

Characterization of Nanochitosan/Nanosilver-Particles from Commercial Low Molecular Weight Chitosan and Extracted Chitosan from Snail Shell: A Comparative Study

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Abstract

The current work aims to characterize silver nanoparticles/nanochitosan composite materials due to their potential industrial and biomedical applications. Chemical reduction of silver nitrate salts produced silver nanoparticles (AgNPs). LMWCS (low molecular weight chitosan) was obtained, and biodegradable chitosan was extracted from snail shells using a standard method. Sodium tripolyphosphate (TPP) was used to produce chitosan nanoparticles (CSNPs) from extracted chitosan and LMWCS. AgNPs-CSNPs were produced by incorporating AgNPs into CSNPs as antimicrobial agents. Characterization of the chitosan doped silver nanoparticles was accomplished using scanning electron microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). The morphological properties of nanochitosan (ScCSNPs and ArCSNPs) and AgNPs-nanochitosan (ScCSNPs-AgNPs and ArCSNPs-AgNPs) indicated porosity and agglomeration, while functional groups -OH, -NH, and C-H were revealed. The presence of AgNPs in the polymeric matrix of nanochitosan was confirmed by a shift in some of the adsorption bands. The XRD and DSC results indicate that the nanochitosan is crystalline, and they also confirm the presence of AgNPs in the chitosan polymeric matrix. The study found that chitosan extracted from snail shells, which contribute to environmental pollution, could be a good source for the preparation of nanocomposite materials, which are useful in a variety of industrial and biomedical applications.

Introduction

Chitosan and its derivatives hold immense promise in many industrial formulations due to attractive properties such as compatibility, bioadhesiveness, high drug loading and high antimicrobial spectrum. Another advantage is that they can be sourced from shells, crabs and shrimps, which abound in Nigeria. The provision of raw materials for the various industries represents a very important area of chemical research since Nigeria currently contends with heavy import burdens as far as industrial raw materials are concerned. With significant economic value, the shellfish industry is important in the fisheries sector but generates a massive amount of shell, a biowaste, that is environmentally hazardous because it creates an unsanitary environment with a strong odour. This shell, as well as insects and fungus, contains protein, chitin and minerals which are valuable resources that can be harnessed. Chitosan, a deacetylated chitin and a polysaccharide with numerous uses, can further be extracted from the shell waste [1–3]. The steps of extraction basically involve pretreatment of shell followed by deproteination and demineralization. The final step is deacetylation of the chitin to get chitosan [4, 5]. Chitosan, a (1–4) 2-amino-2-deoxy- β -D glucan contains a number of beneficial properties that have piqued the curiosity of scientists over the last two decades. Many researchers have studied chitosan and their derivatives because of their diverse applications in agriculture, engineering, biomedical, and pollution remediation due to their non-toxic, biocompatible, edible and adsorptive properties, and in pharmacology for controlled drug release and wound healing due to their hydrogel properties. Also, recently it is used for disinfection due to its antimicrobial properties [3, 6]. However, the various properties of chitosan have drawn the attention of researchers in recent years, generating significant interest in the field of biomedical

engineering [7]. Also, based on these outstanding properties, chitosan nanoparticles (CSNPs) have defied the questioned effect that nanosized materials have on nature and the human systems. In fact, the well-known polymeric and cationic properties of chitosan nanoparticles (CSNPs) have sparked interest in their application [2]. One of the biopolymers used in the formation of metallic nanoparticles as a reducing agent and a protective polymer is chitosan. CSNPs is believed to be a very promising candidate for use as a biomaterial in food-related applications. Because of their small size and high surface-to-weight ratio, CSNPs have been extensively studied in the biomedical field [3].

As versatile as CSNPs are, using high concentrations of their suspensions or quantity in industrial applications may not be cost-effective. To reduce the effective concentration of CSNPs, a synergy of CSNPs with other materials for various applications has been initiated. For example, CSNPs and the antimicrobial alkaloid berberine have a synergistic effect against *B. subtilis* and *S. aureus* [8], the traditional method of using TPP as an ionic cross-linker was replaced with cinnamaldehyde, which resulted in synergistic antibacterial activity [9], with only 5% (v/v) doping of chitosan nanoparticles into the cellulose films, a maximum *E. coli* inhibition of 85% was achieved [10]. Some metals and metal oxides that have beneficial properties were also considered in this synergistic exploitation of CSNPs. A few of these are Ag, ZnO, TiO₂, MgO and CuO. They possess strong antibacterial activity against a variety of bacterial pathogens [11].

