International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 08 Issue: 04 | April - 2024 SJIF Rating: 8.448 ISSN: 2582-3930

Isolation and Encapsulation of Chitosan Silver Nanoparticle Extracted from Squid Pen and its Characterization

[1]Varshini Rajaganesh, [2]Dr. R. Renuka,
[1]PG Student, [2]Associate professor,
[1]Department of Biochemistry,
[1]Sri Ramakrishna College of Arts and Science for women, Coimbatore, India.

Abstract: The main study focuses on the extraction of chitosan from chitin isolated from the exoskeleton of a squid pen and the synthesis of silver chitosan nanoparticles. Chitosan is an amino polysaccharide prepared by processing the squid pen which involves demineralization, deproteinization, and deacetylation. Chitosan is a versatile natural polysaccharide, its second most abundant natural polymer. Previous studies have found that chitosan is biocompatible, biodegradable, and non-toxic, which has wide applicability in the pharmaceutical field, food industries, drug delivery, cancer treatment, and biological imaging and diagnosis. The chitosan and silver chitosan nanoparticles yield was found to be 25% and 51.25%. The conformation of silver chitosan nanoparticles was done through UV spectrum, FTIR, and XRD.

Keywords: Chitosan, Chitin, Biopolymer, Characterization, UV spectrum, FTIR, XRD.

I INTRODUCTION

Nanotechnology is a field of science that deals with altering and generating molecular materials in the range of one to a hundred nanometres(nm)[1]. The tantalizing potential of nanotechnology is to fabricate and combine nanoscale approaches and the properties of nanoparticles show better results than the bulk particles like increment in the surface area and physical strength [2]. Nanoparticles are classified into two categories, Organic nanomaterials, and inorganic nanomaterials. The organic nanomaterials are fabricated from carbon compounds whereas the inorganic is from non-carbon compounds. Organic materials are used for nanoparticle construction of lipids and polymers of natural or synthetic origin that are exploited as drug delivery vehicles. Inorganic materials are used for nanoparticle construction of carbon, silica, and metals such as gold, silver, and iron oxide [3].

Bioactive compounds are those non-living materials used in various sectors such as the agricultural field, biomedical, pharmaceutical, and food technology. Many of these compounds include chitin, and chitosan which are used as an effective alternative compound for replacement of chemicals [4]. The fish wastes are discarded which mainly include muscle trimmings (15–20%), skin and fins (1–3%), bones (9–15%), heads (9–12%), viscera (12–18%), and scales (5%) daily as waste materials from fish markets, fish processing industries, canteens, or restaurants [5]. This abundant waste may pose environmental issues due to its easy deterioration. Squids are widely accepted seafood commodities because of their peculiar palatability, sensory properties, and better yield percentage of meat for consumption [6]. Consumers mostly accept marine lipids because of their high content of omega-3 fatty acids and low content of omega-6 fatty acids [7]. The squid possesses a chitin-rich exoskeleton called pen or galidus, an internalized shell that serves as a site of attachment for important muscle groups and a protective barrier for the visceral organs. However, chitin-rich compounds are non-edible and considered as waste that provides a valuable and biologically sustainable compound in recent research and development. Pens from various species of squid contain by weight, 25-49% chitin and 43-75% protein with very low ash content [8].

Chitin (β-(1–4)-poly-N-acetyl-D-glucosamine) is widely distributed in nature and is the second most abundant polysaccharide after cellulose. Chitin, which occurs in nature as ordered macro fibrils, is the major structural component in the exoskeletons of crustaceans, crabs, and shrimps, as well as the cell walls of fungi [9]. Chitosan is obtained from the alkaline deacetylation of chitin. It is a cationic polymer composed of (1–4)-2-amino-2-deoxy-β-D-glucan that due to its' pH sensitivity, biocompatibility, and bioactive functions has attracted more attention than its base polymer chitin [10]. Chitosan can be used in many industrial applications due to its extending properties of biocompatibility, biodegradability, antimicrobial, emulsifying, and chelating agent [11]. The chitosan extracted from the fish scales of Papuyu fish has been proven to improve the removal of iron in the groundwater from 11.80 mg/L to 3.43 mg/L, by evidencing higher efficiency in coagulation/flocculation treatment than the commercial chitosan from shrimp shell [12]. Comparing the other sources of chitosan, the Squid pen has a high yield of chitosan so it will be used as a source of chitosan for this study. The chitosan and chitosan-based nanoparticles possess wide applications in agriculture, food, medicine, pharmaceuticals, cosmetics, and other sectors of industries [13]. Researchers have extensively studied chitosan NPs for various applications in medicine and pharmaceutics. The material is biocompatible and allows encapsulation and chain grafting of the drugs and active ingredients. Remarkable features such as preventing enzymatic

