



Preparation And Characterization Of Ceftiofur-Loaded Zinc Oxide Nanoparticles (Zno Nps)

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ABSTRACT

Nanotechnology is a scientific field focused on the manipulation of matter at the nanometer scale. Nanoparticles have achieved significant attention in drug-delivery applications because of their small size and large a surface-area-to-volume ratio, which enable improved drug loading and controlled release. These features offer the potential to overcome limitations associated with conventional drug formulations and enhance therapeutic efficiency. In this research, we used sol-gel method to produce zinc oxide nanoparticles, using zinc acetate as a precursor. Ceftiofur, the antibiotic of interest, was subsequently loaded into the nanoparticles, and the resulting samples were freeze-dried. Both blank and ceftiofur-loaded nanoparticles were characterized using dynamic light scattering to determine particle size, polydispersity index (PDI), and zeta potential, and transmission electron microscopy to assess morphology. The prepared blank zinc oxide nanoparticles exhibited an average diameter of 245.8 nm, a PDI of 0.621, and a zeta potential of +26.23 mV. The ceftiofur-loaded nanoparticles showed a slightly larger diameter of 288.13 nm, a PDI of 0.609, and a zeta potential of +27.16 mV. Transmission electron microscopy revealed that both nanoparticle types were nearly spherical in shape, confirming successful synthesis and drug loading.

INTRODUCTION

Antibiotics are frequently used in animal production to combat sickness and stimulate growth. Approximately 70% of antibiotics are reportedly utilized in food-producing animals worldwide. As a result of the rising demand for animal protein, it is predicted that the use of antibiotics in livestock production would only keep rising. Research has demonstrated that antibiotic residues in food can harm humans. Thus, antibiotic contamination of animal products

has emerged as a serious concern to public health (Zhang et al., 2021). Furthermore, the existing approach to antibiotic administration presents several challenges. These include the requirement for a sizeable and knowledgeable workforce capable of diagnosing and dosing numerous animals, the need for repetitive drug administration to maintain effective plasma concentrations, and in certain instances, the necessity for higher doses to achieve therapeutic levels at the targeted site. These latter conditions can lead to potentially harmful medication levels in the bloodstream

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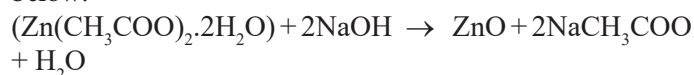
and adverse effects (Ahmed & Kasraian, 2002). The effects of nanotechnology have advanced to a higher level in recent decades. Metal nanoparticles and their composites, in particular, have received a great deal of attention from researchers and are used in a widely (Khalil et al., 2017; Mishra et al., 2017) Metal oxide nanoparticles have also demonstrated potential and a wide range of applications in the biomedical field, including cell imaging, anticancer drug/gene delivery and biosensing. Many human tissues, including the muscle, brain, bone and skin contain zinc, which is often recognized as an essential trace element. Zinc participates in metabolic processes, and plays a crucial part in the formation of nucleic acids and proteins, neurogenesis, hematopoiesis, and other enzyme systems (Ruszkiewicz et al., 2017). Due to their wide bandgap and high exciton binding energy, zinc oxide nanoparticles (ZnO-NPs) possess unique physical and chemical characteristics. According to Ruszkiewicz et al. (2017), it is among the most prominent metal oxide nanoparticles and is used extensively across several fields. Zinc oxide is a versatile material because of its distinctive chemical and physical characteristics, including its high electrochemical coupling coefficient, strong chemical stability, a broad spectrum of radiation absorption, and high photo-stability (Segets et al., 2009). Cephalosporins are a class of antibiotics known to attach with the penicillin-binding proteins (PBPs), which prevents the transpeptidation of the peptidoglycan cell layer and, as a result prevent the production of the bacterial cell wall. Ceftiofur (CEF), a third-generation cephalosporin, has received approval for the treatment of specific infectious diseases. Notably, this antibiotic has demonstrated successful outcomes in addressing respiratory problems in bovines, swines, and poultry (FDA, 1992). Its effectiveness has also been observed in horses and various other livestock. Furthermore, ceftiofur has been used for the treating mastitis in dairy cattle (Folz et al., 1992). There are multiple methods available for characterizing nanoparticles. Morphology and shape can be determined through the use of Transmission Electron Microscopy (TEM), while Zeta size and Zeta potential can be estimated using Dynamic Light Scattering (DLS). Additionally, release kinetics and drug encapsulation efficacy are utilized to assess the amount of drug contained within the nanoparticles and its subsequent release.

