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Research Article

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Multifunctional chitosan nanoparticles: Zn²⁺ adsorption, antimicrobial activity, and promotion of aquatic health

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Abstract: Chitosan nanoparticles (CSNPs) are one of the versatile materials that has potential applications in multidomains. However, these diverse applications are not seen in CSNPs produced from a single source. Owing to the above, in this study, we synthesized CSNPs using the ionotropic gelation method and characterized them with UV-visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) techniques for multi-application investigations. SEM and TEM analyses confirmed the spherical shape of the produced CSNPs with their size ranging from 10 to 40 nm. CSNPs were utilized for Zn²⁺ ion adsorption from aqueous solutions, optimized using response surface methodology. Optimal reaction conditions were pH 7, 60 min of contact time, and 100 mg/L initial concentration, and a loading capacity of 156.2 mg/g was achieved. The model was significant with p-value <0.0001 and lack-of-fit >0.1. CSNPs exhibited antimicrobial activity with zones of inhibition of 25 mm for Escherichia coli and 24 mm for Staphylococcus aureus. Feeding trials with CSNP-supplemented diets in tilapia showed enhanced growth, reduced mortality, and improved red blood cell and white blood cell counts. These findings

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Chitosan Nanoparticles: Synthesis, Characterization, and Applications





demonstrate the multi-functional potential of CSNPs prepared from one source, highlighting their applicability in sustainable practice.

Keywords: chitosan, nanoparticle, adsorption, antimicrobial, diet supplement, sustainability

1 Introduction

Nanotechnology has evolved significantly in almost all domains of science, engineering, technology, and medicine [1]. The unique size- and shape-dependent properties have made the nanomaterials most promising in almost every domain [2]. In general, metal nanoparticles (NPs) are found to have several unique applications in various domains. Similarly, organic- or polymer-based NPs are finding prominence in medicine, wastewater treatments, and biomedical and food supplements.

Chitosan (CS) is a natural polymer that is abundantly available from plant sources. It is eco-friendly and economical in nature and its conversion to NPs will have significant applications. A very useful review of futuristic applications of chitosan nanoparticles (CSNPs) is reported by Murat and Karin [3]. Of the numerous applications, application in wastewater treatments is popular, and numerous investigations

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are performed on the removal of heavy metal ions and synthetic dyes using CSNPs. CSNPs with a mean size range of 40–100 nm were successfully employed in the adsorption of lead ions from aqueous solution with a loading capacity of 398 mg g⁻¹ [4]. CSNPs prepared via the ionic gelation method were successfully used in the adsorption of eosin Y anionic dye with a high loading capacity of 3.3 g g^{-1} [5]. In another study, CSNPs extracted from shrimp shells were evaluated for the removal of Fe²⁺ and Mn²⁺ ions from aqueous solution with loading capacities of 116.2 and 74.1 mg g⁻¹, respectively [6]. An insightful review on CSNPs as a potential nanosorbent for the removal of pollutants is reported by Benettayeb et al. [7].

Antimicrobial applications of CSNPs are another interesting area of investigation that has attracted the researchers. CSNPs prepared via the ionic gelation method were evaluated for antibacterial activity against Escherichia coli, Salmonella choleraesuis, Salmonella typhimurium, and Staphylococcus aureus, and the minimum inhibitory concentration values were less than $0.25 \,\mu g \,m L^{-1}$ [8]. In another study, CSNPs were evaluated as antimicrobial agents against Klebsiella pneumoniae, E. coli, S. aureus, and Pseudomonas aeruginosa and found to be superior to CS and chitin [9]. A comparative investigation against bacteria and fungi was performed for CSNPs and found that CSNPs were effective against Grampositive and Gram-negative bacteria compared to fungi [10]. A review on the antibacterial activity of CSNPs against various Gram-positive and Gram-negative bacteria has been reported by Chandrasekaran et al. [11], who highlights the role of CSNPs in biomedical applications.

The CSNPs not only find applications in wastewater treatment or biomedical application but have significant role in the food industry as well. CSNPs are safe to use as dietary supplements to fish to improve their growth and enhance their production and health [12]. CSNPs are biocompatible and non-toxic materials used in therapeutics, gene transport, biopesticides, as well as efficient fish feed additives [13]. In a study, CSNP-based fish feed administered to *Oreochromis niloticus* (L.) showed enhancement in their health acting as a better immunostimulant and antioxidant agent [14]. It was observed that CS-fortified meals cause significant enhancement in the health status compared to plant-based meals.

