Chitosan and its derivatives as potential biomaterials for biomedical and pharmaceutical applications: A comprehensive review on green extraction approaches, recent progresses, and perspectives

Marwa Hamdi, Haozhi Sun, Lixia Pan, Dandan Wang, Mengxiao Sun, Zhaoning Zeng, Suming Li, Qingkun Dong, Feng Su

European Polymer Journal

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--Manuscript Draft--

Manuscript Number:	EUROPOL-D-24-02812R1
Article Type:	Review article
Section/Category:	Regular Paper
Keywords:	chitin; chitosan; Extraction; Green chemistry; Bioactivity; Biomedical applications.
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	Haozhi Sun
	Lixia Pan
	Dandan Wang
	Mengxiao Sun
	Zhaoning Zeng
	Suming Li
	Qingkun Dong
	Feng Su
Abstract:	Background : Nowadays, the search for new renewable and broad-spectrum natural biopolymers for biotechnological and medical applications has become an absolute necessity. Chitin and its deacetylated derivative, chitosan, are considered interesting and auspicious biopolymers being potentially applied in a wide range of biotechnological sectors, including medicine, food beverages, agriculture, and cosmetics, owing to their enormous ability to undergo changes in structure and mechanical properties to generate new functions (used as a matrix in beads, membranes, gels, etc.) and applications. Scope and Approach: The current review provides a comprehensive report summarizing research on the routine chemical and greener non-conventional extraction methodologies of chitin and chitosan and focuses on the progress in their application over the past two decades, in terms of challenges, opportunities, and future perspectives. Key Findings and Conclusions: Chitosan is an effective material with enormous potential for biotechnology and medicine owing to its biocompatible, biodegradable, and non-toxic traits, besides its antimicrobial potential and low immunogenic potency. To standardize applications in the industrial field considering cost-effectiveness and biocompatibility, the search for innovative recovery and production methods for chitin/chitosan-based materials industrialization is required. Conventional chemical chitin extraction approaches present drawbacks and induce numerous environmental issues. Greener extraction technologies have recently perceived considerable advancement in the polymer chemistry field. This review can serve as a guideline for exploring nature-originated biopolymers as innovative feedstocks for several technologies that show highly appealing potential for application in countless fields.
Response to Reviewers:	Dear Editor European Polymer Journal Date: 07-02-2025 Manuscript ID: EUROPOL-D-24-02812 Subject: Revision of the Manuscript EUROPOL-D-24-02812

Dear Editor,
We found the reviewers' feedback very helpful. We revised our manuscript and included all their recommendations. Please see our responses to each of the reviewers' comments and suggestions with our accompanying justifications. We are confident that the clarifications provided here will address all the reviewers' remarks and we hope that our manuscript will be finally accepted for publication in "European Polymer Journal".
Thanking you,