degradation of drugs [14] and reducing the damage of non-targeted tissue or cells [15] make their use a great asset in drug delivery, cancer treatment, and biological imaging and diagnosis. Besides, the slow biodegradation of chitosan NPs has been reported to ensure controlled and continuous drug release [16].

The novel characteristic features of chitosan not only relieve its properties in the pharmaceutical industry but also in drug targeting and delivery. This research is mainly focused on the synthesis of purified chitosan and silver chitosan nanoparticles to determine the various quality attributes and the confirmation of chitosan was done by UV spectrum, FTIR, and X-ray diffraction (XRD).

II METHODOLOGY

2.1 Materials

Squid pen waste was collected from local fish store Meenu Kadai, VKL Nagar, Kurudam Palayam - Thudiyalur. The squid pens were thoroughly washed with tap water to remove impurities. Fig shows the method of isolation as follows. The pens were dried in sunlight at 60 °C for 48 h before powdering to a homogenized product. Chemicals used for this study are Dimethyl sulfoxide (DMSO), Hydrochloric acid (HCl), Sodium hydroxide (NaOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (vitamin C), silver nitrate ($AgNO_3$), acetic acid (CH3COOH).

2.2 Extraction of Chitosan from Squid Pen

The squid pens were homogenized by treating them with 10% HCl (1:5, w/v) at room temperature for 12 hours to liberate chitin. Then demineralized powder was filtrated and washed with distilled water until reaching a neutral pH of 7 and dried at room temperature *(Saad et al., 2022)*. Through deproteinization, Protein was eliminated from the sample by adding 5% NaOH to the dried squid pen at a solid/liquid ratio of 1:10 (g/mL). The deacetylation of the squid pen was carried out by the addition of 50% NaOH and then boiled at 100°C for 2 hr on a hot plate at a solid/liquid ratio of 1:20 (g/mL). The samples were then cooled for 30 min at room temperature, and then they were washed continuously with 50% NaOH and filtered to drain the solid matter, which is the chitosan. The samples were uncovered and dried at 110°C for 6 h [17].

2.3 Synthesis of Nano-Silver Chitosan

Dissolved 0.2 grams of chitosan into 10 mL of 1% (v/v) acetic acid and stirred at 250 rpm for 3 h in a magnetic stirrer until the chitosan was completely dissolved. To yield cAg-ChNP solution, 10 mL of 2% AgNO3 was gradually added into the chitosan solution while stirring using a magnetic stirrer at a speed of 200 rpm for 24 h. Then the solution was dripped into 25 mL of, 20% NaOH solution. After 15 minutes, when the color changed to brown, indicating the formation of AgNP-chitosan, the solution was centrifuged at 6000 rpm for 10 min. The supernatant was then discarded. The resulting residual spheres were washed twice with 30 mL of double-distilled water (ddH2O) to remove the alkaline solution. The resulting spheres were then dried at 40 ◦C for 1 h to obtain silver nanoparticles–chitosan composite [18].

2.4 Characterization of Silver Chitosan Nanoparticles

The characterization of Ag-CHNP was analyzed by UV electron spectroscopy, FTIR, and XRD.

2.4.1 UV Electron Spectrum: Synthesis of chitosan nanoparticles was assured by measuring the UV–vis spectrum of the reaction mixture. The absorption spectrum was recorded over the range of 200–800 nm using UV–vis spectrophotometer [18].