In Nigeria, snails are a local delicacy that is utilized in making soup. However, the snail shells are not known to serve any useful purpose and form a component of waste materials from kitchens. The utilization of the shells in chitosan production is a very promising waste to wealth research. The extraction of snail shells into high-quality chitosan and subsequent doping with AgNPs becomes vital. Attempts have been made previously to convert waste materials to chitosans [11–17]. Nonetheless, no research of this kind has attempted a comparison of CSNPs synthesized from snail shell waste to CSNPs from LMWC, as well as a comparison of AgNPs-CSNPs synthesized from snail shell waste to AgNPs-CSNPs from LMWC. Therefore, academic activities on CSNPs synthesized from snail shell waste, CSNPs synthesized from low molecular weight chitosan, as well as AgNPs doped CSNPs synthesized from snail shell waste and AgNPs doped CSNPs synthesized from low molecular weight chitosan have become a novelty. Hence, the aim of the current work is to: examine the structural differences in the surface morphology, the FTIR bands, and crystal structure and cell parameters changes of the CSNPs and the AgNPs-CSNPs produced.

Results And Discussion

Figure 1 depicts SEM images of nanochitosan (ArCSNPs) derived from low molecular weight chitosan, nanochitosan (ScCSNPs) derived from snail shell, and their respective silver nanochitosan composites (AgNPs-ArCSNPs and AgNPs-ScCSNPs). The image shows that nanochitosan (Fig. 1a) has rough agglomeration and a porous surface, whereas silver nanochitosan composite (Fig. 1b) has less rough agglomeration with some smooth patches and porous surface structures, indicating that the silver nanoparticles (AgNPs) were aggregated and well distributed into the polymeric matrix. The ArCSNPs (Fig.

1c), on the other hand, had a cracky rough surface with some porosity but a rough membranous phase deposition on the cracky rough surface, as shown in Figure 1d. The presence of AgNPs may have caused the deposition. The reduction and stabilization process by nanochitosan polymeric chains may be responsible for the formation of porous structures. The porous nature of the material is critical in antibacterial and biomedical applications [18, 19]. Britto-Hurtado *et al.* [20] discovered that agglomeration is essential in antibacterial activity while researching "Agglomerates of Au-Pt bimetallic nanoparticles: synthesis and antibacterial activity."

Figures 2(a), 2(b), 2(c), and 2(d) show the FTIR spectra of ScCSNPs, ScCSNPs-AgNPs, ArCSNPs, and ArCSNPs-AgNPs, respectively while Table 1 shows the functional groups associated with corresponding peak wavelength. Figure 2 shows that nanochitosan (ScCSNPs and ArCSNPs) and its AgNPs nanochitosan composite (ScCSNPs-AgNPs and ArCSNPs-AgNPs) shared many peaks of chitosan; the functional group of hydroxyl (OH) stretching vibration, alkane C-H stretching vibration, and N-H stretching vibration [10]. The peaks 2879 cm^{-1} and 3808 cm^{-1} , 3826.2 and 3415.6 cm^{-1} , 3813.072 and 3695.49 cm^{-1} , 3826.2654 and 3685.015 cm^{-1} are assigned to O-H stretching vibration. Figure 2(a, b, c, d) showed that nanochitosan from the different sources and their respective AgNPs composite showed sharp bands between 3354.1 and 3695.494 cm^{-1} , indicating N-H stretching. The band at 2167 and 2026 cm^{-1} , as revealed in Figure 2(a) and 2(b) respectively may correspond to alkanyl C=C stretch. While in Figure 2c and 2d, the C=C stretching and bending vibration are assigned to absorption band at 1610.344 and 904.3848 cm^{-1} respectively, 1992 and 1883.5 cm^{-1} bands can be assigned to C-H stretching from alkane (Fig. 2a and b) which is one of the major absorption bands in chitosan. The absorbance values between 1866.118 cm^{-1} and 1116.067 cm^{-1} were attributed to amine and methyl compounds, CH_3 and C=N stretch, and C-O bond stretch. The peaks around 1420.522 cm^{-1} and 756.3679 cm^{-1} may have been caused by C-H bond bending probably from aromatic and vinyl compounds, respectively (Figures 2c and 2d). Murugan *et al.* [21] observed similar peak at 789 cm^{-1} which was believed to correspond to aromatic C-H bending in bio-encapsulated chitosan-Ag nanocomplex. A significant absorption band of nanochitosan was also observed at 1323 cm^{-1} (Fig 2a), which corresponded to the free $-\text{NH}_2$ group in the glucose amine. C-N has a peak at 818 cm^{-1} , which agrees seriously with similar studies conducted by other scholars [22, 23]. A vibrational mode of the amide (NH) C=O stretching was observed at 1613 cm^{-1} . In comparison to chitosan, the spectra of Ag chitosan nanoparticles composites showed a few alternations. The FTIR spectra of silver nanochitosan (ScCSNPs-AgNPs and ArCSNPs-AgNPs) show a decreasing and shifting of peak to 3415.1 and 3685 cm^{-1} , which is attributed to vibrational stretching of the N-H and O-H groups. This is because hydrogen bonding is enhanced in ScCSNPs-AgNPs and ArCSNPs-AgNPs, implying Ag chelation with both amino and hydroxyl groups of chitosan [23, 24]. The introduction of AgNPs to CSNPs may have resulted in a shift in the peak of the $-\text{NH}_2$ group and C-N at 1434.7 and 1294 cm^{-1} , respectively (Fig. 2b). The shift of band from 1613 to 1621.83 cm^{-1} is attributed to the binding of (NH) C=O group with Ag nanoparticles. In general, the stretching and bending frequency peaks could be attributed to the stretching of chitosan's carbon and hydrogen bonds. The peaks could

