

Original Research Article

Analysis of chitosan nanoparticles synthesized from cowry and oyster shell byproducts using *Cymbopogon citratus* and silver nitrate

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Abstract

Purpose: To determine the properties of chitosan nanoparticles synthesized from *Cymbopogon citratus* and silver nitrate (AgNO₃).

Methods: Chitosan nanoparticles (CNPs) were synthesized using *Cymbopogon citratus* (Poaceae family), aqueous silver nitrate solution (AgNO₃), and chitosan (synthesized via chitin deacetylation separately from oyster and cowry shells). Characterization of both chitosan and its nanoparticles was performed using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM).

Results: X-ray diffraction (XRD) analysis showed that the chitosan extracted from the oyster and cowry shells had crystallite sizes of 30.10 and 37.48 nm, respectively, and crystallinity indices of 68.71 and 60.22 %, respectively. On the other hand, the synthesized chitosan nanoparticles from oyster and cowry shells exhibited crystallite sizes of 18.59 and 19.34 nm, respectively, with crystallinity indices of 91.65 and 83.04 %. The FTIR analysis revealed that the synthesized chitosan exhibited characteristic peaks for C=O stretching, amide I and C=O stretching in the NHCOCH₃ group, whereas the nanoparticles showed O-H stretching overlapping N-H. Scanning electron microscopy (SEM) imaging showed that the extracted chitosan had irregular, rough edges and a coarse grainy structure, while the nanoparticles displayed small, spherical particles with a grainy texture, suggesting increased porosity.

Conclusion: Chitosan nanoparticles have been synthesized through a green process, providing an eco-friendly alternative. Additionally, the structural characteristics of the chitosan nanoparticles suggest their suitability for various industrial applications.

Keywords: Chitosan, Chitosan nanoparticles, FTIR, XRD, SEM, Crystallinity index, Crystallite size, Cowry shell, Oyster shell, Sodium nitrate

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INTRODUCTION

Nanoparticles offer significant advancements in several areas such as drug delivery, imaging,

cosmetics and tissue engineering owing to their unique properties. These nanoscale materials often exhibit enhanced characteristics compared to their bulk counterparts. Nano-sized carriers

improve various pharmaceutical processes, like bioavailability and pharmacokinetics, while also extending drug half-life and reducing the need for frequent administration [1]. Chitosan, a widely used natural polymer, is known for its non-toxic, biodegradable, and physiologically stable properties. Structurally, chitosan is a linear polysaccharide that consists of randomly arranged N-acetyl-D-glucosamine (acetylated) and D-glucosamine (deacetylated) units linked by β -(1 $\rightarrow$ 4) bonds [2].

Chitosan is commercially produced through the chemical deacetylation of chitin, which is found in the shells of crustaceans, insect exoskeletons, and fungal cell walls. The solubility of chitosan in acidic aqueous media and its low stability due to hydrophilicity influence its mechanical and chemical properties [2]. The degree of deacetylation (DD) ranges from 30 – 95 % depending on the source and processing method, which plays an important role in determining the polymer's properties and its biological activity. As a derivative of chitin, the polycationic nature of chitosan makes it valuable for nanoparticle production, with its functional groups allowing for easy chemical modifications through activation or cross-linking [2]. Chitosan nanoparticles (CNPs) have shown promising biological activity.

Some studies suggest that chitosan nanoparticles may be effective for treating viral infections, enhancing drug delivery, and acting as antimicrobial agents [3]. Various physical and chemical methods are employed in synthesizing nanomaterials, and these methods often require high energy, temperature, and pressure, and may release harmful chemicals into the environment. Green synthesis provides environmentally friendly substitutes and allows for the creation of ultrafine nanoparticles < 100 nm, which have gained popularity recently. Secondary metabolites from plant leaf extracts act as reducing agents in the biosynthesis of nanoparticles, serving as reducers, stabilizers, or both in the process of forming nanoparticles. *Cymbopogon citratus*, which is commonly known as lemongrass (Poaceae family), is a popular herb, especially in Southeast Asia [4].

