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Extraction collagen and Nanocollagen Preparation from Iraqi Catfish Skin Using Chitosan Physicochemical Characterization

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Abstract

This study investigated the extraction and preparation of nanocollagen from Iraqi catfish (Siluriformes) skin, utilizing chitosan and sodium tripolyphosphate (STPP) as crosslinking agents. Collagen was extracted through an optimized process involving chemical pretreatment and lyophilization. Nanocollagen was characterized using analytical techniques including Fourier-transform infrared spectroscopy (FTIR), UV-Visible spectroscopy (UV-Vis), X-ray diffraction (XRD), and scanning electron microscopy (SEM). Through FTIR analysis, the resulting functional groups were identified and the chemical reactions were confirmed, UV analysis of collagen showed maximum absorption at 230 nm, XRD spectra confirmed the crystal structure associated with the triple helix conformation, SEM results indicate that ASC collagen appears as a soft white sponge, there is an obvious rough surface with a spherical structure, the results demonstrated significant improvements in the physicochemical properties of nanocollagen compared to raw collagen, including enhanced crystallinity, and particle uniformity. These findings suggest nanocollagen as a promising biomaterial for regenerative medicine and advanced wound care applications.

Introduction

Collagen is a fundamental protein comprising 25-35% of the total protein in the human body, with type I collagen constituting up to 70% of skin tissue [1]. It is a triple-helical protein composed of multiple peptide chains, providing structural integrity and functionality to tissues. With over 29 known types, collagen plays a pivotal role in tissue support and stabilization [2]. Iraqi catfish (Siluriformes) skin serves as a sustainable and unconventional source of high-quality collagen, distinguished by its biodegradability and ease of absorption. Advances in nanotechnology have enabled the transformation of collagen into nanocollagen, significantly enhancing its mechanical, biochemical, and biological properties [3]. Nanotechnology is characterized as any technical progress involving nano-scaled materials that can be utilized in everyday life [4]. It entails the manipulation of matter possessing at least one dimension ranging from approximately 1 to 100 nanometers in size. This technique encompasses the synthesis of nanomaterials applicable in many physical, chemical, and

biological systems, facilitating integration at both microscopic and macroscopic scales [5]. Nanocollagen is standard collagen diminished to the nanometer scale [6]. This 3D biomaterial is optimal due to its nanoscale technology ranging from 1 to 100 nm, which offers a high surface area-to-volume ratio, facilitating efficient penetration into wound sites and effective interaction. When combined with chitosan, a natural polysaccharide with antimicrobial properties, nanocollagen offers unique advantages [4], including improved wound healing, tissue regeneration, and controlled drug release. This study explores the extraction of collagen from Iraqi catfish skin, its conversion into nanocollagen using chitosan, and its physicochemical characterization for potential biomedical applications.

Materials and Methods

Sample collection

Parts of catfish were collected in ice box from a local market in Myssan from October, 2022 to April, 2023, and stored at -18°C. The skin of 12 catfish was used in the experiment. The commercial collagen from the scales of fish type II and collagen I were purchased from Baoding Faithful Industry Co., Ltd.CHINA. All chemicals used in the present work were of analytical grade unless otherwise stated.

Collagen Extraction

Catfish skin was cleaned, washed, and subjected to a series of chemical treatments. Non-collagenous protein removal: Soaked in 0.1 M NaOH (30:1 ratio) for 24 hours with replacement every 8 hours. Defatting: Treated with 10% butyl alcohol (30:1 ratio) for 24 hours under continuous agitation. Collagen solubilization: Soaked in 0.5 M acetic acid, followed by filtration to collect the collagen-rich solution. Precipitation was achieved using 2.6 M NaCl in 0.05 M tris-HCl buffer (pH 7). The precipitate was centrifuged at 10,000 rpm under cooling conditions, dialyzed against acetic acid and distilled water, and lyophilized for preservation [7]

Nanocollagen Preparation

Nanoparticles were synthesized using chitosan and Sodium Tripolyphosphate (STPP) following ionic gelation Chitosan was dissolved in 1% acetic acid and stirred for activation. Collagen was dissolved in Dimethyl Sulfoxide (DMSO) and combined with chitosan at a 1:1 molar ratio. Crosslinking was induced by adding STPP (1:2.5 ratio, w/w) and stirring at 151 °C temperature for 6 hours. Nanoparticles were collected via filtration, washed with ethanol and deionized water, and freeze-dried for further analysis [8].

