 2023; Tabassum *et al*., 2023; Ismikhanov *et al*., 2024). However, upon binding with ZnO nanoparticles, a noticeable red shift was observed, with the peak shifting to approximately 320 nm. This shift indicates that the electronic environment of the aromatic residues has been significantly altered due to the formation of a nanoparticle-protein complex. Moreover, a significant reduction in absorbance intensity at this new peak position suggests that ZnO nanoparticles induce partial denaturation of BSA. This occurs likely through the disruption of key stabilizing interactions like hydrogen bonds and hydrophobic forces. The new signal at 320 nm also supports the formation of a protein corona around the ZnO nanoparticles, which plays a crucial role in modulating their behavior and compatibility within biological environments **(Figure 2)**.

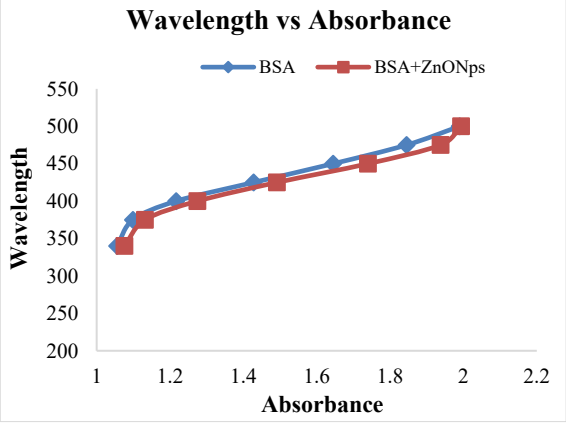

Figure 2. Plot of ZnO with BSA and without BSA protein

Interaction of Zn NPs with marketable surfactant(Sunlight). When we measure the Zn NPs' interaction with marketable surfactant at 370-400 nm, we see that the absorbance value increases from 0 ppm to 100 ppm **(Table 1)**. This is due to the complex formation between surfactant molecules and Zn NPs and also changes the absorbance properties of the system. It was also explained that surfactant may act as a stabilizer, preventing nanoparticle aggregation and as well as increasing optical density due to more dispersed particles.

Table 1. Zn NPs with Marketable surfactant (Sunlight) interaction data with colorimeter

Sl. No.	Concentration(ppm)	Absorbance
1.	00	1.024
2.	10	1.025
3.	25	1.029
4.	50	1.033
5.	100	1.038

An increase in absorbance between 370–400 nm suggests that more ZnNPs are dispersed in the solution, leading to greater light absorption, smaller, well-dispersed nanoparticles absorb more strongly in this range. The surfactant is effectively stabilizing the ZnNPs and preventing aggregation.

The surfactant interacts with ZnNPs by adsorbing onto their surface, providing steric or electrostatic stabilization, preventing agglomeration, and keeping the particles small and evenly distributed.

Conclusion

This study effectively synthesized and thoroughly characterized zinc oxide nanoparticles (ZnO NPs) utilizing both green and conventional chemical synthesis methods. The comparative analysis of these synthesis techniques revealed that both approaches could yield stable and well-dispersed ZnO NPs with desirable physicochemical properties. The green synthesis method, in particular, offers an environmentally friendly and cost-effective alternative, minimizing the use of hazardous chemicals and making it more suitable for biomedical applications.

The interaction between the synthesized ZnO NPs and bovine serum albumin (BSA), a standard model protein, was investigated to evaluate their biocompatibility. The results demonstrated a strong binding affinity between ZnO NPs and BSA, with negligible structural alterations to the protein. This suggests that the nanoparticles maintain their stability while interacting with biological macromolecules, which is crucial for their use in biomedical fields. Such favorable interaction profiles support the potential application of ZnO NPs in drug delivery systems, biosensing platforms, and other biomedical technologies.

Additionally, spectroscopic analysis showed an increase in absorbance from 370 nm to 400 nm upon the introduction of a surfactant to the ZnO nanoparticle solution. This shift in the absorption peak is indicative of successful interaction between the ZnO NPs and the surfactant molecules. The presence of the surfactant enhances nanoparticle dispersion by reducing agglomeration, leading to improved colloidal stability. The colorimetric data further confirmed this observation, showing a significant increase in absorbance due to the more efficient interaction of light with uniformly distributed and stabilized nanoparticles.

Looking ahead, further studies are warranted to explore the interaction of ZnO NPs with a broader range of biomolecules, such as nucleic acids, lipids, and other proteins, to gain deeper insights into their biological behavior. Moreover, systematic investigations into their cytotoxic effects and long-term biocompatibility are essential to ensure the safe and effective clinical application of these nanomaterials in therapeutic and diagnostic domains.

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