Materials And Methods

Synthesis of zinc oxide nanoparticles

ZnOPs were prepared using the sol-gel method employed by Hasnidawani et al., (2016). Initially, 2g of zinc acetate

was dissolved in 15ml distilled water under constant agitation for 60 min. Separately, a sodium hydroxide solution (8gm in 10ml) was prepared and integrated into the zinc precursor. The prepared suspension was titrated with 100ml ethanol dropwise while stirring until a white precipitate was obtained. The solution was kept undisturbed at 37° in the incubator overnight. On the next day, the solids were filtered and washed with methanol and distilled water to get rid of any residue. The final product was dried at 150°C and pulverized using a mortar and pestle. The overall chemical reaction that occurs is stated below:



Preparation of ceftiofur-loaded zinc oxide nanoparticles

For loading ceftiofur into zinc oxide nanoparticles, 0.11gm of C-tab was added to 50ml distilled water, then 140 mg zinc oxide nanoparticles were added to it and stirred for 2 hrs using a magnetic stirrer with hot plate maintained at 40°C. to this 154mg ceftiofur was added and mixed for another 2 hours at room temperature using a magnetic stirrer (Anand & Varghese, 2015). After complete mixing, the solution was freeze-dried and used in the experiment.

Dynamic Light Scattering (DLS)

The Dynamic Light Scattering (DLS) technique was used to examine hydrodynamic diameter (i.e., size), particle size distribution, and zeta potential of the nanoparticles. DLS measurements were done using a Zetasizer Nano ZS manufactured by Malvern Instruments in Malvern, UK. The DLS analysis employed a red laser with a wavelength of 632.8 nm, operating at a detection angle of 250° and 173°. The measurements were conducted in a circular aperture glass cell, and an incubation period of 60 seconds was applied.

Morphology of Nanoparticles

To assess the shape of ZnOPs, transmission electron microscopy (TEM) was used. The samples were analyzed using the High-resolution Transmission Microscope (HRTEM), specifically the JEOL model JEM 2199 Plus, which was equipped with a 1024x1024 pixel digital camera. To prepare the TEM sample, one milliliter of the solution was promptly centrifuged, and the resulting pellet was then resuspended in deionized water. The resuspended sample

was subsequently applied onto support grids, allowed to dry, and then subjected to examination.

Results And Discussion

Polydispersity index (PDI), Particle size, and Zeta potential analysis

The average particle size of blank zinc oxide nanoparticles (ZnO NPs) was 245.8 ± 5.233 nm with an average PDI of 0.621 ± 0.012 . The average particle size of ceftiofur-

Table 1: Particle size (Z-average), polydispersity index (PDI), and Zeta potential average of blank and ceftiofur loaded zinc oxide nanoparticles (ZnO NPs)

Sample name	Z-average (nm)	PDI	Z -potential average (mV)
Blank ZnO NP	245.8 ± 5.233	0.621 ± 0.012	26.23 ± 0.145
Loaded ZnO NP	288.13 ± 4.942	0.609 ± 0.006	27.16 ± 0.120

Values given are Mean \pm SE of the results.

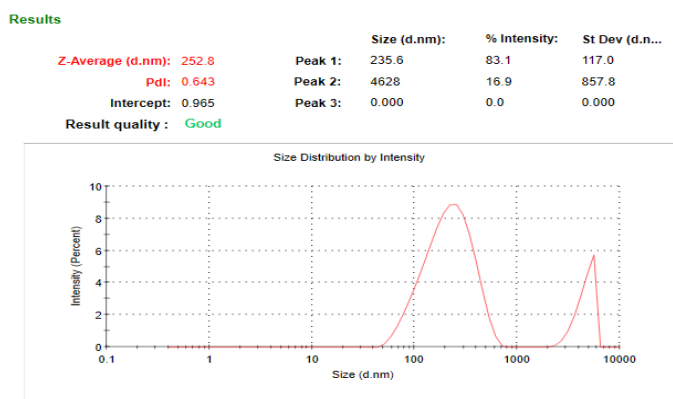


Fig. 1: Particle size of Blank Zinc oxide nanoparticles

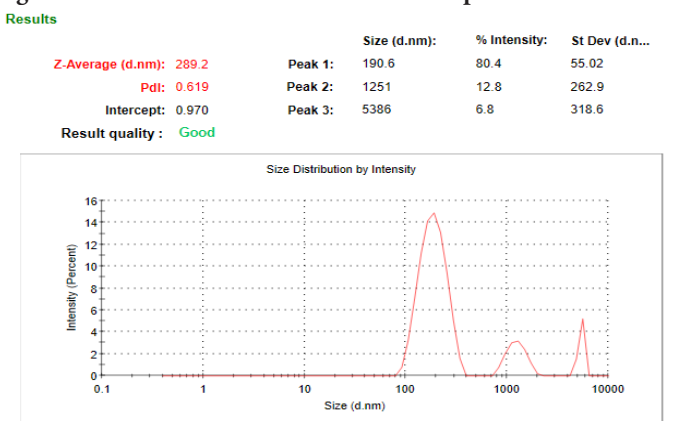


Fig. 2: Particle size of ceftiofur-loaded Zinc oxide nanoparticles

loaded zinc oxide nanoparticles (CEF ZnO NP) came to be 288.13 ± 4.942 nm with an average PDI of 0.609 ± 0.006 as depicted in **Table 1**. A similar study was conducted by Krishnamoorthy and Varghese (2015) and after sonication, the average size of zinc oxide nanoparticles was obtained to be 347nm with C-tab as a stabilizer. A particle size of 244nm and 256nm was obtained by green synthesis of zinc oxide nanoparticles done by Vignesh et al. (2021). Mendes et al., (2024) synthesized ZnO nanoparticles with different shapes and mean averages of 243 and 108 nm **Fig. 1 and 2** represent the DLS image of the size of blank and loaded ZnO NPs.