Studies have reported several pharmacological properties of *Cymbopogon citratus* such as antioxidant, anti-inflammatory and anti-mutagenicity properties [4]. The difficulty in developing a reliable method for fabricating CNPs in the 200 to 300 nm range has been established [5]. However, Sharifi-Rad *et al* [6] reported that CNPs within 10 to 80 nm range show significant potential in fields such as

nanomedicine, biomedical engineering, and pharmaceuticals. This highlighted the need for effective methods to biosynthesize CNPs with ultrafine sizes. This study investigated the characteristics of CNPs obtained by green synthesis using leaf extract of *Cymbopogon citratus*.

EXPERIMENTAL

Chemicals

The chemicals and reagents used were of analytical grade, sourced from Merck Chemicals and SD Fine Chemical Ltd both in Mumbai, India.

Source and preparation of crustacean shells

The oyster and cowrie shells were collected from Warri waterside in Delta State, Nigeria, with an average weight of 100 g. They were packaged in polyethylene bags and transported to the laboratory. Upon arrival, the shells were rinsed properly with tap water, followed by treatment with boiling water at around 98 °C. After cleaning, the shells were oven-dried at 60 °C until they reached a constant weight. The dried shells were then ground into a fine powder using a high-speed blender (Philips Blender, Mumbai, India) and sieved through a 50 μ m mesh for uniformity [7].

Preparation of *Cymbopogon citratus* leaf extract

Leaves of *Cymbopogon citratus* were harvested from the Botanical Garden, University of Nigeria, Nsukka. The leaves were identified and authenticated in the Department of Plant Science and Biotechnology (voucher number: TN6228). The leaves were washed with tap water and distilled water, air-dried for 4 days, ground into fine powder and stored in an airtight container. The dried powder (1 g) was mixed with 100 mL distilled water, and heated at 70 °C for 15 min using a heating mantle, then filtered with Whatman No. 1 filter paper. The extract was then stored in the refrigerator for further use [8].

Production of chitosan from Cowry and Oyster shells

Chitosan production followed the procedure outlined by Onosakponome *et al* [9] with slight modification. The dried cowry and oyster shells (100 g) were treated separately with 2 M hydrochloric acid (HCl) for 24 h to remove minerals. The demineralized shells were then washed with distilled water and treated with 1 M sodium hydroxide (NaOH) at 60 °C for 24 h to

remove proteins. The sample was decolorized by treating the chitosan with a 0.1 % hypochlorite solution for 48 h at room temperature, followed by washing and drying at 60 °C. Deacetylation was achieved by heating the chitosan in 40 % NaOH at 100 °C under reflux for 6 h. The resulting chitosan was washed and dried at 60 °C to a constant weight, and the percentage yield (Y) was calculated using Eq 1.

$$Y (\%) = (W_1/W_0)100 \dots\dots\dots (1)$$

Where W_1 is weight of chitin or chitosan obtained and W_0 is initial weight.

Production of nanoparticles

Filtered *Cymbopogon citratus* leaf extract (10 mL) was mixed with 1 mM solution of silver nitrate (AgNO_3) solution. This mixture was stirred at 600 – 700 rpm on a magnetic stirrer for 48 h, and centrifuged at 8,000 rpm for 10 min to obtain silver nanoparticles (AgNPs). The supernatant was discarded, and the pellet was washed three times with absolute ethanol, followed by deionized water. The pellet was then dried in a hot air oven at 70 °C for 2 h. The resulting powdered AgNPs were stored in an airtight container for chitosan nanoparticle production.

Production of chitosan nanoparticles (CNPs)

Chitosan nanoparticles (CNPs) were produced following the method of Owczarek *et al* [10] with a slight modification. Chitosan (0.5 g) from cowry and oyster shells were separately dissolved in 1 mL of 1 % glacial acetic acid and 49 mL of distilled water. The previously synthesized AgNPs were then added to these chitosan solutions and stirred using a magnetic stirrer for 24 h. The synthesized chitosan nanoparticles were allowed to centrifuge at 10,000 rpm for 10 min. The resulting nanoparticle pellets were washed thrice with absolute ethanol and later suspended in deionized water. The CNP pellets were further used for different structural analytical studies.

Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared (FTIR) spectroscopy was employed to analyze the chemical structure and functional groups of synthesized chitosan and CNPs. Recording of infrared spectra was done using a Jasco FTIR 460Plus IR spectrometer (Japan) over 650 to 4000 cm^{-1} , with a resolution of 4 cm^{-1} and 100 accumulations at room temperature. A JASCO Spectra Manager software was employed in

processing the resulting data. For the FTIR analysis, samples were mixed with potassium bromide (KBr), and 16 scans were collected at a resolution of 4 cm^{-1} . The degree of deacetylation (DD) was calculated using Eq 2 [9].

$$DD = 100 - (A_{1655}/A_{3450})100/1.33 \dots\dots\dots (2)$$

The absorbances at 1655 cm^{-1} (A_{1655}) and 3450 cm^{-1} (A_{3450}) correspond to the amide-I band, indicating the presence of the N-acetyl and hydroxyl groups respectively. These peaks were used as reference points to correct for disc thickness. The factor "1.33" represents the ratio of A_{1655} to A_{3450} for fully N-acetylated chitosan.

X-ray diffraction analysis

X-ray diffraction (XRD) was performed using a Rigaku D/Max-III-C diffractometer (Japan). The samples were subjected to an X-ray beam at 40 kV and 20 mA, using non-monochromated Cu K α radiation in a 2 θ configuration at room temperature. The relative intensity was measured over a range of 2 to 70° (2 θ degrees). The crystallinity index (CrI) of chitosan and CNPs was determined using the Segal method as shown in Eq 3 [11].

$$\text{CrI} (\%) = \{(I_c - I_a)/I_c\}100 \dots\dots\dots (3)$$

where I_c represents the maximum peak intensity (crystalline portion), while I_a corresponds to the minimum intensity between the main peaks. The crystallite size (D) of each sample was calculated using Scherrer equation (Eq 4) [12].

$$D (\text{nm}) = k\lambda/\beta\cos\theta \dots\dots\dots (4)$$

where k is a constant (0.94), λ denotes the wavelength, β is the peak width at half-height, and θ is the corresponding angle of diffraction.

Scanning electron microscopy and energy dispersive x-ray analysis (SEM-EDXA)

Surface morphology of chitosan and CNPs was analyzed using a scanning electron microscope (JEOL JSM – 630, Japan) at an operating voltage of 20 kV. Prior to imaging, the dried samples were coated with a thin gold layer in a vacuum using a sputter coater.

Statistical analysis

Data was analyzed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA) software. Determinations were done in triplicates, expressed as mean $\pm$ standard

deviation (SD) and compared using the student t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Properties of chitosan and CNPs extracted from oyster and cowry shells

This synthesis was facilitated by the enhancement of hydroxyl group oxidation in chitosan by silver ions. The formation of CNPs was indicated by the appearance of a brownish-black solution, which results from the excitation of surface plasmon resonance (SPR) of the silver ions, which is a signature of nanoparticle formation. There was no significant difference in the yield of CNPs and chitosan from oyster and cowry shells ($p > 0.05$; Table 1). Furthermore, there was no significant difference in the degree of deacetylation (DD) of CNPs and chitosan from oyster and cowry shells ($p > 0.05$; Table 2).

Fourier transform infrared spectroscopy (FTIR) spectra

Figure 1 A and B and Figure 2 A and B show the FTIR spectra for chitosan and CNPs extracted from oyster and cowry shells respectively. These spectra highlighted the characteristic wavelengths of the extracted chitosan and its nanoparticles (Table 3; Figure 1).

The chemical structures of chitosan from oyster and cowry shells, and that of the produced CNPs, were confirmed through FTIR analysis. Chitosan from oyster shells exhibited characteristic peaks between 4000 and 500 cm^{-1} corresponding to NH (711.9 and 872.2 cm^{-1}), C-

O-C (1080.9 cm^{-1}), and CH_2 in CH_2OH (1457.4 cm^{-1}). Chitosan from cowry shells displayed functional groups associated with NH (711, 871, and 872 cm^{-1}) stretching vibrations, C-N stretching (1405.2 cm^{-1}), and N-H bending for primary amides (1792.8 cm^{-1} ; Figure 1 A and B). For CNPs, characteristic peaks for oyster-derived nanoparticles were observed at 3347.1 cm^{-1} (O-H stretching overlapping N-H), 1785.4 cm^{-1} (C=O stretch, N-H bending for primary amides), 1636.3 cm^{-1} (C-N stretching), and 857.3 and 711.9 cm^{-1} (NH). For the cowry-derived nanoparticles, the peaks were at 3321.1 cm^{-1} (O-H stretching), 1792.8 cm^{-1} (C=O stretch, N-H bending), 1640.0 cm^{-1} (C-N stretching), and 872.2 cm^{-1} (NH; Figure 2 A and B).