Owing to the multiple application potential, this study aims to synthesize CSNPs via the ionic gelation method and explore their application in adsorption of Zn²⁺ ions from aqueous solution, evaluate the antimicrobial efficacy of CSNPs, and further prepare them as a fish feed meal additive and evaluate their influence on fish growth and health.

2 Materials and methods

2.1 Synthesis of CSNPs

The CSNPs were synthesized using the ionotropic gelation method, with minor modifications to the concentrations of CS and sodium tripolyphosphate (STPP). Initially, 3 g of CS powder (commercially purchased) was set aside, while a solution of 1 mL acetic acid mixed with 800 mL of distilled water was prepared and stirred with a magnetic stirrer for 20 min. The CS powder was gradually added to the stirring solution, and the mixture was maintained at 450 rpm. To ensure optimal conditions, the pH was adjusted to 4-5 by adding a sodium hydroxide (NaOH) solution prepared by diluting two NaOH pellets in purified water. This solution was stirred continuously for 12 h to achieve complete dissolution and stability of the CS. Concurrently, an STPP solution was prepared by dissolving 0.40 g of STPP in 100 mL of distilled water using a motorized stirrer for 30 min to ensure complete dissolution. Subsequently, the STPP solution was added dropwise to the CS solution under instantaneous magnetic stirring for 40 min, creating a 0.4% (w/v) STPP solution. The resulting CS-TPP solution was centrifuged at 8,000 rpm and 4°C for 45 min, and the resulting pellets were washed thoroughly with distilled deionized water to remove impurities. The cleaned pellets were then dried in a hot air oven and stored for further use. These dried CSNPs were characterized for their properties and utilized in feed preparation, demonstrating their potential for practical applications.

2.2 CSNP characterization

The CSNPs were characterized using UV–visible spectroscopy (Shimadzu, Japan) in the range of 200–500 nm to find the maximum absorption peak to confirm preliminary formations. Fourier transform infrared (FTIR) spectroscopy (Avatar330, Thermo Nicolet) was employed to identify the surface functional groups in the range of 4,000–400 cm⁻¹ via the KBr pellet method. Powder X-ray diffraction (XRD; Siemens D5000 X-ray diffractometer) was utilized to obtain the diffraction peaks of the CSNPs in the 2 θ range of 5° and 100°. Scanning electron microscopy (SEM; SUPRA 55, Carl Zeiss NTS GMBH) was performed to assess the surface morphology of the CSNPs. Transmission electron microscopy (TEM) was performed to visualize the size and shape of the CSNPs, and it was performed on a Cu grid containing carbon coating.

2.3 Batch adsorption studies

The adsorption of Zn^{2+} ions by CSNPs was performed in batch adsorption by varying the independent variables. The optimization of independent variables was performed using the central composite design (CCD) of response surface methodology (RSM). All the experiments were performed for 50 mL test solutions in an orbital shaker at 100 rpm. A full factorial design with 20 experiments was used to make it efficient for fitting data and building models, and important factors and their details are given in Table 1. The construction of CCD and building of models were performed with Design Expert 13 software.

The adsorption efficiency and loading capacity of CSNPs were calculated using the following equations:

% Zn²⁺ ion adsorption =
$$\frac{C_0 - C_1}{C_0} \times 100$$
, (1)

Adsorption capacity =
$$\frac{C_0 - C_1}{m} \times V.$$
 (2)

2.4 Antimicrobial assay

The antibacterial properties of CSNPs were tested using the agar well diffusion method on Mueller-Hinton Agar (MHA) medium. Sterilized MHA was poured into sterile Petri dishes under aseptic conditions and left to solidify for 1 h at room temperature. Overnight cultures of E. coli (Gram-negative) and S. aureus (Gram-positive) were standardized to approximately 108 CFU/mL, matching the 0.5 McFarland standard, and evenly spread over the MHA surface using sterile cotton swabs. Sterile wells, 7–8 mm in diameter, were created in the agar using a cork borer. CSNP suspensions were prepared in sterile distilled water, and 50 µL of each suspension was added to the wells. The plates were kept at room temperature for 30 min to allow diffusion of the NPs into the agar and then incubated at 37°C for 24 h to facilitate bacterial growth and interaction with the NPs. After incubation, the zones of inhibition (ZOIs) around the wells were measured with a calibrated ruler to evaluate the CSNPs' antibacterial effectiveness. Each experiment was performed in triplicate to ensure reproducibility and statistical reliability.