RESPONSE TO REVIEWERS' COMMENTS:
REVIEWER #1
*** First of all, we would like to thank Reviewer#1 for all the valuable and fruitful comments and time spent reviewing this manuscript. We have made sure to address all the comments and the responses are provided below. All the suggested changes have been addressed and highlighted in YELLOW COLOR.
1. Graphical abstract: Improve the image quality of the graphical abstract. They are probably too small.
*** Thank you for this fruitful comment. The graphical abstract was improved as recommended by the reviewer.
2. Line 73: Chitin is a foremost constituent of the fungi extracellular matrix, shellfish, and insects. This sentence is incorrect; in insects and crustaceans, chitin is part of the exoskeleton.
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3. Line 91: This assertion contradicts what is said in line 88.
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8. Regarding green methods: why haven't biological methods been explored? What new features does the work bring compared to Mohan et al., (2022)?
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	Mohan et al. (2022). Compared to Mohan et al. (2022) focusing on the extraction of chitin/chitosan, the new features could be recognized as follows: The current review provides a comprehensive report summarizing research on the routine chemical and greener non-conventional extraction methodologies of chitin and chitosan and focuses on the progress in their application over the past two decades, in terms of challenges, opportunities, and future perspectives. 9. On the application side, what are the new features that the work brings compared to the most recent but also the older reviews on the subject? *** Thank you for this fruitful comment. This review delves into the mechanisms underlying chitin/chitosan's biological activity and provides a comprehensive overview of their derivatives in fields such as tissue engineering, wound healing, drug delivery, cosmetics, etc. However, despite the wealth of studies on chitin/chitosan, there exists a notable trend of homogeneity in research, which could hinder the comprehensive development of these biomaterials. This review was aspired to provide an extensive overview of recent updates on the possible applicability of chitin and chitosan for biotechnological applications. Special attention is given to the chitin/chitosan biology, extraction procedures, physicochemical characterization and their biomedical applications. Furthermore, limitations, challenges, and future recommendations were discussed. Overall, this review highlights the potential role of chitin/chitosan in various biomedical platforms, thereby contributing to the continued advancement of chitin and chitosan in the field of healthcare. 10. Some of the pictures, although they form the most interesting part of this work, are of poor quality and the inscriptions are difficult to read. *** Thank you for this fruitful comment. Most of the figures were improved for better clarity and visibility, as recommended by the reviewer. 11. A total review of form and content is recommended, as well as identifying and making explicit the novelty and purpose of the work. *** Thank you for this fruitful comment. As explained in the previous comments, this review can serve as a guideline for exploring nature-originated biopolymers as innovative feedstocks for several technologies that show highly appealing potential for application in countless fields. The aforementioned concepts are summarized in this review and are reinforced by current publications on corresponding research areas. Particularly, this review article comprehensively discussed: i) Different sources for chitin and chitosan production to impart comprehensive information; ii) Routine chemical and greener non-conventional extraction methodologies of chitin and chitosan; iii) Chemical structures, modifications, and bio-functional properties; iv) Chitin and Chitosan journey from biomaterials to advanced functional materials; v) Application of chitin and chitosan over the past two decades, in terms of challenges, opportunities, and future perspectives.

青岛科技大学
Qingdao University of Science & Technology

Dear Editor

European Polymer Journal

Date: **07-02-2025**

Attached is our revised review entitled "***Chitosan and its Derivatives as Potential Biomaterials for Biomedical And Pharmaceutical Applications: A Comprehensive Review on Green Extraction Approaches, Recent Progresses, and Perspectives***" by **Hamdi *et al.*** for publication in your journal.

We found the reviewers' feedback very helpful. We revised our manuscript and included all their recommendations. These are listed below with our accompanying justifications. We are confident that the herein-provided clarifications will address all the referees' remarks. We hope our manuscript will finally be accepted for publication in the European Polymer Journal.

During the submission of the revision, a new author "Qingkun Dong" was added in the author list who highly contributed to the preparation of the revised version of the manuscript.

We would appreciate your understanding and kind acceptance of this modification in the author list.

We look forward to hearing from you.

Kindest Regards,

Corresponding Author

Dr. Marwa HAMDI

Qingdao University of Science and Technology

青岛科技大学
Qingdao University of Science & Technology

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Highlights

- Different natural sources for chitin and chitosan production were summarized to impart comprehensive information;
- Routine chemical and greener non-conventional extraction methodologies of chitin and chitosan were detailed;
- Structural characterization, modification, and bio-functionality of chitin and chitosan were briefly discussed;
- Chitosan and its derivatives are endowed with a plethora of marvelous properties with diverse biotechnological applications;
- Challenges, opportunities, and future perspectives of these fascinating biopolymers were suggested.