Table 1: Chitosan and chitosan nanoparticle yield (%)

Mollusk shells	Chitosan yield (%)	Chitosan nanoparticle (%)
Oyster	43.4 $\pm$ 2.00	34.7 $\pm$ 2.00
Cowry	40.1 $\pm$ 2.00	32.9 $\pm$ 2.00

Morphological characteristics

This research utilized scanning electron microscopy (SEM) in examining the morphology and nanostructures of chitosan and CNPs derived from oyster and cowry shells (Figure 3 A - D). The average chitosan and CNPs sizes ranged from 18.59 to 37.48 nm (Figure 4 A - D). The SEM imaging revealed that chitosan from oyster shells exhibited irregular and rough edges, while chitosan from cowry shells displayed a coarse, grainy structure, indicating a significant increase in porosity. Aggregation was not observed in CNPs.

Table 2: Properties of chitosan and chitosan nanoparticles extracted from oyster and cowry shells

Mollusk Shells	Chitosan			Chitosan nanoparticles		
	DD (%)	Crl (%)	Crystalline size (nm)	DD (%)	Crl (%)	Crystalline size (nm)
Oyster	74.81 $\pm$ 2.00 ^a	68.71 $\pm$ 3.00 ^a	30.10 $\pm$ 4.00 ^a	94.49 $\pm$ 3.00 ^a	91.65 $\pm$ 2.50 ^a	18.59 $\pm$ 1.50 ^a
Cowry	75.70 $\pm$ 2.50 ^a	60.22 $\pm$ 2.00 ^b	37.48 $\pm$ 1.80 ^b	96.29 $\pm$ 1.50 ^a	83.04 $\pm$ 2.00 ^b	19.34 $\pm$ 1.20 ^a

Values are expressed as mean $\pm$ SD (n = 3). ^a $P < 0.05$ vs cowry shells

Table 3: FTIR bands of chitosan and chitosan nanoparticles extracted from mollusk shell waste

Mollusk Shells	FTIR wavelength (cm^{-1})	
	Chitosan	Chitosan nanoparticles
Oyster	711.9, 857.3, 1457.4, 1785.4, 2117.1	711.9, 857.3, 1446.2, 1636.4, 1785.4, 3347.1
Cowry	711.9, 872.2, 1080.9, 1405.2, 1792.8, 2113.4	872.2, 1408.9, 1640.0, 1792.8, 2117.1, 3321.1

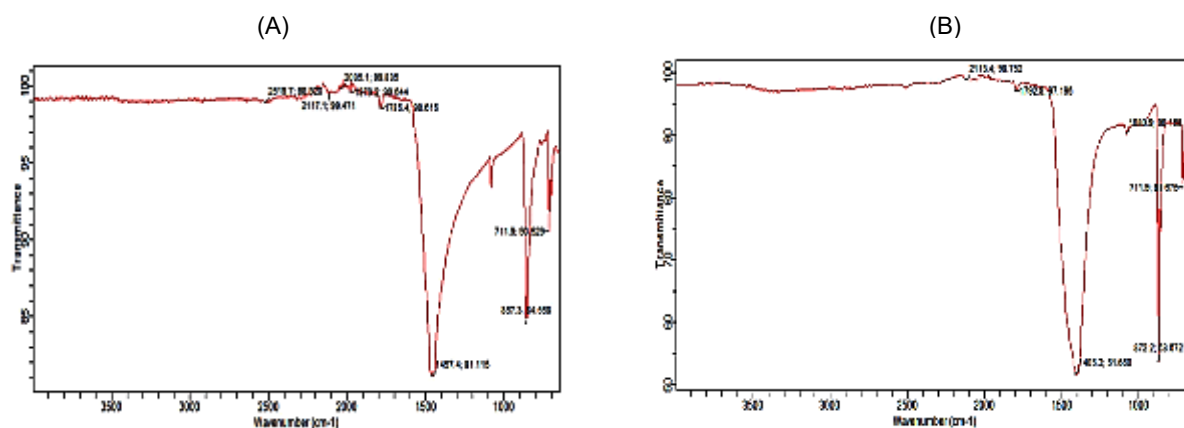


Figure 1: FTIR spectra for chitosan extracted from (A) Oyster shell (B) Cowry shell

