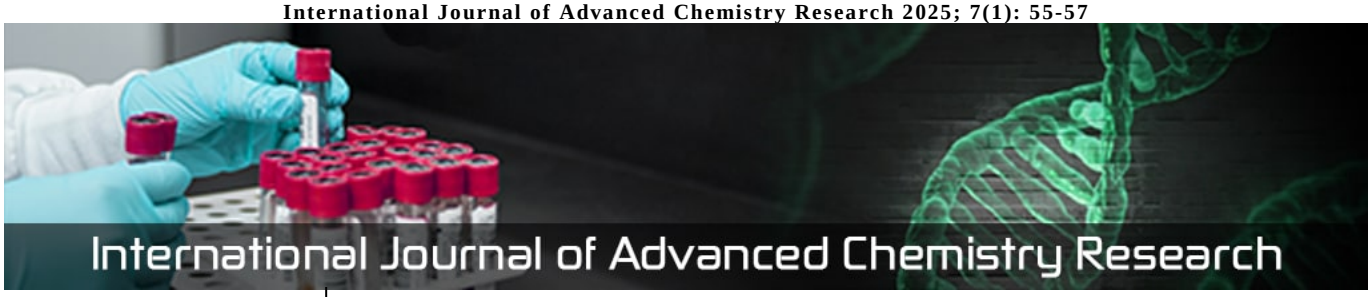

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Size and morphometric analysis of silver and chitosan nanoparticles for application in veterinary medicine

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Abstract
The highly tunable physical and optical properties of the Nanoparticles (NP's) is the ability to bind to an ever expanding ligands that have catapulted the biological tissues had created a interest in the field of nanoparticles research. In our present study we were aimed to evaluate the size and morphometry of the NP's intended for treatment purposes in animals. Our study had revealed that the particles size ranging from 100 - 200 nm will be of optimal use in medical field and can be tried as an alternative to therapeutic agents.

Keywords: Nanoparticles, optical properties, ligand binding, biological tissues, particle size, medical applications, therapeutic agents

Introduction
Nanomedicine is an emerging therapeutic and diagnostic approach in animal disease investigations and management for the effective implementation of nanotechnological systems in veterinary medicine to augment fertility and to have residues free animal foods. Nanoparticles (NP's) are incredibly small particles, typically ranging from 1 to 200 nanometers in size that can be synthesized or can be engineered according to the requirement to perform special tasks. Most commonly the nano scaled materials and external cum internal drug delivery forms had proven to be effective in the diagnosis, treatment, and prevention of both infectious and non-infectious animal diseases (Gurunathan *et al.*, 2018) [4]. In the present paper, we aimed to analyze the size and morphology of Silver, Zinc and Chitosan NP's intended for therapeutic application in the field of veterinary medicine. The size of the NP's were assessed by the Zeta potential and the morphology was assessed by the Transmission Electron Microscopy (TEM).

Materials and Methods
Dynamic Light Scattering particle size was assessed by the zeta potential analyser by the instruments DLS - Malvern Panalytical Ltd, UK. Make. DLS measures precisely the size and distribution of small particles in a solution or suspension by analyzing their Brownian motion as per the recommendations adopted by Bhattacharjee *et al.* (2016) [2].

Dynamic light scattering particle size measuring procedure
- Initially, the DLS analyzer was switched on prior to the start of to warm up the laser.
- The zeta cells were loaded as per the manufacturer's recommendations; Syringe loaded with 1 mL of pre-loaded sample in within the port of cell.
- The orientation of the cells must be on upside-down (ports oriented down) and after placing the cells NP's need to be injected to reach the half of the loop formed at the bottom of the cell, checking that no bubbles are formed into the cell.
- After loading the cells are returned to vertical position (ports up) and the samples will be injected till the cells reach the ¾ capacity volume

- The sample is filled to the maximum of volume (total volume) 0.8 mL or 0.75 mL depending on the cell).
- In DTS1070 model cells the maximum level is marked by a "MAX FILL line sign". Care was taken not to fill the sample beyond the "MAX FILL line sign".
- The electrodes were checked completely for its immersion in the medium without any bubbles
- Cells were inserted into the instrument according to the instruction of the manufacture
- For DTS 10701 cells Malvern logo was oriented towards the top or in front of the instrument
- Positive standard samples were loaded in NIST-1980 cell
- For performing the measurement; Open a new measurement file in the Malvern software by opening File->new- >measurement file, then select the zeta potential option
- Create the SOP using the software from File->new->SOP->zeta potential or open an existing one from File->Open->SOP->zeta potential to select an existing SOP file
- For routinely analysis disperse the sample in 10 mM NaCl and measure the zeta values at 25°C.
- For 10mM NaCl and for Malvern negative standard, follow the SOPs described by the manufacturer's instruction and report the step by step in this session.