Characterization on nanocollagen

Fourier Transform Infrared Spectra of Collagens

FTIR was performed using potassium bromide KBr pellets were obtained from discs containing 2mg of dry collagen was mixed with 200mg potassium bromide (KBr) and passed as disk with wavelength 400-4000 cm^{-1} in SHIMADZU (Japan) model instrument FTIR [9].

X-ray diffractometer (XRD)

A thin film of uniformly water suspended of each type of nanoparticles was prepared on a glass slide and kept for drying. X-ray diffraction (XRD) pattern was recorded by employing x-ray diffractometer at 2θ/θ scanning mode (operational voltage 40 kV and current 30 mA, Cu K(a) radiation λ = 1.540)[10]. Data were recorded for the 2θ range of 10 to 80 degrees with a

step of 0.0200 degree. The result obtained from the XRD pattern was interpreted with standard reference of Joint Committee on Powder Diffraction Standards (JCPDS card number04-0783) for the characterization of C-CsNPs. The particle size of the prepared samples was determined by using Debye–Scherrer equation [11] as follows :

$$D = \beta.\cos\theta / k.\lambda \quad \text{is } \lambda \text{ Where D is the crystal size,} \quad \ldots\ldots\ldots\ldots ((2)$$

Wavelength of X-ray, the θ is the diffraction angle (Braggs angle) in radians and β is the full width at half maximum (FWHM) of the peak in radians. The measurements were investigated at the Al-Khora Company in Baghdad.

Scanning Electron Microscope (SEM)

Morphological properties of the membranes such as surface porosity, roughness and texture were studied. Structure analysis of extracted collagen carried out using Scanning Electron Microscope (SEM). The sample was coated with gold using auto fine ion coater (JEOL JFC-1600) and the structure was viewed under scanning electron microscope (TESCAN, VEGA III) using 20 Kv as the accelerating voltage.

Collagen Ultraviolet-Visible Spectroscopy

The UV-Vis absorption spectrum of ASC, PSC was recorded using SELECTA, SPAIN spectrophotometer in the range of 200 – 300 nm. The collagen samples were prepared by dissolve 2 mg in 0.5 M acetic acid [11, 12].

Results and Discussion

FTIR analysis

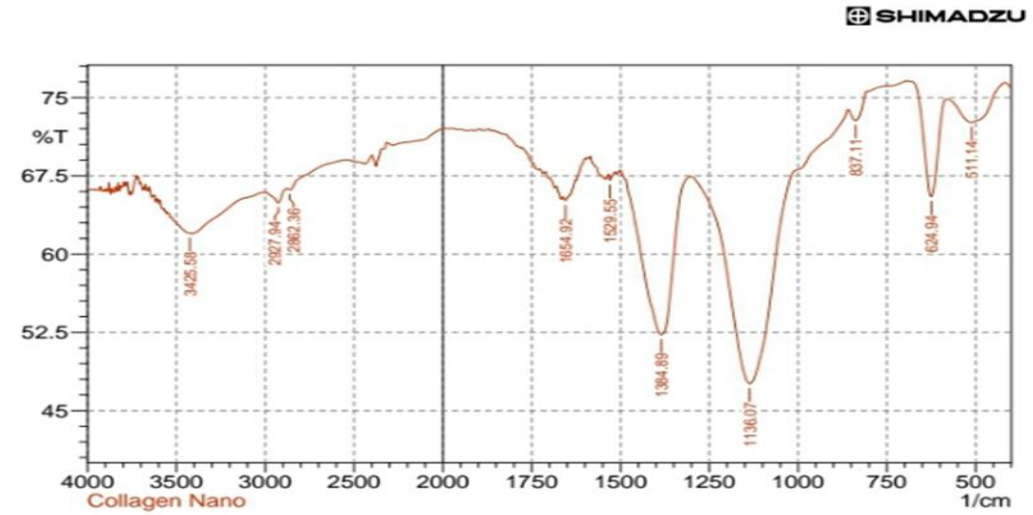
FTIR spectroscopy was employed for identify the vibrational spectra as well as chemical groups present in the isolated collagen. The findings indicated that the Amide A peak manifested at 3315.24 cm^{-1}, signifying stretching vibrations of the N-H group. The Amide B peaks were detected at 3079.05 cm^{-1} and 2934.84 cm^{-1}, associated with the asymmetric stretching of the CH_2 group. The Amide I peak at 1648.87 cm^{-1} suggested that collagen fibers are enriched with pyridinoline-type cross-links. Amide II peaks were detected at 1535.85 cm^{-1}, 1451.88 cm^{-1}, and 1400.23 cm^{-1} for ASC [13], indicating CN and NH vibrations. Amide III peaks were found at 1334.08 cm^{-1}, 1241.60 cm^{-1}, and 1163 cm^{-1}, corresponding to coupling vibrations of NH and CH_2. Absorption peaks at 1082 and 1032 cm^{-1} were attributed to C-OH stretching vibrations of carbohydrates bound to collagen, suggesting the presence of carbohydrates attached to hydroxylysine residues by O-glycosidic bonds as show table 1 Spectral Peak Positions in Fourier Transform Infrared of Acid Solubilized Collagen.

