مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد السابع والعشرون - المجلد العاشر - علوم الكيمياء - نيسان 2026 م
doi.org/10.52866/esj.2026.10.27.13

Synthesis of nano-chitosan from blue crab using a modified gelation method and study its characterisation

Mohammed Mahdi Sami[1a] , Mataz J. Jamai[1b],
Fatima T. Abed Al_jabbar[2c] , Estabraq A. Abd Alwahed[1d]
1) Department of Remote Sensing–
College of Remote Sensing and Geophysics-Al-Karkh University of Science
2) Department of Geophysics College-
College of Remote Sensing and Geophysics-Al-Karkh University of Science
a) Mohammedmahdi81@kus.edu.iq , b) mutaz.alwesy@kus.edu.iq ,
c) fatimaturrky@gmail.com , d) estbrqabbas@yahoo.com

Abstract :

Background: Chitin of arthropod exoskeletons is the most abundant biopolymer found after cellulose. Chitosan is a biopolymer derived from chitin by strong alkaline treatments. Nano-chitosan (NC) offers enhanced physicochemical properties compared to its bulk counterpart due to its high surface area-to-volume ratio, improved solubility, and ability to interact more effectively with biological systems

Material and methods: Chitosan was prepared in three steps: demineralisation, deproteination, and deamination in strong alkali media. Nano-chitosan (NC) was prepared using 1% acetic acid, with a solution of tripolyphosphate added dropwise under magnetic stirring at room temperature, at a 2.5:1 (v/v) ratio, of TPP vs chitosan. The result of the participant was dried for 24h at 60 °C.

Results: A 8.3 gram of chitosan was prepared from 50 gram of exoskeleton. 93% of chitosan was converted to NC, The UV-Vis spectra of NCs observed at 228 nm. The XRD analysis exhibits broad pick at 2Θ = 28.2, and 21.8 with d-spacing 3.1 ˚A, and 3.9 ˚A. The morphology has exhibited the spherical shape of nano-chitosan with root mean square 19.5 nm.

Conclusion: Each 50 grams of crude exoskeleton produces 8.3g of chitosan (16.3% yield). 93% of chitosan was converted to nano-chitosan without using any dispersing agent, which requires a further filtration step.

Keyword: chitin, chitosan, nano-chitosan, FTIR, XRD, SEM .

تحضير النانو - كيتوزان من السلطعون الأزرق باستخدام طريقة الهلام المعدلة ودراسة خصائصه

محمد مهدي سامي ، معتز جليل. جمعة ، فاطمة تركي عبد الجبار ، إستبرق عبد الواحد
قسم االتحسس النايي - كلية التحسس النائي والجيزوفيزياء - جامعة الكرخ للعلوم

مستخلص:

الخلفية: الكيتين في هياكل المفصليات هو أكثر البوليمرات الحيوية وفرة بعد السليلوز. الكيتوسان هو بوليمر حيوي مشتق من الكيتين بواسطة معالجات قلوية قوية. يوفر النانو-كيتوسان (NC) خصائص فيزيائية وكيميائية محسنة مقارنة بنظيره التقليدي بسبب ارتفاع نسبة السطح إلى الحجم، وتحسن الذوبان، وقدرته على التفاعل بشكل أكثر فعالية مع الأنظمة البيولوجية.

المواد والطريقة: تم تحضير الكيتوسان على ثلاث خطوات: إزالة المعادن، وإزالة البروتينات، وإزالة الأمين في وسط قلوي قوي. تم تحضير النانو-كيتوسان (NC) باستخدام حمض الخليك 1٪ مع محلول ثلاثي فوسفات البولي، حيث تم إضافته قطرة قطرة تحت التحريك المغناطيسي في درجة حرارة الغرفة بنسبة 2.5:1 (حجم/ حجم). ثم تم تجفيف الناتج لمدة 24 ساعة عند 60°م.

النتائج: تم تحضير 8.3 غرام من الشيتوزان من 50 غرام من الهيكل الخارجي. تم تحويل 93٪ من الكيتوسان إلى النانو-كيتوسان. تم ملاحظة أطياف UV-Vis للنانو-كيتوسان عند 228 نانومتر. أظهر تحليل XRD قمة عريضة عند الزاويتين 2Θ = 28.2 و 21.8 مع تباعد d بمقدار 3.1 Å و 3.9 Å. أظهرت الصور المورفولوجية الشكل الكروي للنانو-كيتوسان مع جذر متوسط مربع يبلغ 19.5 نانومتر.

الكلمات المفتاحية: الكيتين، الكيتوسان، النانو-كيتوسان، FTIR، XRD، SEM .