2.4.2 Fourier Transform Infrared Spectroscopy: FTIR measurements were used to identify the possible biomolecules associated with Chitosan formation. The FTIR spectra were recorded in the range of 400-4000 cm-1 by the KBr pellet method. 0.1 gm of potassium bromide was ground to fine paste in mortar and pestle for 2 min and a small amount of liquid sample was mixed into it [19].

2.4.3 XRD Analysis: An XRD characterization was conducted using Cu and Kα as radiation sources, and Ni as a filter with an energy of 30 kV/30 mA. For XRD analysis, Ag- ChNPs samples were placed on glass substrates. The XRD pattern was taken at room temperature with its Tesla angle (2θ) of $10^\circ \leq 2\theta \leq 70^\circ$ [20].

III. RESULTS AND DISCUSSION

3.1 Extraction of Chitosan and Synthesis of Silver Chitosan Naoparticle

The chitosan was extracted from a chitin-rich squid pen through alkaline deacetylation using various chemical processes by demineralization and deproteinization. Figure 1 shows the exoskeleton of the squid pen and Figure 2 shows the formation of Silver chitosan Nanoparticle (Ag – ChNP) using a 20% concentration of NaOH, which produced better particles with better spherical shape and homogeneity. The chitosan yield was found to be 45% after purification of the total squid pen taken. This study also determined the percentage yield of chitosan and Ag - ChNP, as shown in Table 1.

Figure 1. Chitin-rich Exoskeleton of Squid pen

Table 1. Percentage yield of chitosan and silver chitosan nanoparticles using NaOH

Particles	NaOH [%]	Product [g]	Percentage yield (%)
Chitosan	20	45	25%
Silver Chitosan nanoparticle	20	0.8 ± 0.4	51.25%

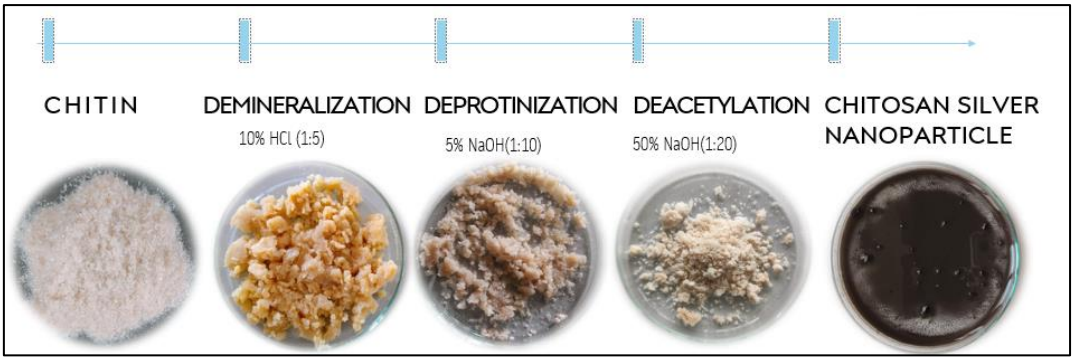

Figure 2. Processes of synthesis of silver chitosan nanoparticle

3.2 Characterization of Silver Chitosan Nanoparticles

3.2.1 Ultra-Violet Electron Spectrum

The peak values are observed in the range of 200 – 800 nm for the silver chitosan nanoparticle are shown in Table 2 and Figure 3. The UV study evaluates the identification of the sample that had better absorption activity in the wavelength between 320 nm and 200 nm at the peak absorbance of 3.505. As observed in the spectra of [21], the ChAgNP plot has absorption peaks at 236 and 261 nm. Also, the result from [22], states that silver nanoparticles absorb at a wavelength of approximately 410 nm which indicates that the addition of NaOH does not significantly affect the diameter of the synthesized silver nanoparticles [23].