Transmission electron microscopy (tem) for morphology analysis of np's

Transmission Electron Microscopy (TEM) (JEM-2100Plus: A new 200kV Transmission Electron Microscope, Pvt, Ltd., JEOL Benelux, Tokyo) was performed to assess the nanoparticle size, grain size, size distribution, and morphology pattern in liquid dispersion as reported by Filippov *et al.* (2023) [3].

Procedure for TEM analysis

- Formvar-filmed 400 mesh Cu grids (Graticules Optics Limited, UK) was used as a platform to prepare standard methods
- Using forceps, two Formvar-filmed grids was inserted into each labeled mPrep/g capsule.
- Each mPrep/g capsule containing grids was attached to an mPrep/f filter coupler and placed on a channel of a Pipetman Neo® 8-channel P200 pipettor attached to TEM
- 40 µl of aqueous nanoparticle suspension was pipetted into a row of 8 microplate wells to accommodate 8 grid-containing mPrep/g capsules.
- After adjusting the volume of the pipettor to 35 µl, the nanoparticle solution was aspirated into the mPrep/g capsules, and held inside the capsule for 30 minutes of adsorption, and then dispensed to waste
- Non-adherent nanoparticles were rinsed out using 8 changes (8 x 35 µl) of DI water by aspirating and dispensing to waste (Figure 2).
- After air-drying for 5 minutes to enhance particle adsorption to the filmed grids, 35 µl of 50% ethanolic 2.5% uranyl acetate stain was aspirated into each mPrep/g capsule, held inside the capsule for 10 minutes using the lab stand support, and then dispensed to waste.
- The stain was rinsed out with 8 changes of distilled water.
- Each mPrep/g capsule was then separated from its mPrep/f coupler and placed uncapped in the mPrep capsule/grid box to air-dry the grids, and then closed and stored until TEM imaging.
- Grids were removed from the mPrep/g capsules with forceps and imaged with an FEI Tecnai™ T12 at 80 KeV and recorded to an FEI Ultrascan™ Camera

Results and Discussion

Particle and size characterization of NP's

The assessment nanoparticles were evaluated for its size by the zeta potential in Dynamic Light Scattering process (DLS) and the physical appearance was characterized by the Transmission Electron Microscopy (TEM).

The particle size (diameter-nanometer) of Ag NP's and CS NP's was determined by Zeta potential was 152.2, 185.4, respectively. The size and distribution of CS NP's for exerting efficient therapeutic efficacy ranges between 124.1-402.3 nm (Zoe *et al.*, 2023) [9]. Similar to the findings of Zoe *et al.* (2023) [9] our study also demonstrated the size of 148.8 nm for CS NP's.

Vo-Van *et al.* (2023) [7] reported that to achieve desired therapeutic efficacy the size and shape of the Ag NP should be within 200 nm size and must be spherical in shape. In our study the size of the Ag NP was 152.2 nm and our study findings concurs with the findings of Vo-Van *et al.* (2023) [7]. Further, the TEM analysis for the Ag NP's and CS NP's morphometry showed 149.8 and 194.41with a standard deviation of 12.67 and 19.14, respectively and tabulated in Table. 21. Morphometric analysis revealed presence of round shape of NP's for the Ag NP's and CS NP's.

Spherical cum round morphology of NP's are often preferred for its uniformity in size distribution, increased stability, better surface interaction for achieving therapeutic and diagnostic targets. Uniform size was considered to be an essential factor for drug loading and delivery systems and also for contrast enhancement in diagnostic imaging procedures (Gurunathan *et al.*, 2018) [4].

To achieve desired therapeutic applications of NP's they must penetrate deep into the the biological barriers *viz*., for tumor therapy (Rodrigues *et al.*, 2024), reducing the inflammatory process (Ashraf *et al.*, 2024), removal of bacterial infection (Gurunathan *et al.*, 2014) [10] and to promote tissue regeneration size (Kushwaha *et al.*, 2022) [5]. The size and distribution of NP's was considered to the crucial step for NP's delivery process to the targeted tissues for diagnostic and therapeutic purpose. The larger size of NP's will have therapeutic difficulties like the poor penetration owing to larger size and less conducive for deep tumor penetration and uniform drug distribution into the target organs. Hence NP's ranges between 50-200 nm will be optimal for achieving desired diagnostic and therapeutic properties (Xu *et al.*, 2023) [8].

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