Introduction

Arthropods are the largest animal family with the largest species in the world. Arthropods have an exoskeleton that is periodically shed as they grow. The commercial utilization of crustaceans comprised 58% shrimps, 27% crabs, and 5% lobsters (Moghal, M., et al, 2015). Chitin of arthropod exoskeletons is the most abundant biopolymer found after cellulose. It has significant attention in various fields industry due to its biocompatibility, biodegradability, non-toxicity, and unique functional properties (Shanta Pokhrel, et al, 2015). Chitosan (β -(1,4) glucosamine) is a biopolymer derived from chitin by strong alkaline treatments. It is non-toxic, liner, heterocyclic, solid, white to light yellow, and insoluble in water but soluble in organic acids. Chitosan is largely used in anti-microbial bacterial, biotechnology, cosmetics, and drug delivery (Alam p ., 2018). Nano-chitosan (NC)offers enhanced physicochemical properties compared to its bulk counterpart due to its high surface area-to-volume ratio, improved solubility, and ability to interact more effectively with biological systems (Nurul A., 2021). This nanoscale form of chitosan has found widespread applications across industries, including biomedicine, pharmaceuticals (Mohebbi, S.; et al, 2018), drug delivery (Baghdan, E.; et al 2018), agriculture (Morin C. N., et al, 2018), food technology (Li, H., et al, 2019), and environmental protection (Cristina C., et al, 2017). Chitosan is a semi-crystalline biopolymer with a molecular structure that contains both hydrophobic and hydrophilic groups. The hydrophilic arises from the D-glucosamine residues, which cause of polycationic nature of chitosan in acidic conditions that allows for ionic bonds with charged surfaces, with high susceptibility to water purification, drug delivery, and antimicrobial coatings applications (Desai N, et al, 2023; Joseph S.M., et al, 2021). Several methods are available to use for the synthesis of nano-chitosan (NC), resulting in varying in sizes, properties, and shapes. These methods can be broadly classified into chemical, physical, biological, and approaches (Kou S.G., et al 2021). Chemical methods, that are widely used include

ionic gelation, precipitation, and cross-linking, of NC by using a tripolyphosphate (TPP) in an aqueous solution. The positive amino groups interact with the negative charges of the TPP, leading to the formation of nanoparticles (Barikani M., et al, 2010). Cross-linking involves scattering chitosan in an oil phase and crosslinking with glutaraldehyde (Ryan C., et al, 2017). Physical methods primarily involve top-down techniques, where chitosan is broken down into smaller fragments through mechanical forces. Ultrasonication with high frequency is one of the most common methods used, ultrasonic waves also, are also applied to the chitosan solution, causing particle size reduction (Dwi Setyawan, et al, 2023). The biological synthesis of NC is done by using enzymes or microbial systems to produce NC. This a time-consuming but green method, eliminating the need for toxic reagents or harsh conditions (Maestrelli F., et al, 2018). Several properties of NC make them favorable in the industry, these properties include increased surface area, enhanced solubility, use as antimicrobial activity, non-toxicity, and biocompatibility (Rofeal M., et al, 2022). Due to its diverse and enhanced properties, NC has found a wide array of applications across various sectors such as Drug Delivery, Wound Healing (Gupta, A., et al 2019), Agriculture, Water Treatment (Zhang J., et al 2019), While nano-chitosan holds immense promise, there are still several challenges that need to be addressed for its widespread adoption. One major challenge is the scalability of production methods. Current synthesis techniques are often labor-intensive and expensive, making it difficult to produce NC at an industrial scale. Additionally, more research is needed to fully understand the long-term safety and environmental impact of NC, particularly in biomedical and agricultural applications.

Materials and methods

Blue crab *(pelagicus)* is bought from a local market in Iraq -Baghdad, initially harvested from the Shatt Al-Arab River in Al-Basrah city in December. HCl, NaCl, Ethanol, and tripolyphosphate (TPP) were manufactured by CDH India. The shells were cleaned of any soft tissues, washed, dried at 200

°C for 10 minutes, and ground into fine powder.

Demineralization was done by adding 50 gm of crab powder in 500 ml of (1.0 N) HCl solution with constant stirring at boiling point for 2 hours. At the end of 2 hours, the crude was washed, neutralized, and dried. The demineralized powder (34.7g) was mixed with 600 mL of (1.0 N) NaOH and heated at 80 °C under reflux for 6 hours. The deproteinized powder (chitin) was converted to chitosan in which a 12.7 g of sample remaining from the previous step was mixed with 254 ml of (1N) NaOH under reflux and stirred for 6 hours at boiling point. After the deacetylation, the NaOH was drained off by tap water. Next, the pure chitosan (8.30g) was stirred with 96% ethanol for 1 hour, followed by washing with deionized water to neutral pH and dried in an oven at 60 °C for 30 h. Chitosan was identified by using FTIR.