Table 2. Effect of UV Spectra analysis of silver chitosan nanoparticle

WAVELENGTH (nm)	ABSORBANCE
210.5	3.505
220.5	3.175
270.0	0.619
322.5	0.404
407.5	0.649
394.5	0.635
794.5	0.409

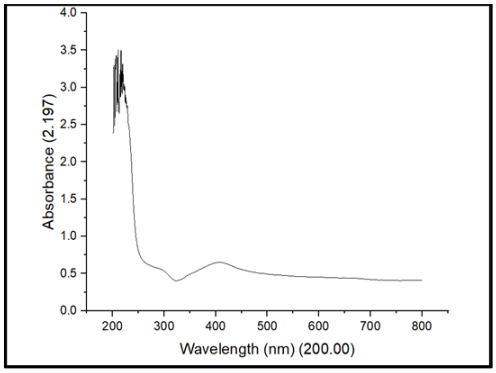

Figure 3. UV Spectra Analysis of Silver Chitosan Nanoparticle

3.2.2 Fourier Transform Infrared Spectroscopy

To classify functional groups of silver chitosan nanoparticles, we performed FTIR spectroscopy in the range of 4000−500 cm-1, as shown in Figure. The peak of Ag-ChNP obtained between 4000−500 cm-1 showed the presence of functional groups such as alcohols, phenols, alkyl halides, alkynes, and aliphatic and aromatic compounds. The low absorption peak observed at around 2800 to 1600 cm−1 could be appointed to hydroxyl (OH) groups; also, some other bands were observed at around 1400, 1200, and 600 cm−1. The table below shows the peak absorption with respective to its functional group.

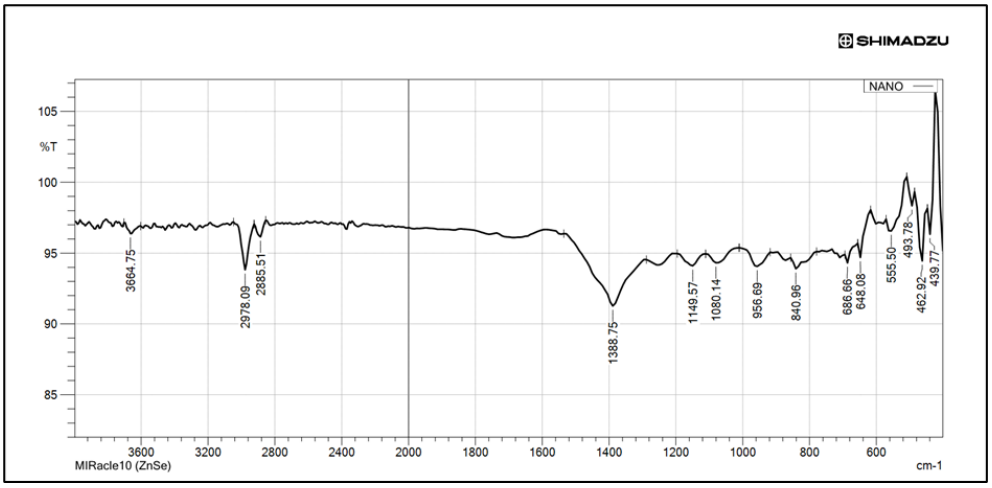

Figure 4. FTIR Analysis spectra of silver chitosan nanoparticle

3.2.3 Xray Diffraction

The figure shows the XRD pattern of Ag-ChNP. The XRD analysis showed that the numbers of Bragg reflections with 2θ values of 38.290, 44.500, 64.760, and 77.800 correspond to h, k, I value (111), (200), (220), and (311) respectively for silver particles. The numbers of Bragg reflections with 2 values of 26.65, 32.79, 38.06, and 47.07 correspond to h, k, and I value (110), (111), (200), and (211) respectively for silver oxide particles. The two characteristics of silver nanoparticles (111), (200), and (220) are consistent with those found in the Joint Committee on Powder Diffraction Standards (JCPDS) database. These indicate that the resulting nanoparticles are silver and silver-oxide nanoparticles, as the positions and relative intensities of all the diffraction peaks are consistent with the cubic and crystalline pattern of silver. The results also show that the resulting spectrum tends to be wider (broad); this might be caused by the chitosan polymer. The XRD studies of [24] chitosan showed a peak at 20.04° (113.92 counts/s). Similar results were obtained in previous studies with five sharp crystalline reflections [25]. The sharper peaks at 20.92° for chitosan obtained in the present study are evidence of a denser crystalline structure. Another study reported that the stronger reflection for chitosan at around 30−35° is almost similar to those obtained in the present study [25].