Graphical Abstract

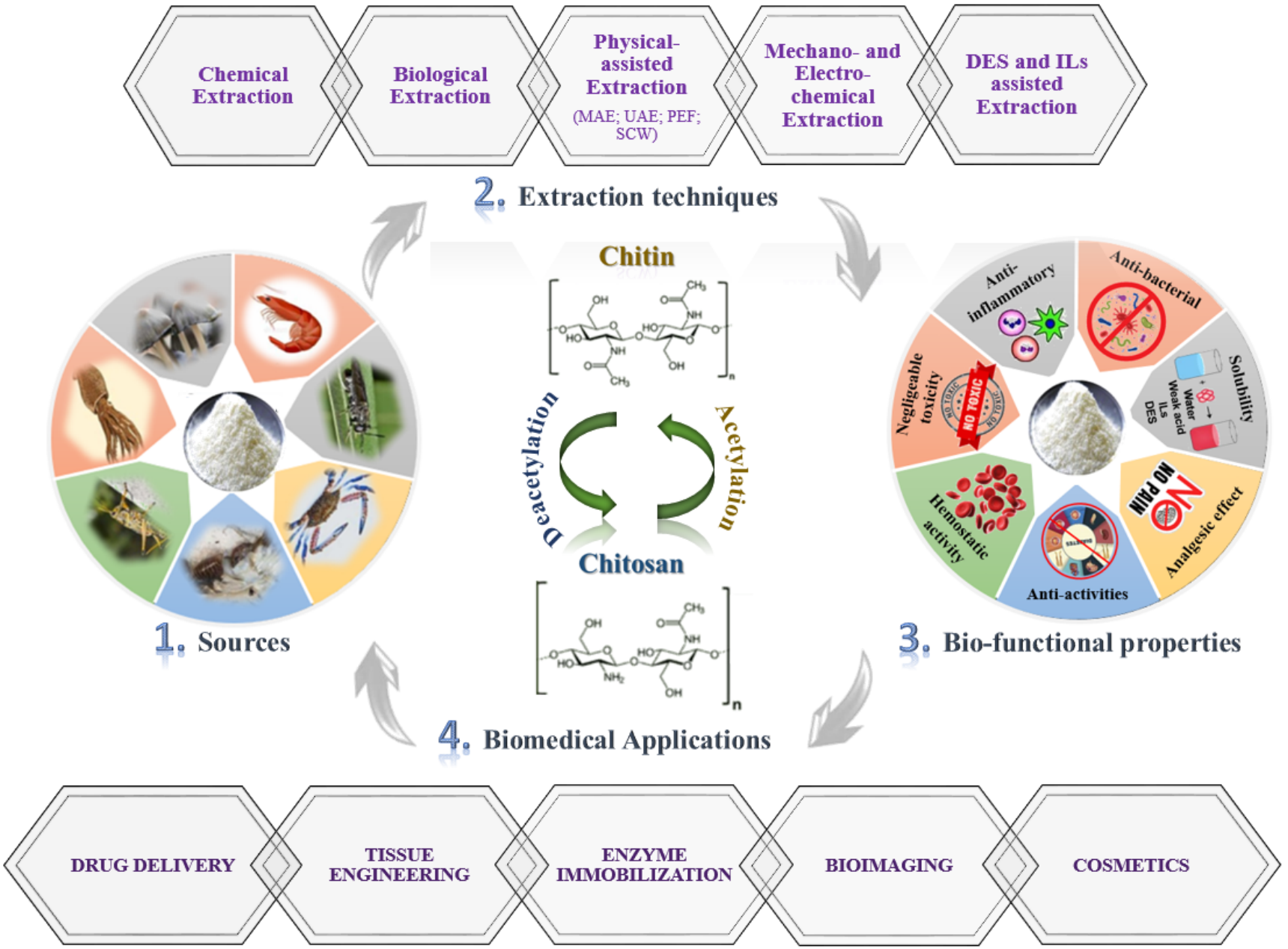


Figure captions:

Figure 1. Chemical **(A)** and Crystal **(B)** structures of chitin and chitosan.

Figure 2. Number of publications (1961-2025) in the "*ScienceDirect*" database in all research fields with the keywords "*Chitosan Applications*" **(A)**. Examples of potential application sectors of chitin and its derivative, chitosan **(B)**.

Figure 3. Routine extraction approaches of marine-derived chitin and its derivative chitosan with a schematic illustration of the hierarchical structure of crustacean shells (Raabe et al., 2005). Copyright 2005 Elsevier.

Figure 4. Principles and Flow diagrams of the Microwave irradiation **(A)**, Ultrasound vibration **(B)**, Subcritical water **(C)** (Li et al., 2014), Pulsed Electric Field **(D)** (Sridhar et al., 2021), and Electrochemical **(E)** (Mohan et al., 2022) extraction apparatus. Copyright (2014) Elsevier, Copyright (2021) Springer, Copyright (2022) Elsevier.

Figure 5. Schematic overview of Ionic Liquids (ILs) **(A)** and Deep Eutectic Solvents (DES) **(B)** synthesis routes. Similarities and differences in the preparation steps of ILs and DES. Easier preparation of DES, compared to ILs that require a large number of synthesis steps leading to environmental impacts **(C)**. Summary of eco-friendly extraction methods for chitin and chitosan from different biomass waste sources using DES or ILs **(D)**.