Chitosan nanoparticles were prepared using the modified ionic gelation method (Hoang N.H., 2022) due to the interaction between positively charged amine groups and negatively charged TPP (Linda J., and Taty R., 2019). A weight of 2g chitosan was dissolved in 100 ml, 1% acetic acid with stirring until a clear solution appeared at room temperature, and the pH was raised to 4.5 by using 1N NaOH. A weight of 0.700 g TPP was dissolved in 100 ml of distilled water. The TPP solution was added dropwise to the chitosan solution under magnetic stirring at 800 × g at room temperature at a 2.5:1 (v/v) ratio, followed by ultrasonication for 15 minutes. The resulting solution was centrifuged at 6000 ×g for 5 min, the supernatant was discarded, and the participant was dried for 24h at 60 °C. The yield of NC prepared from 2g of bulk chitosan is 1.86; in other words, about 93% of the bulk chitosan was converted to NC. The nanochitosan was characterised by XRD, UV-Vis, AFM, and SEM.

Results and discussion

Various absorption bands within the 500-4000 cm^{-1} range were recorded in the FTIR of chitin, chitosan (Cs), and nano-chitosan (NCs), Table 1. Vibrations of compounds above illustrated in Figure 1-a, 1-b, and 1-c respectively were approximately similar (within

a range of functional groups) O-H out of a plane (free), C-C pyranose, C-OH, C-O, C-N, C-O-C, C-H pyranose, CH_2 alcoholic, free C-H, and O-H. The chitosan and nano-chitosan show vibrations of N-H and -NH_2 function groups that were absent in chitin compound vibration. The chitin vibration shows absorbance at 1373 cm^{-1} due to -CH_3, which was absent in chitosan and nano-chitosan. The nano-chitosan compound includes vibration at 880 cm^{-1} due to the P-O-P group and 1211 cm^{-1} due to the P=O group absent in chitosan.

Table 1: function group wavelength of FTIR absorbed in chitin, chitosan and nano-chitosan

Function group	Chitin	Chitosan	Nano-chitosan	Reference of function groups vibration
O-H free	617	617	617	-
N-H free		671		-
P-O-P			880	-
C-C pyranose	895	896	883	808
C-OH	959	1026	1026	1020-1200
C-O	1043	1068	1068	1045-1380
C-N	1075	1098	1098	1025-1200
C-O-C	1075	1153	1153	1050-1250
P=O			1211	1220-1250
C-H pyranose	1332	1320	1320	
CH3	1373			1390-1395
Amide II	1573			1506-1572
C=O amide	1673			1500-1700
C-H	2879	2879	2879	2850-2950
O-H alcoholic	3271	2918	2918	2500-3300
NH_2		3384	3409	3350-3500

These results are same that found in numerous articles who purified chitin and synthesis chitosan and nano-chitosan (Olajede A., *et al,* 2018; Choiri N., 2017; Manuela R. A., *et al,* 2016).