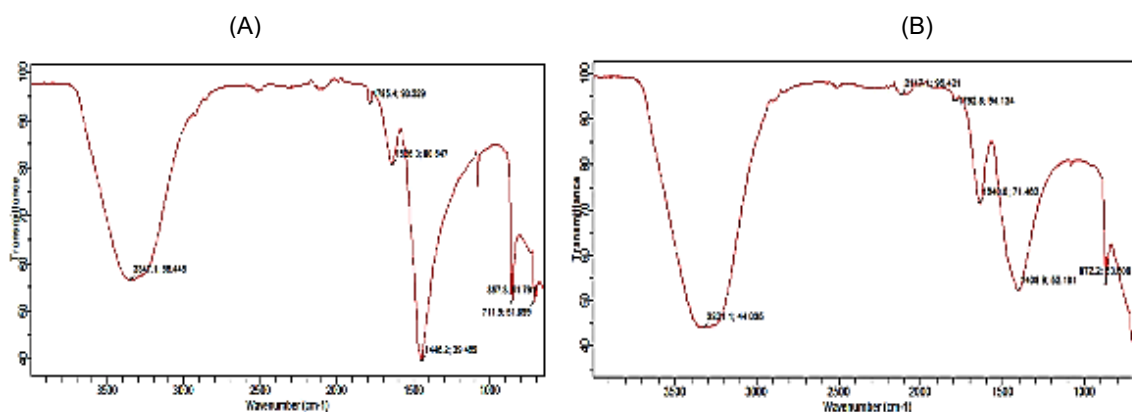


Figure 2: FTIR spectra for chitosan nanoparticles extracted from (A) Oyster shell (B) Cowry shell

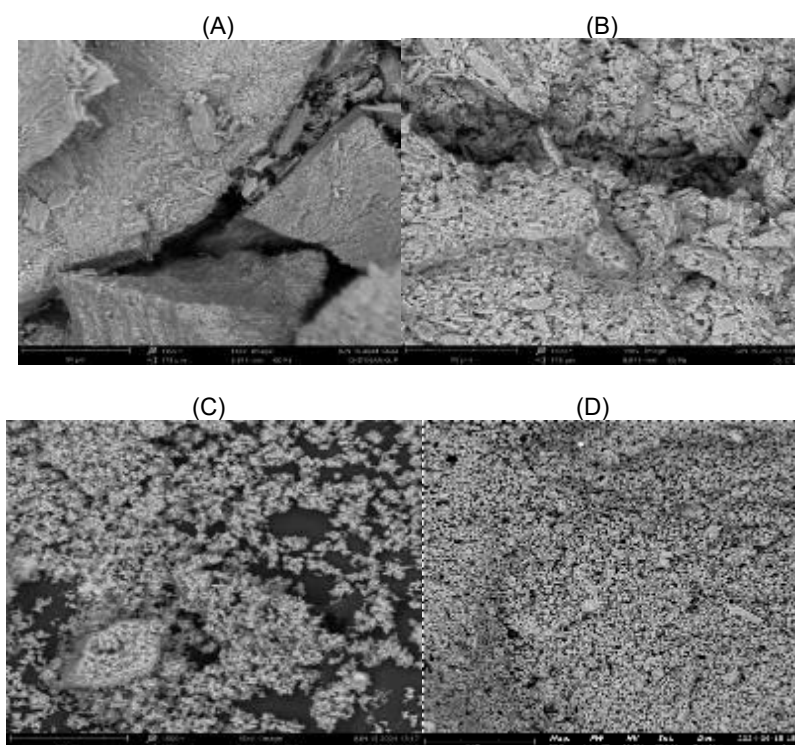


Figure 3: Scanning electron microscopy (SEM). Micrograph of chitosan and chitosan nanoparticles from Cowry and Oyster Shells (A) Chitosan from Oyster shell (B) Oyster shell Chitosan Nanoparticles (C) Chitosan from Cowry shell (D) Cowry shell Chitosan Nanoparticles