Table 3. FTIR Spectra of silver chitosan nanoparticles

Peak obtained by FTIR spectra	Frequency	Bond	Functional group
3664.75	3640–3610 (s, sh)	O–H stretch, free hydroxyl	Alcohols, Phenols
2978.09	3000–2850 (m)	C–H stretch	Alkanes
2885.51	3000–2850 (m)	CH_2 stretch	Alkyl
1388.75	1440-1395	O-H bend	Aliphatic bending group
1149.57	1250–1020 (m)	C–N stretch	Aliphatic amines
1080.14	1250–1020 (m)	C–N stretch	Aliphatic amines
956.69	1000–650 (s)	=C–H bend	Alkenes
840.96	850–550 (m)	C–Cl stretch	Alkyl halides
686.66	700-610	-C(triple bond) C-H Bend	Alkynes
648.08			
555.50	690–515 (m)	C–Br stretch	Alkyl halides (Bromine)
493.78			
462.92			
439.77			

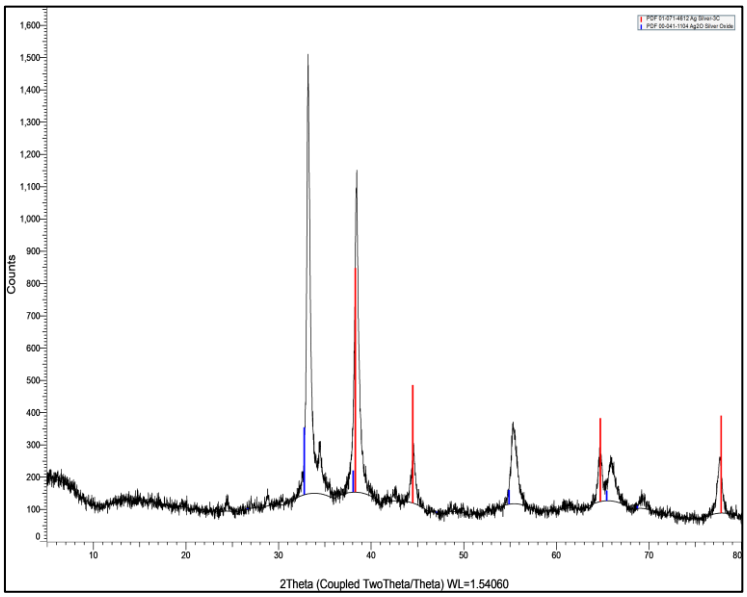

Figure 5. XRD result of deposited silver and silver oxide nanoparticles.

IV CONCLUSIONS

This study shows the production of chitosan from the exoskeleton of squid would successfully act as a biopolymeric compound in various applications. Present study aims to produce silver nanoparticles encapsulated at low concentrations of chitin without using any additional harmful chemical or physical methods. The effect of the concentration of metal ions and concentration of extracted chitosan quantity was evaluated to optimize the route to synthesis of silver nanoparticles. The percentage yield of chitosan and Ag-ChNP was found to be 25% and 51.25%. The UV study evaluates the identification of the sample that had better absorption activity in the wavelength 210.5 at the peak absorbance of 3.505. The interactions between the silver and chitosan nanoparticles showed the presence of functional groups through the characterization of functional groups by FTIR analysis, whereas its presence was quantified alcohols, phenols, alkyl halides, alkynes, and aliphatic and aromatic compounds in between 4000−500 cm-1. The XRD analysis of silver chitosan nanoparticles confirmed the formation of silver particles and silver oxide particles in the cubic and crystalline pattern compared with the Bragg reflection values. The FTIR and XRD studies confirm the production of chitosan and silver particles and showed that it can be used in various fields like the pharmaceutical industry, food packaging, water treatment, drug delivery, etc.