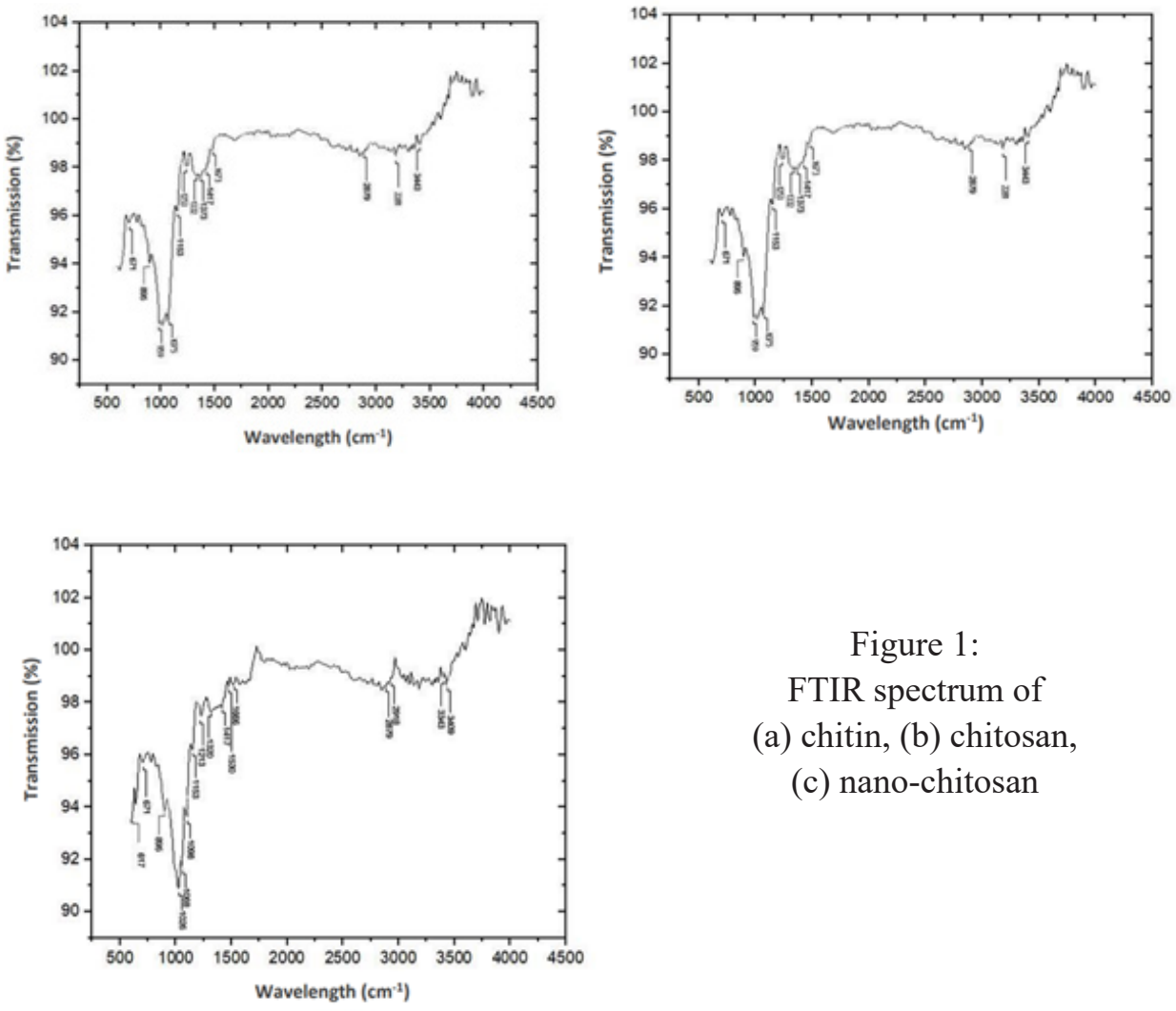

Figure 1:
FTIR spectrum of
(a) chitin, (b) chitosan,
(c) nano-chitosan

The UV-Vis spectra of N-Cs with Cs and their corresponding Tauc plot is observed in Figure 2 (a, and b)). The results illustrate that NCs (a) are observed at 228 nm, while the bulk Cs (b) have absorption at 222 nm.