Table 1: Spectral Peak Positions in FTIR

Region	ASC	Assignment
Amide A cm^{-1}	3315.24 cm^{-1}	The elongation of the bond N-H is related to the OH 3440-3400 cm^{-1}
Amide B cm^{-1}	3079.05, 2934.84 cm^{-1}	Asymmetric elongation of CH2
Amide I cm^{-1}	1648.87 cm^{-1}	C=O carbonyl group stretching 1690-1600 cm^{-1}

Amide II cm $^{-1}$	1535.85, 1451.88, 1400.23 cm^{-1}	N-H stretching vibration 1480 - 1350 cm^{-} Curving CH_2
Amide III cm $^{-1}$	1334.08, 1241.60 1163.56 cm^{-1}	Coupling NH by elongation of CH_2 1300-1180 cm^{-1}
- cm $^{-1}$	1082.36 cm^{-1}	C-O
- cm^{-1}	1032.21 cm^{-1}	PO_4

Fourier transform infrared of CSNPs loading collagen refer to ten peaks 511.14, 624.94, 837.11, 1136.07, 1384.89, 1529.55, 1654.92, 2862.36, 2927.94, 3425.58 cm^{-1}, shown in figure (1) the 624.94 cm-1band, is attributed to the vibration of C-C 1384.89 cm^{-1} band is related to the vibration of C-N, while 1654.92 cm^{-1} band corresponds to the stretching vibration of carbonyl (C=O) bond . 2862.36 cm-1 band associated with to the stretching vibration of C-H. The final value of 3425.58 cm^{-1} denotes the stretching vibrations of the hydroxyl group. This change in the bonds and the interaction kinetics between the compounds is evidence of the collagen link to CSNPs and the formation of the Nanocapsule. Figure 1 indicates the presence of the transformation process, that is, the compounds were bound and the rest were withdrawn thus obtaining a highly effective capsule with a low concentration.



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	511.14	72.623	2.891	582.5	414.7	21.881	1.524
2	624.94	65.499	10.045	686.66	582.5	15.097	2.504
3	837.11	72.806	1.804	860.25	806.25	7.165	0.333
4	1136.07	47.587	22.266	1301.95	860.25	91.43	24.632
5	1384.89	52.278	15.409	1500.62	1303.88	43.478	10.197
6	1529.55	67.052	0.664	1533.41	1516.05	2.965	0.032
7	1654.92	65.111	0.355	1662.64	1649.14	2.5	0.016
8	2862.36	66.156	0.27	2877.79	2574.97	50.971	-0.501
9	2927.94	64.919	1.227	3007.02	2877.79	23.663	0.431
10	3425.58	62.004	0.026	3510.45	3423.65	17.743	0.079

Fig. 1: *Fourier transformation infrared spectroscopy (FTIR) Spectra Pattern of chitosan nanopartical loaded with collagen From Iraqi catfish skins. Term 400 – 4000 cm -1 infrared waves.*

Uv-Vis analysis

The UV-Vis examination of acid-soluble collagen (ASC) derived from fish skin exhibited a peak absorption at 230 nm, associated with peptide linkages, with no significant absorption in the typical UV region (280 nm) [14] as show in figure 2.