also indicate that a carbonyl group formed amino acid residues that capped AgNP, implying that molecules capping chitosan-Ag nanoparticles contained both free and bound amide groups [21, 25].

Table 1: Functional groups associated with corresponding peak wavelength

Functional groups	ScCSNPs	ScCSNPs-AgNPs	ArCSNPs	ArCSNPs-AgNPs
	(cm ⁻¹)	(cm ⁻¹)	(cm ⁻¹)	(cm ⁻¹)
O-H stretch from alcohol	3808.5	3826.2	3813.072	3826.265
N-H stretch	3354.1	3685	3565.898	3415.675
O-H stretch from carboxylic acid	2879.2	3415.6	3695.494	3685.015
C-H stretch	2751.9	3130.5		
O-H stretch from alcohol	2594	1994.1	3396.427	3291.782
C=N Stretch	2534	1874.9		
C=O stretch	2450	2452.4		
C=C stretch	2167	2026		
C-H stretch from alkane	1992	1883.5		
C-H from aldehyde, CH ₂ from methylene			2936.345	2812.209
N-H stretch	1613	1621.83	3565.898	3415.675
NH ₂ glucosamine	1323	1143.47		
C-N stretching	818	1294		
N-CH ₃ stretch, S-H from thiols			2785.879	2452.465
C≡C from alkyne, C-H aromatic bending			2205.112	1883.586
N=C=S from thiocyanate, C=C stretch			2033.158	1621.838
CH ₃ from methyl			1866.118	1434.784
C=C stretch, C=N stretch			1610.344	1294.012
C-H bend			1420.522	
C-O stretch			1116.067	
C=C bend			904.3848	
C-H aromatic bend			756.3679	

Figure 3 depicts the X-ray diffraction analysis result of nanochitosan and its respective silver nanoparticles composite. The results demonstrated the crystallinity of the nanochitosan and its silver nanochitosan obtained from snail shells and commercially purchased chitosan. The peak of nanochitosan was observed at $2\theta = 8.1^\circ$ (ScCSNPs) and 20.1° (ArCSNPs), which is closer to the characteristic peak of $2\theta = 9.1^\circ$ and 19.9° mentioned by Dara *et al.* [26]. The same peak was observed in their AgNPs nanochitosan composite. As also observed by Zahedi *et al.* [27] and Dara *et al.* [26], the peak at $2\theta = 20.1^\circ$ indicates the high degree of chitosan and their crystal lattice constant a corresponding to 4.4. As shown in Figure 3, peaks at $2\theta = 33.6^\circ$, 43.33° , and 46.5° appear on the spectra of ScCSNPs-AgNPs that do not appear on the spectra of Sc-CSNPs but are devoid of peaks at 64.7° and 69.1° . Also, the sharp peaks at $2\theta = 27.6$, 28.9 , 32 , and 33.9° on ArCSNPs pattern are absent on ArCSNPs-AgNPs. The presence of AgNPs in the polymeric matrix of the nanochitosan may explain the appearance and disappearance of these peaks. The Bragg reflection's numbers with 2θ values of 43.33° and 64.7° referring to (200) and (220) respectively indicate the spherical and crystalline nature of Ag NPs [1, 28]. The sharp diffraction peaks represent a large particle, whereas the broad diffraction peaks represent a smaller particle. The XRD pattern of AgNPs nanochitosan revealed the polymeric nanoparticles' crystalline nature.