Table 1: Independent variables, their levels, and symbols used in this study for Zn²⁺ ion adsorption by CSNPs (data from authors' own research)

Variables	Symbol	Levels		
		-1	0	+1
рН	А	4	6	8
Contact time	В	30	45	60
Initial concentration	C	50	100	150

2.5 Diet preparation and experimental desian

Fingerlings of O. niloticus (L.) were obtained from nursery ponds and acclimatized to laboratory conditions in an indoor spun-glass aquarium for 2 weeks. The aquarium was aerated continuously, and a controlled light source maintained a 12-h light-dark cycle. A total of 30 fish, with an average weight of 16.21 ± 0.24 g, were randomly distributed among three experimental groups, with 10 fish housed in each 3L aquarium tank, replicated four times. The groups included Experiment 1, where fish were fed a commercially available feed; Experiment 2, serving as the control group; and Experiment 3, where fish were provided with a CS-enriched feed. All aquariums were equipped with compressed air pumps to ensure proper aeration. The fish were fed their respective diets twice daily, at 9:00 AM and 2:00 PM, for 45 days, with feed provided until the fish were satiated. Each day, settled waste and half of aquarium water were removed and replaced with fresh, aerated tap water from a holding tank. Mortality records were maintained daily, and any deceased fish were promptly removed from the tanks to ensure optimal experimental conditions.

2.6 CSNPs loaded with fish feed

The synthesized CSNP pellets were dispersed in distilled water to prepare a uniform 1% solution that provides moderate concentration of CSNPs. This suspension was loaded into a 20 mL syringe and sprayed evenly onto the prepared fish feed. The CS-coated feed was thoroughly mixed and then dried in a hot air oven at 100°C. Once dried, the CS-enriched feeds (as described in Table 2) were provided to the fish in experimental tanks 1–3 for a duration of 1 week. At the end of the observation period, the fish were weighed, and their lengths were measured to evaluate their growth. The initial and final weights, as well as the initial and final lengths of the fish, were recorded to assess the impact of the CS-loaded feed on their growth and development.

3 Results

3.1 Synthesis of CSNPs

The ionic gelation approach was used in the current study to create CS nanosuspension. The above-mentioned procedures are advantageous for CSNP synthesis since they are quick and gentle, requiring no organic solvents or high temperatures. It hinges on the ionic connection between

 Table 2: Ingredients and chemical analysis of the experimental diets

 containing CS-loaded NPs (% dry weight) (data from authors' own

 research)

Components	Control	Formulated feed	CSNPs (1%) formulated feed
Chicken meal	16.0	16.0	16.0
Fish meal	11.5	11.5	11.5
Soya bean meal	32.0	32.0	32.0
Groundnut cake	19.5	19.5	19.5
Wheat bran	11.0	11.0	11.0
Corn oil	1.0	1.0	1.0
Codfish oil	2.0	2.0	2.0
Vitamins	1.0	1.0	1.0
Minerals	2.0	2.0	2.0
Starch	4.0	4.0	4.0
TOTAL	100	100	100
Elemental analysis	(%)		
Solid matter	92.9	93.5	93.8
Protein content	32.6	33.4	34.7
Crude fat	6.9	6.9	7.1
Ash content	5.1	5.7	5.9
Insoluble fiber	5.2	5.5	5.8
Nitrogen-free extract	50.11	50.25	50.77
Total energy (kcal/100 g)	451.6	457.2	460.8

the positively and negatively loaded amino groups in the acidic CS solution and the aqueous STPP solution. The molecules with opposing charges can form inter-molecular crosslinks due to the ongoing magnetic churning. TPP was gradually included into the CS solution to produce the ionotropic gelation of CSNPs.

The synthesized CSNPs had monodispersed NPs of around 100 nm in size and consistently low polydispersity index (PDI) values. The stability and structural uniformity of NPs were assessed using the PDI. In this study, low PDI values denote higher levels of monodisperse CSNPs and higher levels of particle equilibrium, whereas higher values suggest lower levels of particle equilibrium and CSNP accumulation. Opacity of the synthesized CSNPs was verified by the presence of opalescence, which was evaluated for physico-chemical traits like crystalline structure, thermal potential, ultra-structural morphology, and the dimension of particles.