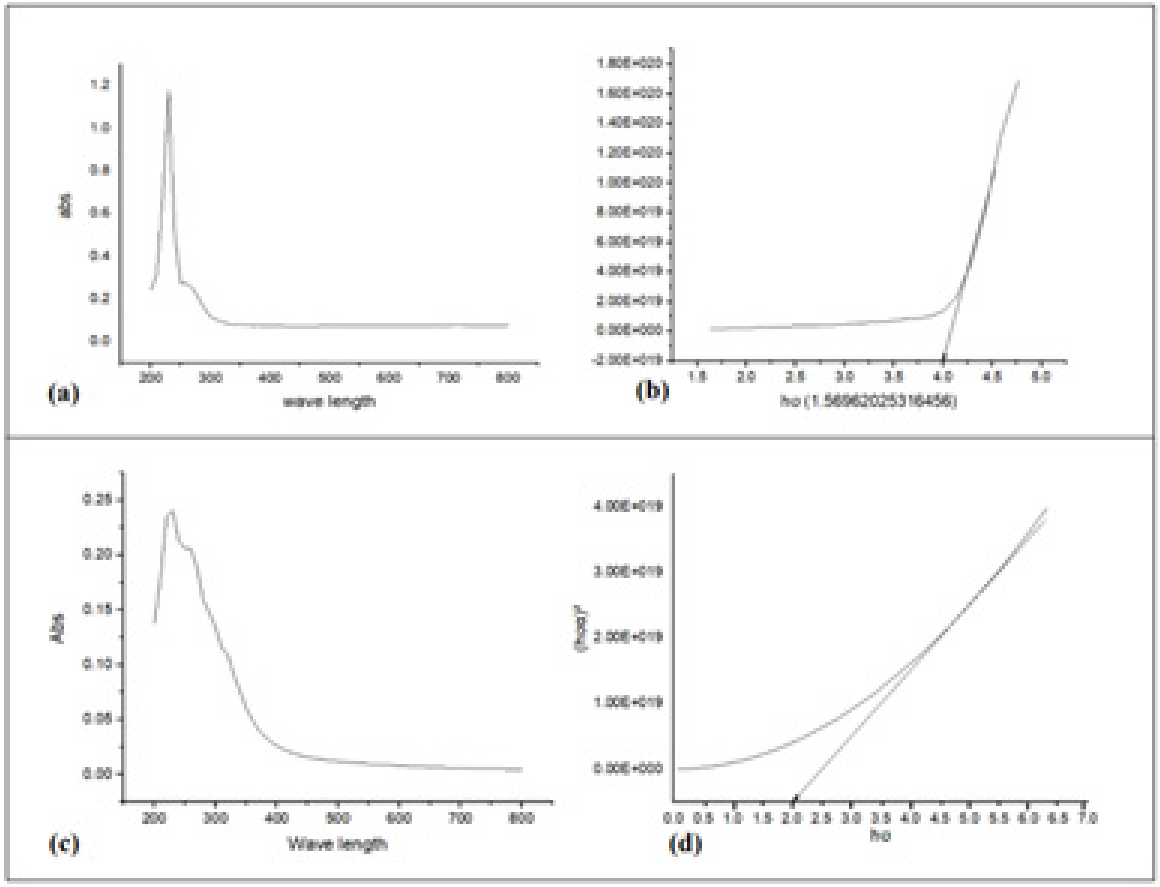

Figure 2: The UV-Vis spectra of a) nano-chitosan (b) TAUC plot from nano-chitosan c) UV-Vis spectra of chitosan, d) TAUC plot of chitosan.

The XRD analysis of cross-linked chitosan with PPT at 2.5:1 ratio, x-ray diffraction study exhibits broad pick at 2Θ = 28.2, with d-spacing 3.1 ˚A, and 21.8 with d-spacing 3.9 ˚A Figure 3, which were shifted to a higher diffraction angle 2Θ =20.2 and 20.9, (Farhatun N., et al, 2019; Gokila S., et al, 2017; Shuja B. A., et al, 2019), this diffraction is due to transforming amorphous chitosan into crystalline or increase crystallinity form after reaction with TPP (Olajide A., et al, 2018; Vijayalakshmi K., et al, 2015). The change in packing position may reflect the change in the molecular level or tighter polymer packing. There are other broad diffraction bands at 10.3, 24.3, 26.5, and 48.3 with low intensity due to plenty of -OH and -NH_2 groups in the chitosan structure, forming stronger inter and intramolecular hydrogen bonds (Vijayalakshmi K., et al, 2015).

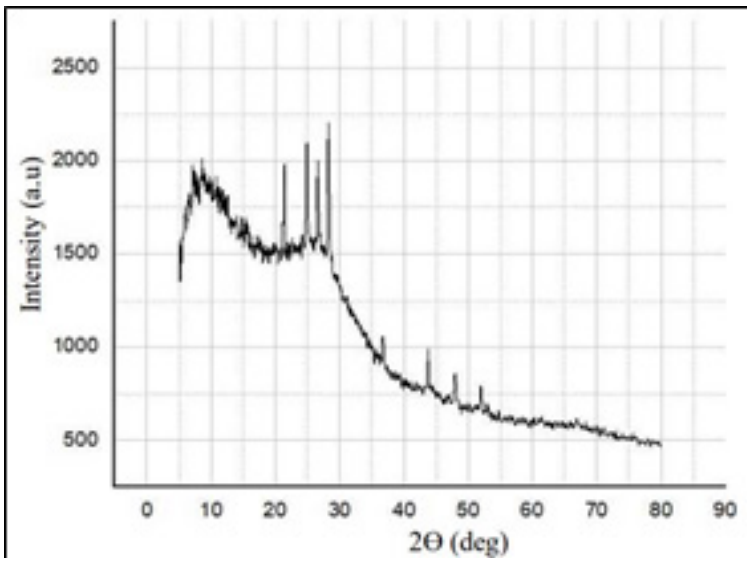

Figure 3: XRD of nano-chitosan prepared from chitosan extract

The morphology of the nano-chitosan was analyzed through AFM, as shown in Figure 4-a. The morphology of NCs has exhibited the spherical shape of nano-chitosan. The nanoparticles were separately and homogenously synthesized. The average roughness of the NCs was calculated by AFM analysis to be 16.9 nm, and the root mean square (r.m.s) was equal to 19.5 nm, with a size distribution of 84 nm 4-b.