Figure 4 shows the DSC thermograms of nanochitosan (ScCSNPs and ArCSNPs) and its respective silver nanoparticles composite (ScCSNPs-AgNPs and ArCSNPs-AgNPs) for a heating rate of $10^\circ\text{C}/\text{min}$ with various compositions. Except for ScCSNPs (Figure 4a), which showed heat release (exothermic) as a result of crystallization, all samples showed a glass transition (T_g) temperature, indicating good miscibility between nanochitosan matrix at the start of the heat flow. This resulted in a crystal melting temperature of 60°C . The appearance of T_g in Figure 4a for ScCSNPs-AgNPs, as well as the difference in T_g size in Figure 4b, may be due to the incorporation of AgNPs into the nanochitosan matrix, which can be attributed to the higher mobility of the polymer macromolecules [29, 30].

Methods

Snail shell was obtained from Eke-Awka market, washed and dried under the sun for 6 hours before oven drying. Low molecular weight Chitosan (LMWC) was gotten from Central Drug House (CDH).

2.1 Preparation of Chitin and Chitosan

Chitosan was prepared according to the method described by Okoya *et al.* [31]. The snail shell was ground into powder with a dry grinder and sieved through a 2 mm sieve. The sieved powdered snail shell was deproteinized by weighing 50 g of the shell powder into a 500 cm^3 beaker and 200 cm^3 of 4 % (w/v) KOH was added with constant stirring for 6 hours at 80°C and filtered. The residue was washed with distilled water until it was free of base, then dried for 2 hr at 100°C . The deproteinized snail shell residue was then placed in a 250 cm^3 conical flask with 100 cm^3 of 3 % (v/v) 1 M HCl and demineralized for 3 hr

at 30°C on a magnetic stirrer. The content was filtered, and the residue was washed until it was acid-free. The acid-free residue was then dried for 1 hr at 90 °C. Chitin, a snow-white residue, was obtained. For deacetylation, the chitin was poured into a 250 cm³ conical flask. A 50 percent (w/v) NaOH solution was added, stirred for 4 hr at 30°C, and filtered. After filtration, the chitosan residue was washed until the filtrate was neutral, then dried at 90°C for 1 hr and stored for further use.

2.2 Preparation of CSNPs from LMWC

Chitosan (2 g) was dissolved in 50 cm³ of 1% acetic acid with constant stirring. The pH of the resulting solution was maintained at 4 using 1M HCl. A 100 mg of sodium tripolyphosphate was dissolved in 100 cm³ of 1M NaOH. The solution of the sodium tripolyphosphate in 1M NaOH was added to the mixture of the acidified chitosan in drops with the aid of syringe until complete formation of precipitates. The mixture was homogenized at 1000 rpm for 5 min using a homogenizer. The mixture was filtered using a filter paper of 0.22 µm. The nanochitosan was collected as the residue [32].

2.3 Preparation of Nano silver particles

Silver nanoparticles were prepared using the chemical reduction method described by Rashid *et al.* [33], with some modifications. A 40.77 mg of sodium borohydride and 18.16 mg of silver nitrate were weighed and dissolved in 200 cm³ of ice-cold water at 5 °C in a separate beaker. Equal volumes of sodium borohydride solution and silver nitrate solution (25 cm³) were mixed as follows: the sodium borohydride solution was placed in a volumetric flask with a magnetic stirrer, which was then placed on a heater. The silver nitrate solution was poured into a burette and allowed to drip out in drops, titrating the sodium borohydride solution to a golden-brown colour. To determine the presence of silver nanoparticles, 9 cm³ of the binary mixture was diluted with 1 cm³ of distilled water and subjected to UV scanning.