Figure 6. Proposed applications of DES and ILs on chitin and chitosan-related processes (Özel and Elibol, 2021). Copyright (2021) Elsevier.

Figure 7: Chitin, chitosan, and their derivatives/oligomers (Le Roux, 2012).

Figure 8: Chitosan target reactive groups, possible modification reactions, and structures of some examples of the resulting derivatives.

Figure 9: Major chitin, chitosan, and its derivatives, myriad anti-activities, and health-related benefits **(A)** in biomedical applications **(B)**.

Figure 10: Schematic illustration of four proposed hypotheses for the antimicrobial action mechanism of chitosan in Gram-positive and Gram-negative bacteria: Due to its polycationic feature, chitosan interferes and breaks the cell membrane, inducing the leakage of intracellular components (disturbance of membrane fluidity), binds to the bacterial DNA/mRNA (interfering with transcription and translation), blocks nutrient and gas exchange (inhibition of bacterial growth), and interacts with metal ions (chelating agent) (Ul-Islam et al., 2024). Permission for reproduction of figures. Copyright (2024) Elsevier.

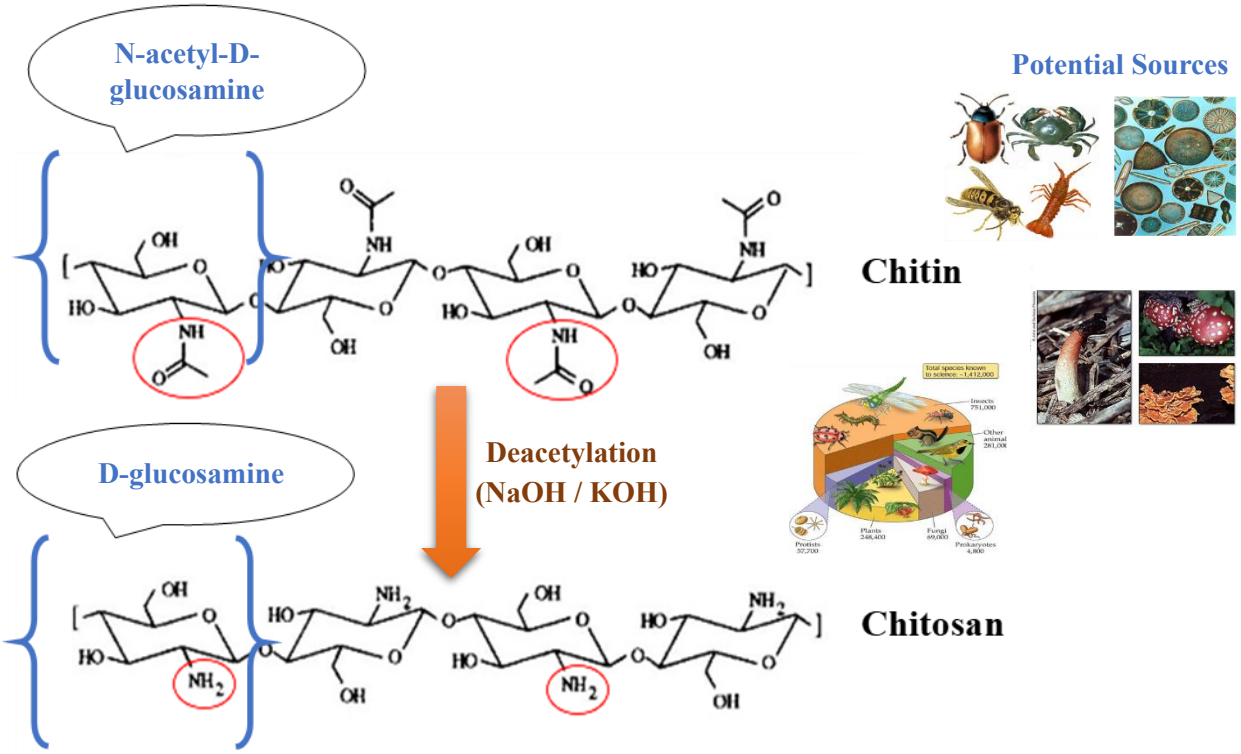
Figure 11. Cytotoxic study **(A)**. Wound contraction during the wound healing treatment period **(B)**. Histology of wounds treated with chitosan hydrogels-based patches on the 13th day after the operation **(C)**. *Group 1:* control rats treated with physiological serum; *Group 2:* rats treated with the MEBO® healing reference; *Group 3:* rats treated with Chitosan-based hydrogel patches; *Group 4:* rats treated with Chitosan-Protein Isolate composite hydrogel patches; *Group 5:* rats treated with Chitosan-Protein Isolate composite Carotenoids-loaded hydrogel patches (Hamdi et al., 2020). Permission for reproduction of figures. Copyright (2020) Elsevier.