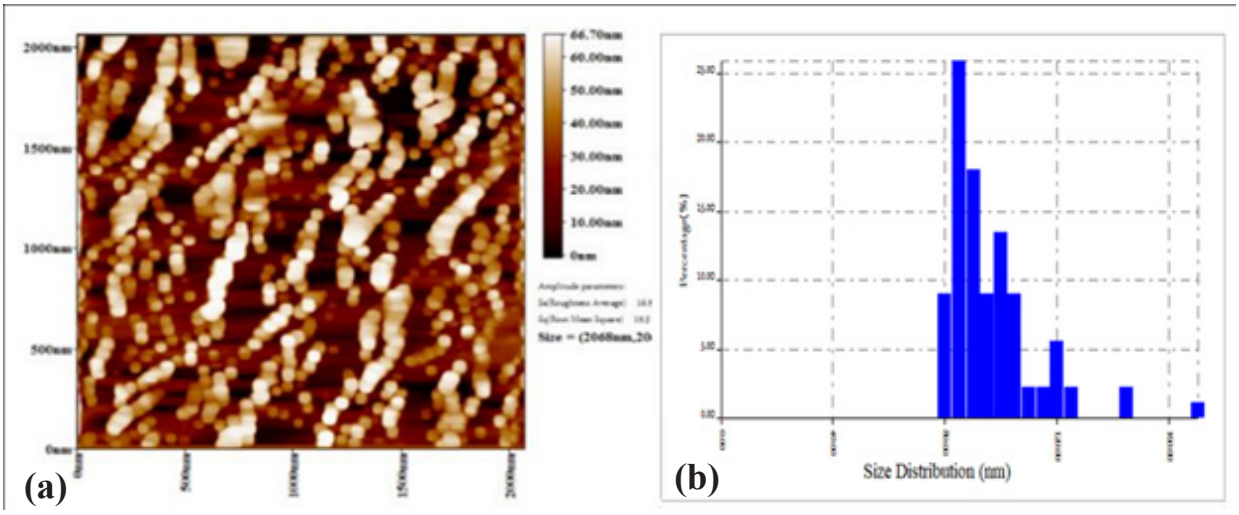

Figure 4: micrograph of
(a)Top ography and (b) size distribution image of chitosan nanoparticles.

The morphology of nano-chitosan was observed using scanning electron microscopy (SEM), and the results are shown in Figure 5. The SEM image highlights clusters of NC, and the smallest particle size labeled L1 = 18.13 nm" indicates the size of an observed feature, likely a particle or surface roughness. This figure shows a broader view of the NC clusters. The particles appear to have a porous structure, sponge-like, with some larger aggregates of NC visible. The surface shows complex texture and roughness, with both small and large particles agglomerated.

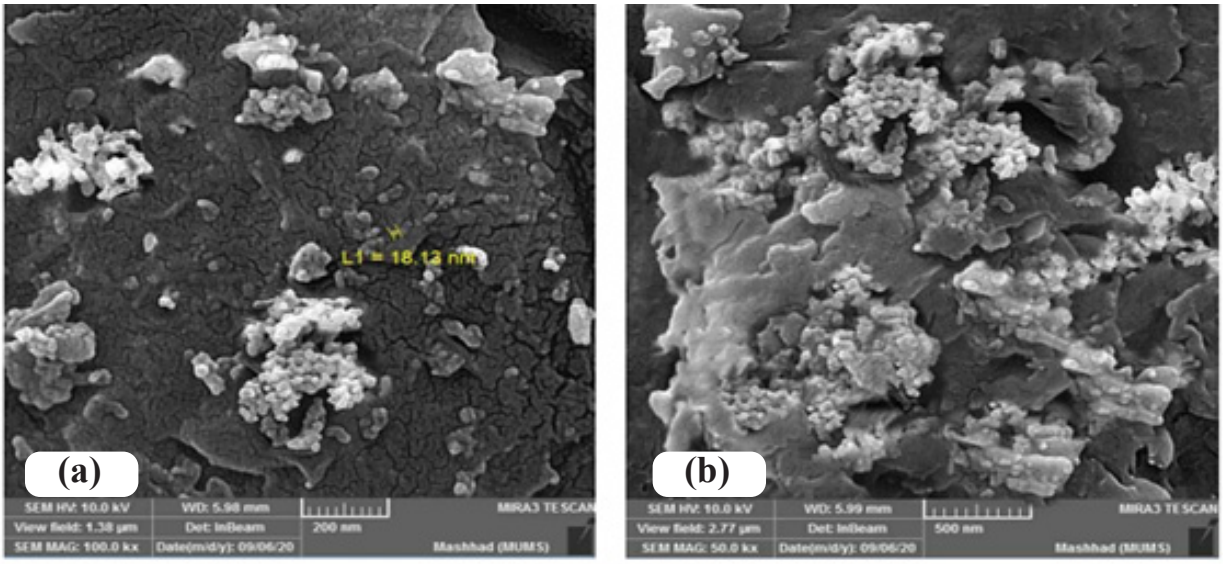

Figure 5: SEM micrograph of nano-chitosan represents a cluster of nano-particles. L1 indicate the smallest size of nano-chitosan, a) magnification: 100.0 kx (100,000×), scale bar: 200 nm, accelerating voltage: 10.0 kV, working distance (WD): 5.98 mm b) magnification 50.0 kx, scale bar: 500 nm, accelerating voltage: 10.0 kV, working distance: 5.99 mm.