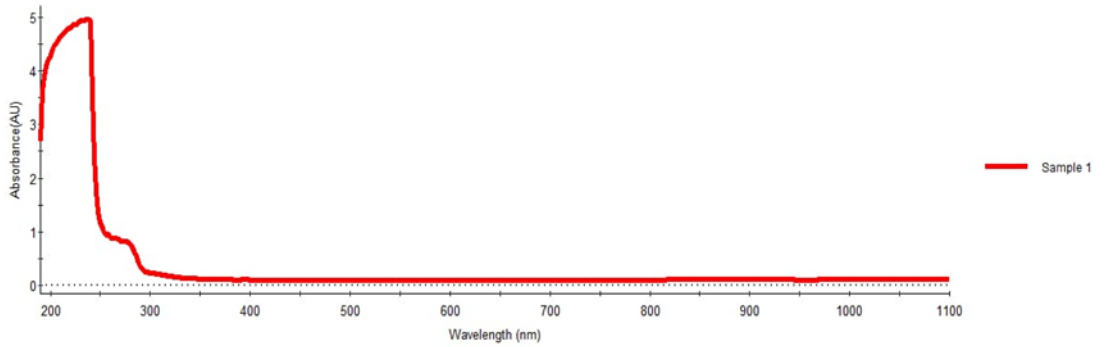


Fig. 2: *UV/Vis Absorption Spectrum of acid soluble collagen (ASC) Extracted from Iraqi Catfish skin*.

UV-Vis spectra of collagen with chitosan nanoparticles (CSNPs) indicated a decrease in absorbance at 430-442 nm, confirming successful nanocapsule formation and collagen loading, As show Figure (3) and Table (2)

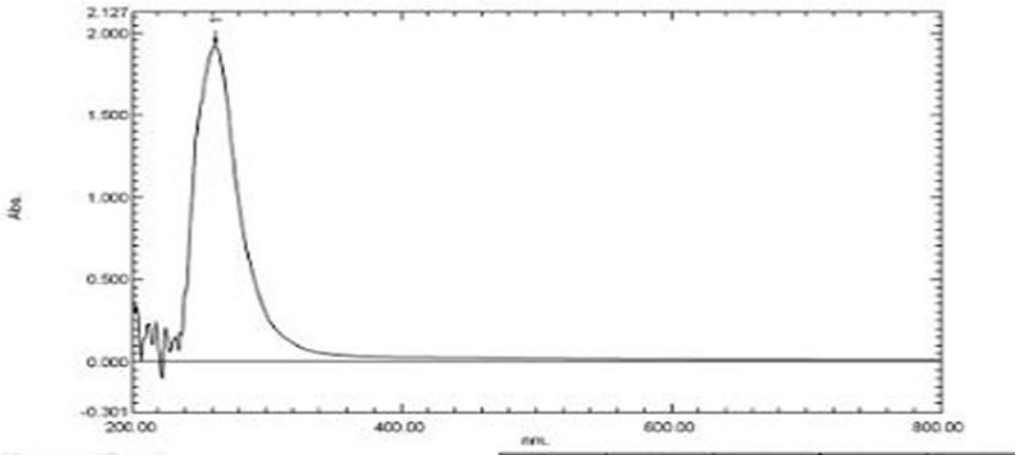


Fig. 3: *UV-Visible spectral analysis of Chitosan Collagen Nanoparticles from Iraqi catfish skins*

Table 2: Wavelength and absorbance of Chitosan Collagen Nanoparticles

Peak No.	Wavelength /nm	Absorbance
1	442.00	0.006
2	357.00	0.369
3	261.00	1.924
4	204.00	0.169

XRD analysis

The results shown **in Figure (4)** X-ray diffraction of standard collagen lyophilized acid-soluble collagen ASC derived from catfish skin, after obtaining the reading from the apparatus, it was determined that the diffraction angles (2θ) of the ASC were around 11.5094, 12.7304, and 13.7204 degrees correspondingly. The values that corresponded to the sharp peak for the ASC were 7.6814, 7.1094, and 7.0434 Å. In conjunction with the triple helix shape, the molecular structure of collagen was verified, and ASC exhibited three peaks. Crystal and amorphous structures are also associated with the triple helix conformation and the spacing among molecular chains relative to the distance among skeletons. Zhang et al. examined collagen sourced from carp scales and determined a minimum d-spacing of 11.87 Å for the sharp peak and 4.48 Å for the broad peak [15]. In addition, another study on collagen

extracted from salmon skin showed similar diffraction patterns, supporting the preservation of the triple-helical structure of collagen after extraction [7]. Thus, the XRD results of collagen extracted from Iraqi catfish skin are consistent with previous studies [13, 2], indicating the preservation of the characteristic triple-helical structure of collagen, which is essential for its biological functions. The X-ray diffractograms indicate that the morphological characteristics of the two collagen forms are directly associated with the material's phase structure. X-ray diffraction is commonly utilized to assess the distribution and orientation of collagen fibrils in mineralized tissues of fish.