3.2 Characterization of CSNPs

3.2.1 UV-vis absorption spectroscopy

The optical properties of CSNPs were analyzed using UV–visible spectroscopy within a wavelength range of 200–500 nm. A sharp absorption peak was observed at 240 nm in the UV–vis



Figure 1: UV-vis spectra of CSNPs prepared via the ionic gelation method (data from authors' own research).

spectra, which is due to $n-\sigma^*$ of the $-NH_2$ and -OH groups (Figure 1). The band around 280 nm is due to the $n-\pi^*$ of the acetyl group of CS. The bands observed in CS bulk as well as minor shifts in wavenumbers due to the nanosize nature of CS confirm the formation of CSNPs. Similar observations were reported for the biologically synthesized CSNPs [15].

3.2.2 FT-IR spectroscopy analysis

The molecular structures of CSNPs were analyzed using FT-IR spectroscopy, as shown in Figure 2. The FT-IR spectra revealed characteristic peaks, indicating specific molecular vibrations and functional groups. A broad absorption band at 3,358 cm⁻¹ corresponds to the –OH groups of the CSNPs, and a small peak at 3,252 cm⁻¹ overlapped with the –OH peak indicates the –NH stretching. The peak at 2,873 cm⁻¹ is due to the symmetrical stretching of -CH groups [16]. Bands at 1,647 and 1,587 cm⁻¹ correspond to the amide I C=O stretching and amide II C-N stretching vibrations, respectively, and these bands were shifted compared to the CS bulk, and this is due to the formation of NPs and their interactions with the cross linker TPP used in this study [17,18]. The peak at 1,145 cm⁻¹ corresponds to the glycosidic linkage group of CSNPs. The peak at 695 cm⁻¹ is primarily due to N–H bending that arises due to NH₃⁺ in the ionic gelation method. These findings confirm the presence of various functional groups, validating the molecular structure and composition of the synthesized CSNPs.

3.2.3 Field emission SEM analysis

SEM was employed to analyze the microstructures of the synthesized CSNPs, focusing on their size, shape, and



Figure 2: FT-IR spectrum of CSNPs prepared via the ionic gelation method. Inset: FT-IR spectrum of CS (data from authors' own research).

dispersion patterns (Figure 3). The SEM images revealed that the CSNPs exhibited a spherical shape with a homogeneous nanostructure. The particles were uniformly distributed and of nanoscale size, indicating that the interaction between CS and TPP molecules successfully facilitated the formation of well-defined CSNPs. The observed morphology aligns with the findings from previous studies, confirming the reliability of the synthesis method in producing consistent particle structures [19,20]. These results highlight the potential of CSNPs for diverse applications across various fields, including tissue engineering, environmental remediation, biomedical imaging, and drug delivery systems. This consistent particle morphology provides a strong foundation for exploring the functional capabilities of CSNPs in these domains.

3.2.4 High-resolution TEM (HR-TEM) analysis

HR-TEM analysis confirmed the size and microscopic structure of the synthesized CSNPs, prepared under optimal conditions (Figure 4). The NPs were spherical, with sizes



Figure 3: SEM images of CSNPs prepared via the ionic gelation method (data from authors' own research).



Figure 4: TEM image of CSNPs prepared via the ionic gelation method (data from authors' own research).

ranging from 10 to 40 nm. HR-TEM images revealed welldefined lattice fringes, indicative of a high degree of crystallinity in the samples [21]. The precise dry size of the CSNPs was measured through this technique, providing detailed structural insights. These findings highlight the strong interaction dynamics between CS and TPP, which play a crucial role in the formation and stability of CSNPs.

3.2.5 XRD measurement

XRD analysis was utilized to assess the morphological characteristics and crystallite dimensions of CSNPs. The XRD patterns of the CSNPs are presented in Figure 5. The crystalline quality of the CSNPs was demonstrated by a prominent diffraction peak at a 2θ range of 10° and 20°. The small and less intense peak at 10° is due to the hydrated crystalline structure of the CSNPs, confirming the reduction in crystallinity due to nanoscale compared to the bulk CS [22]. The intense peak around 20° is due to the crystalline structure of CS, showcasing the regular arrangement of the polymeric chains [23]. The presence of a broader band further indicated an imperfect crystal structure, likely due to the synthesis and processing conditions, especially the use of TPP. These findings provide insight into the crystalline properties and structural behavior of CSNPs.