V REFERENCES

[1] Badawy, M. E. I., Lotfy, T. M. R., & Shawir, S. M. (2019). Preparation and antibacterial activity of chitosan-silver nanoparticles for application in preservation of minced meat. Bulletin of the National Research Centre, 43(1). https://doi.org/10.1186/s42269-019-0124-8.

[2] Srinivas, P. R., Philbert, M. A., Vu, T. Q., Huang, Q., Kokini, J. L., Saos, E., Chen, H., Peterson, C. M., Friedl, K. E., McDade–Ngutter, C., Hubbard, V. S., Starke–Reed, P., Miller, N. H., Betz, J. M., Dwyer, J. T., Milner, J. A., & Ross, S. A. (2010). Nanotechnology Research: Applications in Nutritional Sciences. The Journal of Nutrition, 140(1), 119–124. https://doi.org/10.3945/jn.109.115048

[3] Rajabi, M., & Mousa, S. A. (2016). Lipid Nanoparticles and their Application in Nanomedicine. Current Pharmaceutical Biotechnology, 17(8), 662–672. https://doi.org/10.2174/1389201017666160415155457

[4] Ghormade, V., Deshpande, M. V., & Paknikar, K. M. (2011). Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnology Advances, 29(6), 792–803. https://doi.org/10.1016/j.biotechadv.2011.06.007

[5] Martínez-Álvarez, Ó., Chamorro, S., & Brenes, A. (2015). Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review. Food Research International, 73, 204–212. https://doi.org/10.1016/j.foodres.2015.04.005

[6] Rajabi, M., & Mousa, S. A. (2016). Lipid Nanoparticles and their Application in Nanomedicine. Current Pharmaceutical Biotechnology, 17(8), 662–672. https://doi.org/10.2174/1389201017666160415155457

[7] Loppies, C. R. M., Apituley, D. a. N., & Tentua, E. (2021). Nutrition content and amino acid profile of paper squid (Loligo edulis). IOP Conference Series: Earth and Environmental Science, 797(1), 012020. https://doi.org/10.1088/1755-1315/797/1/012020

[8] Boukhlifi, F., Zahrae, M. F., & Razouk, R. (2018). Chitin/Chitosan's Bio-Fertilizer: Usage in vegetative growth of wheat and potato crops. In InTech eBooks. https://doi.org/10.5772/intechopen.75208

[9] Messerli, M. A., Raihan, M. J., Kobylkevich, B. M., Benson, A. C., Bruening, K. S., Shribak, M., Rosenthal, J. J. C., & Sohn, J. J. (2019). Construction and Composition of the Squid Pen fromDoryteuthis pealeii. The Biological Bulletin, 237(1), 1–15. https://doi.org/10.1086/704209.

[10] Yanat, M., & Schroën, K. (2021). Preparation methods and applications of chitosan nanoparticles; with an outlook toward reinforcement of biodegradable packaging. Reactive and Functional Polymers, 161, 104849. https://doi.org/10.1016/j.reactfunctpolym.2021.104849.

[11] Harugade, A., Sherje, A. P., & Pethe, A. M. (2023). Chitosan: A review on properties, biological activities and recent progress in biomedical applications. Reactive and Functional Polymers, 191, 105634. https://doi.org/10.1016/j.reactfunctpolym.2023.105634.