The average zeta potential of the blank zinc oxide nanoparticles was 26.23 ± 0.145 mV and that of ceftiofur-loaded zinc oxide nanoparticles was 27.16 ± 0.120 mV (**Table 1**) which suggests that C-tab being a cationic surfactant imparted a positive zeta potential to the zinc oxide nanoparticles. A zeta potential of 19.6 mV was obtained by Krishnamoorthy and Varghese (2015) after the addition of the C-tab.

TEM analysis

For further analysis, transmission electron microscopy (TEM) was used. It is a technique employed to obtain high-resolution images of samples at the nanoscale. In TEM, a small beam of electrons is transmitted through a thin sample, and the resulting electron interactions provide information about the sample's structure, composition, and morphology. The technique allows for the visualization of individual nanoparticles, crystal lattice structures, and fine details of biological specimens.

Zinc oxide nanoparticles are present in various shapes like rods, spheres, rectangles, flower, etc. depending on the type of methods used for the nanoparticle synthesis and the reagents used, time of sonication, etc. In our study, almost spherical zinc oxide nanoparticles were obtained after HRTEM analysis. **Fig. 3 and 4** show the shape of zinc oxide nanoparticles at a 50nm scale.

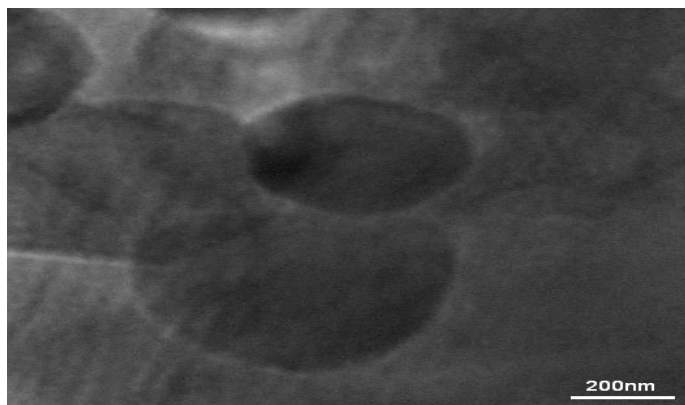


Fig. 3: TEM image of blank zinc oxide nanoparticle at 200nm scale

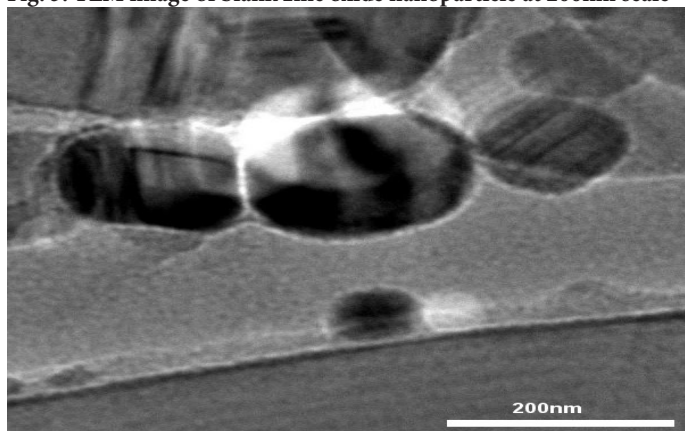


Fig. 4: TEM image of ceftiofur loaded zinc oxide nanoparticle at 200nm scale

A study conducted by Dallatu and Shallangwa (2021) found the shape of prepared nanoparticles to be distinctly spherical. Another study by Hassan et al. (2016) concluded the synthesis of almost sphere-shaped zinc oxide nanoparticles with small agglomeration.

CONCLUSION

Our study successfully synthesized zinc oxide nanoparticles using a sol-gel approach and achieved efficient loading of ceftiofur through a surfactant-assisted dispersion method. The resulting nanoparticles exhibited desirable physicochemical characteristics, including nanoscale size, uniform distribution, stable positive zeta potential, and nearly spherical morphology as confirmed by TEM analysis. These features indicate good structural integrity and colloidal stability of both blank and drug-loaded formulations. Overall, the synthesized ceftiofur-loaded ZnO nanoparticles demonstrate suitable properties for further investigation as a veterinary nanocarrier system, supporting their potential for advanced formulation development and future in-vivo evaluation.

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Conflict Of Interest

The authors declare that they have no conflicts of interest.

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