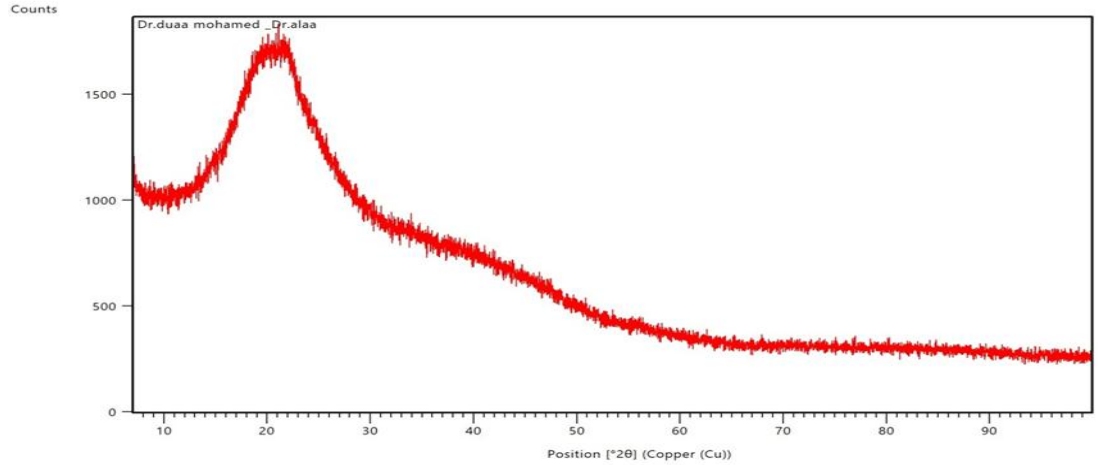


Fig.4 *: X-ray of collagen from skin of catfish*

SEM analysis

The change in structural morphology of fish skin collagen was observed by SEM images in Figure (5). Morphology of fish skin collagen SEM analysis for Acid-Soluble Collagen (ASC). (Figure 5 A) show the collagen ASC appeared as a soft white sponge with size ranging from 30-88 nm. (Figure 5 B) there are obvious rough surface with globular structure The coil-like fibrils were found. and random windings of coil structures which indicates the fibrous nature of the collagen (Figure 5 C) in high magnification appeared regular and uniform network with porous and honey –comb like structure. The cavities and cracks seen in images B and C indicate the fibrous nature of collagen, showing the material's ability to retain its mechanical properties.

This is inconsistent with [16] who measured the SEM for ASC from outer skin of spineless cuttlefish Sepiella inermis. The results of this study are consistent with SEM analysis of collagen extracted from Catla fish skin [17,18], which showed smooth or slightly wrinkled surfaces with plate-like structures, indicating intertwining of collagen fibers.