3.3 RSM-based optimization

CCD was utilized to optimize the adsorption of Zn^{2+} ions using CSNPs. The study focused on three independent variables: pH, contact time, and initial Zn^{2+} concentration. The CCD included 20 experimental runs, consisting of 6 axial points, 6 central points, and 8 cubic points distributed within the design space. Tables 3 and 4 provide the experimental and predicted design matrices as well as the analysis of variance (ANOVA) results for the Zn^{2+} removal process.

Design-Expert 13 software was employed to develop a quadratic model, yielding a second-order polynomial equation (equation (3)) that defines the relationship between the selected factors and the Zn^{2+} removal efficiency, given by



Figure 5: XRD patterns of CSNPs prepared via the ionic gelation method. Inset: CS XRD pattern (data from authors' own research).

Table 3: CCD runs and % adsorption	comparison with experimenta	al and predicted values of Zn ²⁴	ion adsorption by CSNPs	data from authors' own
research)				

		Factor 1 Factor 2		Factor 3	Removal %	
Std	Run	A: pH	B: Contact time (min)	C: Initial concentration (mg/L)	Experimental	Predicted
9	1	4	75	90	29.3	32.7
13	2	7	75	10	99.5	96.3
4	3	9	120	30	56.2	58.8
16	4	7	75	90	93.1	93.1
17	5	7	75	90	93.2	93.1
11	6	7	10	90	47.5	51.9
20	7	7	75	90	93.2	93.1
10	8	10	75	90	37.8	33.4
7	9	5	120	150	78.4	76.5
1	10	5	30	30	52.4	48.8
3	11	5	120	30	69.5	72.2
14	12	7	75	190	79.3	80.5
5	13	5	30	150	22.9	20.7
6	14	9	30	150	37.2	34.9
15	15	7	75	90	93.1	93.1
8	16	9	120	150	64.1	68.4
19	17	7	75	90	93	93.1
12	18	7	150	90	93.2	89.2
2	19	9	30	30	55.7	58.6
18	20	7	75	90	93.1	93.7

$$-26.70A^{2} - 9.95B^{2} - 1.72C^{2} - 5.65AB$$
 ⁽³⁾
+ 1.25AC + 8.10BC.

The influence of each factor (pH, contact time, and concentration) on Zn^{2+} adsorption by CSNPs is reflected in the coefficients of the quadratic equation (equation (3)). Positive coefficients indicate that increasing the factor level enhances adsorption, whereas negative coefficients suggest a reduction in the adsorption efficiency. The equation also accounts for interactions between factors (two-factor terms) and the impact of these interactions on adsorption efficiency (curvature terms). The strong correlation between predicted and experimental results, as shown in Table 3, confirms the validity of the selected model and demonstrates improved Zn^{2+} adsorption in the experiments.

The ANOVA results in Table 4 affirm the robustness of the model for predicting Zn^{2+} removal using CSNPs. The extremely small *p*-value (<0.0001) highlights the model's statistical significance, indicating the results are highly unlikely due to random variation. A high *F*-value of 92.77 further reinforces the model's reliability, as larger *F*-values suggest a very low probability (0.01%) of the findings occurring by chance [24]. Moreover, the lack-of-fit *F*-value exceeding 0.1 indicates minimal unexplained variation within the model. The alignment between predicted and actual values was assessed for accuracy, as shown in Figure 6d. The points are randomly distributed along a straight line, demonstrating a normal data distribution without significant deviations, eliminating the need for

Table 4: ANOVA for the adsorption of Zn²⁺ ions by CSNPs (data from authors' own research)

Source	Sum of	df	Mean	<i>F</i> -value	<i>p</i> -value
	squares		square		
Model	11846.80	9	1316.31	92.77	<0.0001
A – pH	0.6050	1	0.6050	0.0426	0.8405
B – contact time	2539.67	1	2539.67	178.99	<0.0001
C – initial	267.30	1	267.30	18.84	0.0015
concentration					
AB	255.38	1	255.38	18.00	0.0017
AC	12.50	1	12.50	0.8810	0.3700
BC	524.88	1	524.88	36.99	0.0001
A ²	7345.31	1	7345.31	517.69	<0.0001
B ²	1151.20	1	1151.20	81.14	<0.0001
C ²	31.56	1	31.56	2.22	0.1667
Residual	141.89	10	14.19		
Lack of fit	141.86	5	28.37	56.78	0.4521
Pure error	0.0283	5	0.0057		
Cor total	11988.69	19			
R^2	0.9882		Predicted	0.9038	
Adjusted <i>R</i> ²	0.9775		R Adeq precision	28.3943	

data transformation and validating the model. Additionally, correlation coefficients nearing 1 (0.988), along with high adjusted and predicted R-squared values (0.977 and 0.903, respectively), further corroborate the model's effectiveness [25]. An adequate precision value of 28.3 underscores the model's strong predictive capability.