Conclusion

The synthesis of chitosan from crab shell is summarized in deproteination, demineralization, and deacetylation of chitin in high alkali solution. Each 50 grams of crude exoskeleton produces 8.3g of chitosan (16.3% yield). 93% of chitosan was converted to nano-chitosan without using any dispersing agent, which requires a further filtration step.

1. XRD results resulted that chitosan with TPP with ratio of2.5:1 shift-

ed the diffraction peaks toward higher angles, explaining move the crystalline structure.

2. Broad and low-intensity bands at 10.3°, 24.3°, 26.5°, and 48.3° implying extensive inter- and intramolecular hydrogen bonding within the chitosan.

3. AFM analysis recorded average roughness of 16.9 nm with RMS roughness of 19.5 nm, indicating nanoscale surface uniformity.

4. SEM images displayed sponge-like structures with agglomeration of nanoparticles, with the smallest particle observed about 18.13 nm, confirming nanosized dimensions.

Reference

- M. Moghal, V. Pradhan, V. Ladniya. “Studies on crabs (Brachyura): A Review.” *J. Adv. Scientific Res.* **4**, 585–595 (2015).
- S. Pokhrel, P. N. Yadav, R. A. “Applications of Chitin and Chitosan in Industry and Medical Science: A Review.” *Nepal J. Sci. Technol.* **16**, 99–104 (2015).
- P. Alam. “Structures and Composition of the Crab Carapace: An Archetypal Material in Biomimetic Mechanical Design.” In: Kloc M., Kubiak J. (eds) *Marine Organisms as Model Systems in Biology and Medicine. Results and Problems in Cell Differentiation.* Springer, Cham, 569–584 (2018).
- N. A. Ahmad, F. Kadir, N. A. Ahmad, N. A. F. Mahmud, M. F. “The Potential of Insects as Alternative Sources of Chitin: An Overview on the Chemical Method of Extraction from Various Sources.” *Int. J. Mol. Sci.* **21**, 4978 (2020).
- E. Baghdan, S. R. Pinnapireddy, B. Strehlow, K. H. Engelhardt, J. Schäfer, U. Bakowsky. “Lipid coated chitosan-DNA nanoparticles for enhanced gene delivery.” *Int. J. Pharm.* **535**, 473–479 (2018).
- S. Mohebbi, M. N. Nezhad, P. Zarrintaj, S. H. Jafari, S. S. Gholizadeh, M. R. Saeb, M. Mozafari. “Chitosan in Biomedical Engineering: A Critical Review.” *Curr. Stem Cell Res. Ther.* **14**, 93–116 (2019).
- C. N. Morin, E. Lichtfouse, G. Torri, G. Crini. “Fundamentals and Applications of Chitosan.” In: *Sustainable Agriculture Reviews.* Springer, Cham, Switzerland, **35**, 49–123

(2019).

- C. Cristina, V. Dolores, R. G. Maria, S. Du, R. Cortesi, P. D. Magri. "Chitin and Chitosans: Characteristics, Eco-Friendly Processes, and Applications in Cosmetic Science." *Mar. Drugs* **19**, 369 (2017).
- H. Li, D. Wang, C. Liu, J. Zhu, M. Fan, X. Sun, T. Wang, Y. Xu, Y. Cao. "Fabrication of stable zein nanoparticles coated with soluble soybean polysaccharide for encapsulation of quercetin." *Food Hydrocoll.* **87**, 342–351 (2019).
- N. Desai, D. Rana, S. Salave, R. Gupta, P. Patel, B. Karunakaran, A. Sharma, J. Giri, D. Benival, N. Kommineni. "Chitosan: A Potential Biopolymer in Drug Delivery and Biomedical Applications." *Pharmaceutics* **15**(4), 1313 (2023).
- S. M. Joseph, S. Krishnamoorthy, R. Paranthaman, J. A. Moses, C. Anandharamakrishnan. "A Review on Source-Specific Chemistry, Functionality, and Applications of Chitin and Chitosan." *Carbohydr. Polym. Technol. Appl.* **2**, 100036 (2021).
- S. G. Kou, L. M. Peters, M. R. Mucalo. "Chitosan: A Review of Sources and Preparation Methods." *Elsevier B.V.*, Amsterdam, The Netherlands, **169**, 85–94 (2021).
- M. Barikani, H. Honarkar, M. Barikani. "Synthesis and Characterization of Chitosan-Based Polyurethane Elastomer Dispersions." *Monatsh. Chem.* **141**, 653–659 (2010).
- C. Ryan, E. Alcock, F. Buttimer, M. Schmidt, D. Clarke, M. Pemble, M. Bardosova. "Synthesis and characterization of cross-linked chitosan composites functionalized with silver and gold nanoparticles for antimicrobial applications." *Sci. Technol. Adv. Mater.* **18**(1), 528–540 (2017).
- D. Setyawan, T. Amrillah, C. A. C. Abdullah, F. B. Ilhami, D. M. M. Dewi, Z. Mumtazah, A. Oktafiani, F. P. Adila, M. F. H. Putra. "Crafting two-dimensional materials for contrast agents, drug, and heat delivery applications through green technologies." *J. Drug Target.* **31**(4), 369–389 (2023).
- F. Maestrelli, M. Jug, M. Cirri, I. Kosalec, P. Mura. "Characterization and Microbiological Evaluation of Chitosan-Alginate Microspheres for