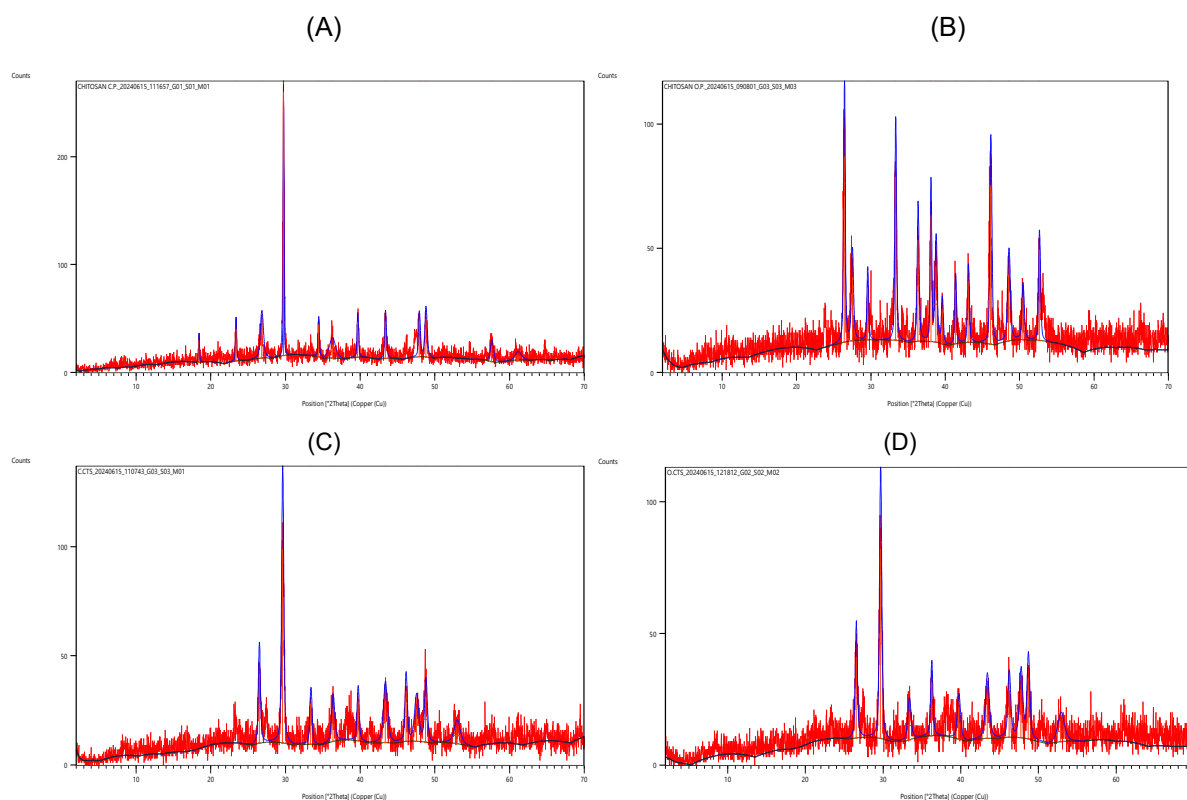


Figure 4: XRD spectra of chitosan and chitosan nanoparticles derived from Cowry and Oyster Shells. (A) Chitosan from cowry Shells (B) Chitosan from Oyster shells (C) Cowry chitosan nanoparticles (D) Oyster chitosan nanoparticles

X-ray diffractograms (XRD)

There was a prominent peak that appeared at 29.7°, with weaker peaks ranging from 34.4° to 61.1° for chitosan from cowry shells (Figure 4 A). The XRD spectrum of cowry-derived chitosan nanoparticles displayed ten peaks, with primary peaks at 29.6° and secondary peaks at 26.4°, 33.3°, 36.4°, 43.4°, 46.1°, 47.5°, 48.8°, and 52.9° (Figure 4 B). For oyster-derived chitosan, three distinct peaks were observed at 26.4°, 33.3°, and 46.1° (Figure 4 C), while the nanoparticles from oyster shells showed a prominent peak at 29.7° along with weaker peaks at 26.5°, 33.3°, 36.4°, 39.7°, 43.4°, 46.2°, 47.7°, 48.7°, and 52.9° (Figure 4 D).

DISCUSSION

In this study, chitosan nanoparticles of considerable yield were synthesized from cowry and oyster shell byproducts. The differences in yield from this study may be attributed to the significant loss of acetyl groups during the deacetylation process. Varma *et al* [13] reported a chitosan yield of 45.01 % from 100 g of *Pinna deltoide* shells. The chitin source and extraction methods utilized are frequently associated to variations in yield [13]. The degree of deacetylation (DD) is a key factor influencing the

physicochemical, biological, and mechanical properties of chitosan, and these depend on the extraction method.