The interaction between independent variables and their impact on the binding of Zn^{2+} ions by CSNPs can be effectively visualized using 3D surface plots, as shown in Figure 6. These plots provide critical insights into the adsorption process. Figure 6a illustrates the combined effect of pH and contact time on Zn^{2+} ion binding. It reveals that as the pH and contact time increase, the binding efficiency also improves, reaching its peak at pH 7. However, beyond pH 7, the efficiency declines, even with extended contact times. At lower pH levels, competitive adsorption by hydronium ions on the active surface sites results in reduced binding efficiency. As the pH increases, this competition diminishes, enabling better adsorption of Zn^{2+} ions. The improvement in efficiency with longer contact times is attributed to the increased opportunity for Zn^{2+} ions to interact with the CSNP surface. The 3D plot clearly demonstrates that both pH and contact time significantly influence adsorption efficiency.



Figure 6: 3D surface plots of independent variable interactions: (a) pH vs contact time, (b) pH vs initial concentration, (c) initial concentration vs contact time, and (d) predicted vs actual values (data from authors' own research).

Table 5: ZoI of CSNPs toward	E. coli	and S.	aureus	bacterial	strains	and
comparison with standards (d	ata fro	om autl	hors' ov	vn resear	ch)	

Species	Concentration (µL)	ZoI (mm)
E. coli	20	25
S. aureus	20	24
Streptomycin disc	_	21

Figure 6b illustrates the interaction between pH and initial concentration. At the optimal pH of 6, the removal efficiency decreases with an increase in initial Zn^{2+} concentration. This decline occurs because the surface active sites on CSNPs become saturated as the concentration increases, reducing the percentage of ions bound. Figure 6c shows the synergistic effect of initial concentration and contact time on Zn^{2+} ion adsorption. While prolonged contact time enhances the adsorption efficiency, increasing the initial concentration diminishes it due to limited availability of active sites.

3.4 Antimicrobial investigation of CSNPs

The antimicrobial activity of the CSNPs synthesized in this study was evaluated against *E. coli* and *S. aureus*, with the results summarized in Table 5. The findings indicate that CSNPs demonstrated significantly superior antimicrobial activity with 25 and 24 mm of ZoI against both *E. coli* and *S. aureus* compared to the standard antibiotic streptomycin at 20 µL concentration. This enhanced activity at low concentrations can be attributed to the interaction of the NPs with the microbial cell membrane, which leads to membrane disruption and leakage of intracellular contents, ultimately compromising the viability of the microbes [9,11].

3.5 Growth and feed conversion efficiency of *O. niloticus*

Table 6 highlights that the CSNP-formulated feed significantly enhanced the growth performance of O. niloticus compared to both commercial and control basal feeds. Fish fed with the CSNP-formulated diet exhibited a higher feed intake (40.43 \pm 0.75) compared to those on the control feed. Throughout the experimental period, the CSNP-supplemented group demonstrated excellent health and growth efficiency, with a 100% survival rate, consistent across all diet groups, including the control and commercial feeds. The improved growth performance was attributed to better intestinal villi development, enhanced nutrient absorption, increased specific growth rate (SGR), and higher feed consumption in the CSNP-formulated diet group [26]. These factors collectively contributed to the observed superior growth in O. niloticus. Additionally, the dietary supplementation with CS-enriched feed improved the feed conversion efficiency and overall productivity, consistent with similar findings reported for Dicentrarchus labrax [27]. This demonstrates the efficacy of CSNPs as a dietary supplement for enhancing fish growth and health.