Figure 12: Schematic illustration of the synthesis and mechanism of action of a multi-functional chitosan thermo-responsive hydrogel combined with black phosphorus nanosheets as an injectable biomaterial for rheumatoid arthritis biotherapy and phototherapy treatment. The resulting formulation was used for treating hyperplastic synovial tissues and a calcium-free phosphorus strategy for enhancing osteogenesis (Pan et al., 2020). Copyright (2020) Elsevier.

Figure 13: Schematic representation for cellulose acetate/chitosan/poly(ethylene oxide) scaffold synthesis process, laccase immobilization, and ABTS degradation. (Salehizadeh et al., 2023). Permission for reproduction of figures. Copyright (2023) Elsevier.

Fig. 1

(A)


N-acetyl-D-glucosamine
Potential Sources
Chitin
Deacetylation
(NaOH / KOH)
D-glucosamine
Chitosan

(B)

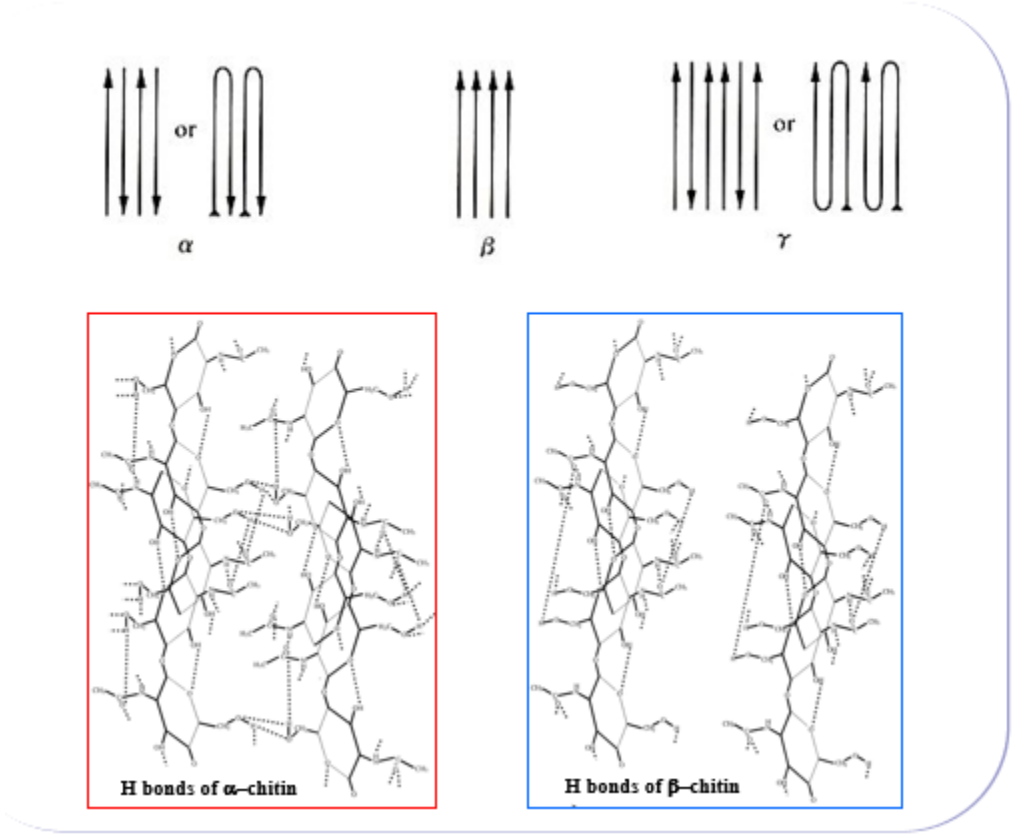
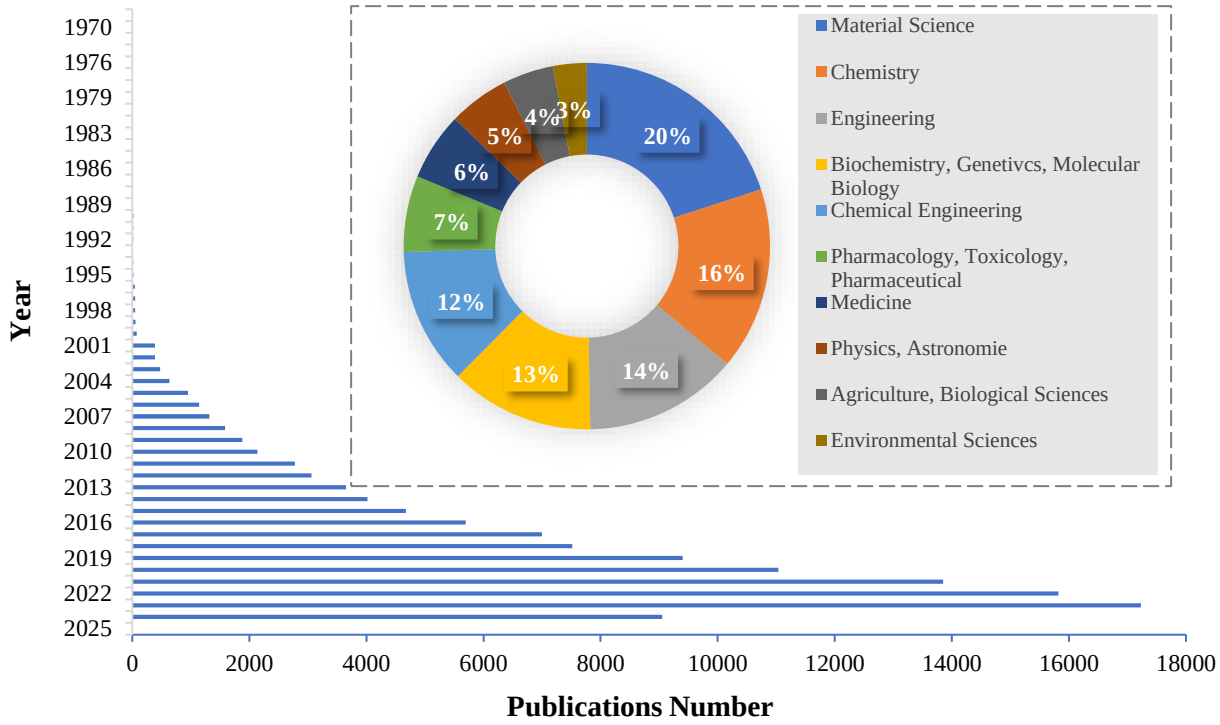