2.4 Impregnation of the CSNPs with AgNPs

CSNPs (0.1 g) was dissolved in a beaker containing 0.1 g of AgNPs and a magnetic stirrer. The mixture was homogenized by placing it on a magnetic stirrer with an electric heater for 5 minutes. After heating, the mixture was filtered, and the residue containing silver nanochitosan was left to dry.

2.5 Characterization of the CSNPs/AgNPs

The particles were characterized using a variety of techniques. To examine the morphological structural changes of samples, the scanning electron microscopy (SEM) model (Phenom ProX, by phenomWorld Eindhoven-the Netherlands) was used. Functional group analysis of the materials was done using a Buck Scientific M530 USA FTIR. The samples' X-ray diffraction (XRD) data were collected using Cu K α radiation ($\lambda = 1.5418$) and the reflection-transmission spinner stage with Theta-Theta settings. The two-theta starting position was 4 degrees, and the two-theta step was 0.026261 at 8.67 seconds per step. The tension was 45VA and the tube current was 40mA. The Goni Scan was used in conjunction with a Programmable Divergent Slit and a 5mm Width Mask [34]. Differential Scanning Calorimetry (DSC)

measurements were taken using a Mettler Toledo STARe SW 11.00 system to scan the synthesised material (2-10 mg) in a Tzero hermetic alumina pan under dry nitrogen atmosphere. The scans were performed at a heating rate of $10\text{ }^{\circ}\text{C min}^{-1}$, with samples heated between $30\text{ }^{\circ}\text{C}$ and $160\text{ }^{\circ}\text{C}$.

Conclusions

SEM, FTIR, DSC and XRD were used to affirm the synthesis of nanochitosan and its silver nanochitosan. SEM and XRD results revealed the porous, agglomeration and crystalline structure of the ScCSNPs, ScCSNPs-AgNPs, ArCSNPs and ArCSNPs-AgNPs. The crystal structure and the presence of AgNPs was also confirmed by DSC analysis. The presence of hydroxyl, amino, and alkyl groups in nanochitosan and its silver nanochitosan counterpart was revealed by FTIR analysis. Even with the presence of AgNPs, the amino group can also serve as available charged sites for interaction with negatively charged bacterial cells, making them suitable for adsorption and antimicrobial application. Chitosan extracted from biowaste (snail shell) can be an effective tool for controlling microbial pathogens, thereby addressing two major public health issues: waste recycling and vector control. According to the findings of this study, nanochitosan and its silver nanochitosan composites have excellent bioactive, biocompatible, and antibacterial properties and can thus be used as a bio-polymeric material in water purification, tissue engineering, biomedical, and therapeutic applications. The study has shown that chitosan extract from snail shell and its silver nanochitosan composite could potentially substitute commercial low molecular chitosan and its silver nanochitosan composite respectively in all the various applications of chitosan and its derivatives. However, academic activity in the area of using natural product extracts as agents of deproteination, demineralization and deacetylation during the extraction of chitosan from biowastes before modification and impregnation with silver nanoparticle is highly recommended.

Declarations

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Competing of Interest

The authors declare no conflicts of interest.

Author Contributions

The study conception and design were performed by [Vincent Nwalieji Okafor]. Material preparation, data collection and analysis were performed by [Vincent Nwalieji Okafor], [Akinyele Abimbola Bankole], [Iwe Assumpta Daberechukwu] and [Mbume Oluchukwu Francisca]. The first draft of the manuscript was written by [Akinyele Abimbola Bankole] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

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

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Figures

Figure 1

SEM Images of synthesized (a) ScCSNPs (b) ScCSNPs-AgNPs (c) ArCSNPS (d) ArCSNPS-AgNPs

Figure 2

FTIR spectrum of (a) ScCSNPs (b) ScCSNPs-AgNPs (c) ArCSNPS (d) ArCSNPS-AgNPs

Figure 3

X-Rays diffraction patterns of nanochitosan (ScCSNPs and ArCSNPS) and its respective silver nanoparticles composite (ScCSNPs-AgNPs and ArCSNPS-AgNPs)

Figure 4

Differential Scanning Calorimetry thermogram of (a) ScCSNPs and ScCSNPs-AgNPs (b) ArCSNPS and ArCSNPS-AgNPs