Cefixime Vaginal Administration." *Carbohydr. Polym.* **192**, 176–183 (2018).

- M. Rofeal, F. Abdelmalek, A. Steinbüchel. "Naturally-Sourced Antibacterial Polymeric Nanomaterials with Special Reference to Modified Polymer Variants." *Int. J. Mol. Sci.* **23**(8), 4101 (2022).
- Gupta, V. Rattan, S. Rai. "Efficacy of Chitosan in Promoting Wound Healing in Extraction Socket: A Prospective Study." *J. Oral Biol. Craniofac. Res.* **9**, 91–95 (2019).
- J. Zhang, C. Wen, J. Gu, C. Ji, Y. Duan, H. Zhang. "Effects of Subcritical Water Extraction Microenvironment on the Structure and Biological Activities of Polysaccharides from Lentinus edodes." *Int. J. Biol. Macromol.* **123**, 1002–1011 (2019).
- N. H. Hoang, T. Le Thanh, R. Sangpueak, J. Treekoon, C. Saengchan, W. Thepbandit, N. K. Papathoti, A. Kamkaew, N. Buensanteai. "Chitosan Nanoparticles-Based Ionic Gelation Method: A Promising Candidate for Plant Disease Management." *Polymers (Basel)* **14**(4), 662 (2022).
- L. Jaya, T. Rahayu. "Characterized and Synthesis of Chitosan Nanoparticle as Nanocarrier System Technology." *Mater. Sci. Eng.* **508**, 508–514 (2019).
- Olajede, O. Afamefuma, O. Oluwasina, T. Abe. "Utilization of Blue Crab Shells for the Synthesis of Chitosan Nanoparticles and Their Characterization." *Songklanakarin J. Sci. Technol.* **40**(5), 1043–1047 (2018).
- N. Choiri, N. Amalina, E. Maiza, D. Mustika, S. Diana, S. Tantang, S. Salmahaminti. "Synthesis of Chitosan from Crab Shell with Encapsulation Method." *J. Eng. Appl. Sci.* **12**(18), 4725–4729 (2017).
- M. R. Alexandru, M. A. Gabriel, A. Dinu, S. Verginica. "Extraction and Characterization of Chitosan from Local Marine Resource." *Naval Acad. Sci. Bull.* **19**(1), 9–12 (2016).
- N. Farhatun, M. Zulkifli, N. Ahmad, F. Sharida, A. S. Nur, H. H. Abu, L. D. "A Potent Antifungal Agent for Basal Stem Rot Disease Treatment in Oil Palms Based on Chitosan-Dazomet Nanoparticles." *Int. J. Mol. Sci.* **20**, 1–15 (2019).
- S. Gokila, G. Thandapani, P. N.

Sutha, A. S. "Removal of the Heavy Metal Ion Chromium(VI) Using Chitosan and Alginate Nanocomposites." *Int. J. Biol. Macromol.* **104**, 1459–1468 (2017).

- A. Shuja, A. Rebar, R. Mariwan, O. G. Abdullah. "Polymer Blending as a Novel Approach for Tuning the SRP Peak of Silver Nanoparticle." *Polymers (Basel)* **9**, 1–12 (2017).
- Olajide, E. Afamefuna, O. Olugbenga, T. O. "Utilization of Blue Crab Shells for the Synthesis of Chitosan Nanoparticles and Their Characterization." *Songklanakarin J. Sci. Technol.* **40**, 1043–1047 (2018).
- K. Vijayalakshmi, B. Devi, P. Sudha, V. J. Anand, A. S. "Synthesis, Characterization and Applications of Nanochitosan/Sodium Alginate/ Microcrystalline Cellulose Film." *J. Nanomed. Nanotechnol.* **7**, 1–11 (2015).