3.6 Hematological analysis

The red blood cell (RBC) and white blood cell (WBC) counts in the control group increased gradually from the first to the third week but showed a significant rise after being fed with an enriched diet formulated in this study starting from the first week. The RBC levels improved notably due to the loaded CSNP diet. While the control group showed gradual improvement, fish treated with the

Table 6: Growth performance and feed utilization of Nile tilapia fed with different diets, including commercial feed, formulated feed, and CSNP formulated feed (data from authors' own research)

Parameters	Control	Commercial feed	Formulated feed	CS formulated feed
Initial weight (g)	17.45 ± 0.19	17.71 ± 0.21	17.56 ± 0.12	17.62 ± 0.22
Final weight (g)	29.26 ± 0.31	29.97 ± 0.38	34.64 ± 0.31	36.7 ± 0.43
Weight gain (g)	11.81 ± 0.23	12.26 ± 0.52	17.08 ± 0.19	19.08 ± 0.38
Weight gain (%)	40.36 ± 1.31	40.90 ± 1.92	49.30 ± 1.04	51.9 ± 1.21
Specific growth rate (g)	25.71 ± 0.09	29.51 ± 0.07	42.64 ± 0.11	47.27 ± 0.07
Feed conversion ratio (g)	0.26 ± 0.31	0.23 ± 0.21	0.17 ± 0.19	0.16 ± 0.08
Total feed intake (g)	100	100	100	100
Protein conversion ratio (g)	0.40 ± 0.05	0.47 ± 0.06	0.61 ± 0.09	0.68 ± 0.31
Survival rate	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

CSNPs/kg diet exhibited a substantial boost in RBC and WBC levels of 20.0 \times 10⁶/µL and 3.2 \times 10³/µL, respectively (Figure S1). WBCs are an essential component of innate immunity in fish and release humoral chemicals like cationic antimicrobial peptides, complement system components, lectins, and cytokines [28]. The higher WBC count exhibited by fish fed with CSNP diet suggests the improved immunity compared to other diets, and this can be attributed to the enhanced activity and proliferation of lymphocytes and macrophages. Further, the cytokine production may be stimulated that promotes immune cell activation. The stress reduction and nutritional enhancement of CSNPs improved the RBC production compared to other diets supplied to the fish. Similar results were reported for adding the nanochitosan/zeolite composite to rainbow trout's diet, which resulted in an increase in serum protein levels [29].

3.7 Future directions

The findings of this study highlight the potential of CSNPs for addressing Zn²⁺ ion contamination in water in a sustainable manner. Furthermore, Zn²⁺ ion-bound CSNPs can be evaluated for their antimicrobial properties, leveraging the synergistic effects of Zn²⁺ ions and CSNPs. The incorporation of Zn²⁺ ion-bound CSNPs into aquatic environments can reduce pathogen loads, improving fish health by minimizing infections. The application of CS formulations in fish feed has already shown benefits, such as promoting healthier growth in O. niloticus. The inclusion of Zn²⁺ ion-bound CSNPs in feed formulations can further enhance the production and activation of immune cells, stimulate macrophage activity, and increase the resilience of O. niloticus to diseases and environmental stress. However, optimizing the Zn²⁺ ion loading onto CSNPs is critical to prevent zinc toxicity and ensure a balanced approach to fish health management. Current investigations are underway to determine the optimal Zn²⁺ ion loading levels that contribute to the overall growth and health of O. niloticus, paving the way for a sustainable and effective practice.

4 Conclusions

The present investigation synthesized the CSNPs via the gelation method and characterized for its surface morphology. The nanoscale of CS was confirmed by SEM and TEM techniques, and the particles were found to be in the range of 10–40 nm with a spherical shape. The CSNPs were

evaluated for their ability to adsorb Zn²⁺ ions from aqueous solution by RSM. The developed model was found to be significant with *p*-value < 0.0001 with higher *F*-value and correlation coefficients. The optimal conditions of pH 7, 60 min of contact time, and 100 mg/L initial concentration resulted in 156.2 mg/g loading capacity of Zn^{2+} ions onto CSNPs. The antimicrobial efficacy was noted to be high compared to the standards, suggesting the potential applications of CSNPs in biomedicine. The exploration of CSNPs in dietary supplements of O. niloticus proved to be prolific with improved health and growth and enhanced WBC and RBC counts. In conclusion, CSNPs offer diverse applications. With careful design, CSNPs can be sustainably utilized to capture zinc ions from aqueous solutions and incorporate them for fortification of diets for fisheries, thereby promoting improved health and growth in aquatic organisms.

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