Degree of deacetylation (DD) of chitosan nanoparticles (CNPs) were higher compared to that obtained from oyster and cowry shells. Kumari *et al* [14] reported DD values of 70, 75 and 78 % for chitosan derived from crab, fish and shrimp shells, respectively. Previous studies have reported a DD of 88 % in *Haliotis tuberculata*, 83.76 % in *Doryteuthis singhalensis* [15] and 89.14 % in oyster shells [7]. Degree of deacetylation (DD) is crucial in determining the appropriate applications of chitosan [9]. Lower DD (50 - 60 %) leads to lower solubility, while higher DD (> 80 %) increases solubility.

Chitosan from oysters and cowry shells in this study, with moderate solubility, makes it suitable for cosmetic products like moisturizers and skincare formulations. Conversely, the high solubility and biodegradability of CNPs, along with moderate mechanical strength, make them ideal for several applications including drug delivery, tissue engineering, wound healing, and edible food coatings. The FTIR wavelength reported for chitosan in this study is consistent. Hamrayev and Shameli [16] revealed that ammonia (NH₂) and hydrogen-bonded O-H

stretching vibrations are responsible for the strong, wide peak between 3300 and 3500 cm^{-1} of CNPs. The FTIR spectra of CNPs from oyster and cowry shells showed some changes compared to chitosan spectra. A broad shoulder at 3347.1 cm^{-1} for oysters and 3321.1 cm^{-1} for cowry, due to O-H stretching overlapping with N-H stretch, suggests the interaction between primary amino and amide groups in chitosan and silver nanoparticles. This shift indicated effective electronic interaction between the silver ions and nitrogen/oxygen-containing biomolecules in chitosan, which promotes nucleation and aggregation of metal nanoparticles during synthesis. The interactions may explain the reduction in intensity of O-H and N-H peaks during the nucleation process. Scanning electron microscopy (SEM) showed that chitosan from cowry shells showed enhanced pore density which improved its capacity as a sorbent for metal ions.

Vairamani *et al* [17] studied the highly porous structure of chitosan derived from cuttlefish. This current study therefore showed that the structure of chitosan varied across species. The CNPs showed no aggregation, suggesting that silver ions effectively dispersed within the chitosan. X-ray diffraction (XRD) analysis provides valuable information on the crystallinity, crystal structure, and size of the materials. The chitosan peaks reported in this present study compares favourably with those reported by Varma *et al* [13] derived from *Pinna deltoidea*. Furthermore, the XRD pattern of CNPs revealed distinct peaks at 43.4 ° and 52.9 °, suggesting a shift from typical chitosan peaks to those of nanoparticles. Olajire and Bamigbade [18] observed similar results, attributing the peak near 30 ° to residual inorganic materials in chitosan.

The diffraction peaks reflected the hydrated crystalline structure of α -chitin, confirming the crystalline phase in CNPs. The oyster-derived CNPs had the highest crystallinity index indicating better crystalline structure and chain alignment compared to cowry-derived chitosan nanoparticles. Chitosan from cowry shells exhibited the lowest crystallinity index suggesting that oyster shell-derived nanoparticles have superior crystallinity. This high crystallinity is beneficial for developing chitin nanofibrils, which are particularly useful in biomedical and cosmetic applications [19].

CONCLUSION

Chitosan and its nanoparticles have been biologically synthesized from cowry and oyster shell byproducts and *Cymbopogon citratus* leaf

extract respectively and then characterized. The obtained CNPs have small particle sizes, and regular spherical shapes, with significantly increased porosity. Analysis of synthesized CNPs suggests that they could be suitable for various biomedical and cosmetic applications.

DECLARATIONS

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None.