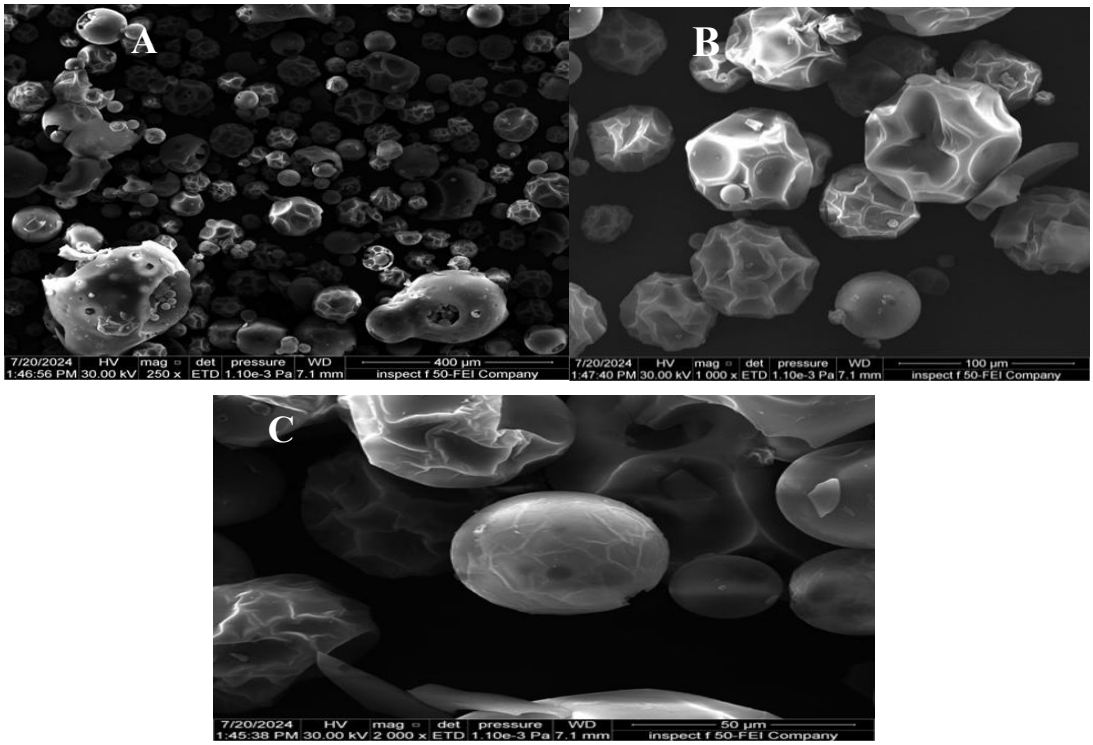


Fig. 5. Scanning Electron Microscope Morphologic of Collagen from Iraqi Catfish Skins.

(A) shows the distribution of particles on a wide scale at low magnification (250x) Particles of different sizes and shapes can be observed, some clustered and others dispersed. This indicates a processing method that results in a relatively uniform distribution. (B) shows the " zoomed in at 1000x, the details become much clearer. The image shows a wavy surface structure with visible cavities and cracks on the surface of the particles. This indicates the characteristics of the extracted collagen, which retains its distinctive fibrous structure." (C) shows the zoomed in at (2000x), the particles appear with very fine details. Smooth surfaces with clear particle boundaries can be seen. This indicates the quality of the collagen and its ability to retain its original structure after processing.

Conclusions

The research effectively illustrated the extraction of collagen from the skin of Iraqi catfish and its conversion into nanocollagen utilizing chitosan. The nanocollagen exhibited superior physicochemical properties compared to raw collagen, including enhanced crystallinity, uniformity, and antimicrobial activity. These results position nanocollagen as a promising biomaterial for regenerative medicine ,particularly in wound healing and advanced biomedical applications.

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استخلاص الكولاجين وتحضير الكولاجين النانوي من جلد السلور العراقي باستخدام التوصيف الفيزيائي الكيميائي الشيتوزان

دعاء محمد جبر *، شروق محمد كاظم
جامعة بغداد معهد الهندسة الوراثية والتقنيات الاحيائية

معلومات البحث:
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الكولاجين، الكولاجين النانوي، الشيتوزان، مطيافية الاشعة تحت الحمراء، الأشعة فوق البنفسجية المرئية،حيود الاشعة السينية ، المجهر الالكتروني الماسح

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الخلاصة:
تضمنت هذه الدراسة استخلاص وتحضير الكولاجين النانوي من جلد سمك السلور العراقي (Siluriformes)، باستخدام الكيتوزان وترايبوليفوسفات الصوديوم (STPP) كعوامل تشابك. تم استخراج الكولاجين من خلال عملية محسنة تتضمن المعالجة الكيميائية والتجفيف. تم تشخيص النانوكولاجين باستخدام التقنيات التحليلية بما في ذلك التحليل الطيفي للأشعة تحت الحمراء لتحويل فورييه (FTIR)، والتحليل الطيفي للأشعة فوق البنفسجية المرئية (UV-Vis)، وحيود الأشعة السينية (XRD)، والمجهر الإلكتروني الماسح (SEM) من خلال تحليل FTIR، تم تحديد المجموعات الوظيفية الناتجة وتأكيد التفاعلات الكيميائية، وأظهر تحليل الأشعة فوق البنفسجية للكولاجين الحد الأقصى للامتصاص عند 230 نانومتر، وأكد أطياف XRD البنية البلورية المرتبطة بالتشكل الحلزوني الثلاثي، وتشير نتائج SEM إلى أن كولاجين ASC يظهر كإسفنجة بيضاء ناعمة، وهناك سطح خشن واضح مع هيكل كروي، وأظهرت النتائج تحسينات كبيرة في الخواص الفيزيائية والكيميائية للنانو كولاجين مقارنة بالكولاجين الخام، بما في ذلك التبلور المعزز، وتوحيد الجسيمات. تشير هذه النتائج إلى أن الكولاجين النانوي مادة حيوية واعدة للطب التجديدي والتطبيقات المتقدمة للعناية بالجروح.