[12] Irawan, C., Nata, I. F., Putra, M. D., Marisa, R., Asnia, M., & Arifin, Y. F. (2018). Biopolymer of Chitosan from Fish Scales as Natural Coagulant for Iron–Contaminated Groundwater Treatment. Jurnal Rekayasa Kimia & Lingkungan, 13(2), 93–99. https://doi.org/10.23955/rkl.v13i2.10601

[13] Morin‐Crini, N., Lichtfouse, É., Torri, G., & Crini, G. (2019). Applications of chitosan in food, pharmaceuticals, medicine, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and environmental chemistry. Environmental Chemistry Letters, 17(4), 1667–1692. https://doi.org/10.1007/s10311-019-00904-x

[14] Huang, X., Du, Y., Yuan, H., & Hu, F. (2009). Preparation and pharmacodynamics of low-molecular-weight chitosan nanoparticles containing insulin. Carbohydrate Polymers, 76(3), 368–373. https://doi.org/10.1016/j.carbpol.2008.10.025

[15] Rieux, A. D., Fiévez, V., Garinot, M., Schneider, Y., & Préat, V. (2006). Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach. Journal of Controlled Release, 116(1), 1–27. https://doi.org/10.1016/j.jconrel.2006.08.013

[16] Panonnummal, R., Gopinath, D., Biswas, R., Sabitha, M., & Jayakumar, R. (2016). Chitosan nanoparticles in drug therapy of infectious and inflammatory diseases. Expert Opinion on Drug Delivery, 13(8), 1177–1194. https://doi.org/10.1080/17425247.2016.1178232

[17] Puvvada, Y. S., Saikishore, V., & Sukhavasi, S. (2012). Extraction of chitin from chitosan from the exoskeleton of shrimp for application in the pharmaceutical industry. International Current Pharmaceutical Journal, 1(9), 258–263. https://doi.org/10.3329/icpj.v1i9.11616

[18] Mirda, E., Idroes, R., Khairan, K., Tallei, T. E., Ramli, M., Earlia, N., Maulana, A., Idroes, G. M., Muslem, M., & Jalil, Z. (2021). Synthesis of Chitosan-Silver nanoparticle composite spheres and their antimicrobial activities. Polymers, 13(22), 3990. https://doi.org/10.3390/polym13223990

[19] Kumari, S., & Rath, P. (2014). Extraction and Characterization of Chitin and Chitosan from (Labeo rohit) Fish Scales. Procedia Materials Science, 6, 482–489. https://doi.org/10.1016/j.mspro.2014.07.062

[20] 46. Leonardi, M., Caruso, G., Carroccio, S., Boninelli, S., Curcuruto, G., Zimbone, M., Allegra, M., Torrisi, B., Ferlito, F., & Miritello, M. (2021). Smart nanocomposites of chitosan/alginate nanoparticles loaded with copper oxide as alternative nanofertilizers. Environmental Science. Nano, 8(1), 174–187. https://doi.org/10.1039/d0en00797h

[21] Canama, G. J. C., Delco, M. C. L., Talandron, R. A., & Tan, N. P. B. (2023). Synthesis of Chitosan-Silver nanocomposite and its evaluation as an antibacterial coating for mobile phone glass protectors. ACS Omega, 8(20), 17699–17711. https://doi.org/10.1021/acsomega.3c00191

[22] Wang, L., Wang, C., Yang, C., Hsieh, C., Chen, S., Shen, C., Wang, J., & Huang, K. (2015). Synthesis and anti-fungal effect of silver nanoparticles–chitosan composite particles. International Journal of Nanomedicine, 2685. https://doi.org/10.2147/ijn.s77410

[23] Mori, Y., Ono, T., Miyahira, Y., Nguyen, V., Matsui, T., & Ishihara, M. (2013). Antiviral activity of silver nanoparticle/chitosan composites against H1N1 influenza A virus. Nanoscale Research Letters, 8(1). https://doi.org/10.1186/1556-276x-8-93

[24] Varma, R., & Vasudevan, S. (2020). Extraction, Characterization, and Antimicrobial Activity of Chitosan from Horse Mussel Modiolus modiolus. ACS Omega, 5(32), 20224–20230. https://doi.org/10.1021/acsomega.0c01903

[25] Rasti, H., Parivar, K., Baharara, J., Iranshahi, M., & Namvar, F. (2017). Chitin from the Mollusc Chiton: Extraction, Characterization and Chitosan Preparation. PubMed, 16(1), 366–379. https://pubmed.ncbi.nlm.nih.gov/28496490