Ethical approval

None.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. Jurak M, Wiącek AE, Ladniak A, Przykaza K, Szafran K. What affects the biocompatibility of polymers? *Adv Colloid Interface Sci* 2021; 294: 102451.
2. Ma J, Wang Y, Lu R. Mechanism and application of chitosan and its derivatives in promoting permeation in transdermal drug delivery systems: A review. *Pharmaceutics* 2022; 15: 459.
3. El-Naggar NE, Shiha AM, Mahrous H, Mohammed ABA. Green synthesis of chitosan nanoparticles, optimization, characterization and antibacterial efficacy against multi drug resistant biofilm-forming *Acinetobacter baumannii*. *Sci Rep* 2022; 12: 19869.
4. Saenthaweesuk S, Munkong N, Parklak W, Thaeomor A, Chaisakul J, Sompam N. Hepatoprotective and

- antioxidant effects of *Cymbopogon citratus* Stapf (Lemon grass) extract in paracetamol-induced hepatotoxicity in rats. *Trop J Pharm Res* 2017; 16(1): 101-107.
5. Bekmukhametova A, Uddin MMN, Houang J, Malladi C, George L, Wuhler R, Barman SK, Wu MJ, Mawad D, Lauto A, et al. Fabrication and characterization of chitosan nanoparticles using the coffee-ring effect for photodynamic therapy. *Lasers Surg Med* 2022; 54(5): 758-766.
 6. Sharifi-Rad J, Quispe C, Butnariu M, Rotariu LS, Sytar O, Sestito S, Rapposelli S, Akram M, Iqbal M, Krishna A, et al. Chitosan nanoparticles as a promising tool in nanomedicine with particular emphasis on oncological treatment. *Cancer Cell Int* 2021; 21(1): 318.
 7. Alabaraoye E, Achilonu M, Hester R. Biopolymer (Chitin) from various marine seashell waste: isolation and characterization. *J Polym Environ* 2017; 26: 2207–2218.
 8. Sousa R, Figueirinha A, Batista MT, Pina ME. Formulation effects in the antioxidant activity of extract from the leaves of *Cymbopogon citratus* (DC) Stapf. *Molecules* 2021; 26(15): 4518.
 9. Onosakponome I, Okwuenu PC, Oparaji EH, Isoje AO, Odesa GE, Awhin EP, Eze SOO, Chilaka FC. Extraction and FTIR spectra of chitin and chitosan produced from *Periwinkle* (*Tympanotonos fuscatus*) under differential decolorization conditions. *Trop J Chem* 2025; 1(1): 50-57.
 10. Owczarek M, Herczyńska L, Sitarek P, Kowalczyk T, Synowiec E, Śliwiński T, Krucińska I. Chitosan nanoparticles-preparation, characterization and their combination with *Ginkgo biloba* extract in preliminary in vitro studies. *Molecules* 2023; 28(13): 4950.
 11. Segal L, Creely JJ, Martin AE, Conrad CM. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Text Res J* 1959; 29: 786–794.
 12. Scherrer P. Determination of the internal structure and size of colloidal particles using X-rays. *Colloid Chemistry. A Textbook* 1912; 12: 387–409.
 13. Varma R, Vasudevan S, Chelladurai S, Narayanasamy A. Synthesis and physicochemical characteristics of chitosan extracted from *Pinna deltoidea*. *Lett Appl Nano Bio Sci* 2021; 11: 4061–4070.
 14. Kumari S, Annamareddy SHK, Abanti S, Rath PK. Physico-chemical properties and characterization of chitosan synthesized from fish scales, crab and shrimp shells. *Int J Biol Macromol* 2017; 104: 1697–1705.
 15. Ramasamy P, Subhapradha N, Thinesh T, Selvin J, Selvan KM, Shanmugam V, Shanmugam A. Characterization of bioactive chitosan and sulfated chitosan from *Doryteuthis singhalensis* (Ortmann, 1891). *Int J Biol Macromol* 2017; 99: 682–691.
 16. Hamrayev H, Shameli K. Synthesis and characterization of ionically cross-linked chitosan nanoparticles. *J Res Nanosci Nanotechnol* 2022; 7(1): 7-13.
 17. Vairamani S, Subhapradha N, Ramasamy P, Raveendran S, Srinivasan A, Shanmugam A. Physicochemical characteristics and antioxidant efficacy of chitosan from the internal shell of spineless cuttlefish *Sepiella inermis*. *Prep Biochem Biotechnol* 2013; 43(7): 696–716.
 18. Olajire AA, Bamigbade LA. Green synthesis of chitosan-based iron silver nanocomposite as adsorbent for wastewater treatment. *Water Resour Indus* 2021; 26: 100158.
 19. Triunfo M, Tafi E, Guarnieri A, Scieuzo C, Hahn T, Zibek S, Salvia R, Falabella P. Insect chitin-based nanomaterials for innovative cosmetics and cosmeceuticals. *Cosmetics* 2021; 8(2): 1-20.