or
α
β
or
γ
H bonds of α–chitin
H bonds of β–chitin

Fig. 2

(A)


Year
1970
1976
1979
1983
1986
1989
1992
1995
1998
2001
2004
2007
2010
2013
2016
2019
2022
2025
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
Publications Number
20%
16%
14%
13%
12%
7%
6%
5%
4%
3%
Material Science
Chemistry
Engineering
Biochemistry, Genetivcs, Molecular Biology
Chemical Engineering
Pharmacology, Toxicology, Pharmaceutical
Medicine
Physics, Astronomie
Agriculture, Biological Sciences
Environmental Sciences

(B)


Chitin / Chitosan
Cosmetics
Dietetics
Medicine
Pharmacy
Nutrition
Food
Agriculture
Environment
Paper
DIETETICS
NUTRITION
☐ Protective gel
☐ Thickening agent
☐ Dental care
☐ Surface coating
☐ Antimicrobial
☐ Retention and Drainage
☐ Strength agent
☐ Protective gel in the stomach, thickener or stabilizer
☐ Water treatment and purification
☐ Air purification
☐ Biosensors
☐ Heavy metals adsorption
☐ Cholesterol-lowering agent
☐ Biomaterials (tissue regeneration)
☐ Bioresorbable sutures
☐ Adhesive
☐ Healing dressings
☐ Seed coating
☐ Plant disease control
☐ Plant growth stimulants
☐ Resistance to abiotic and biotic stress
☐ Food additive
☐ Food protective
☐ Food preservative
☐ Packaging, edible films, coating materials

Fig. 3

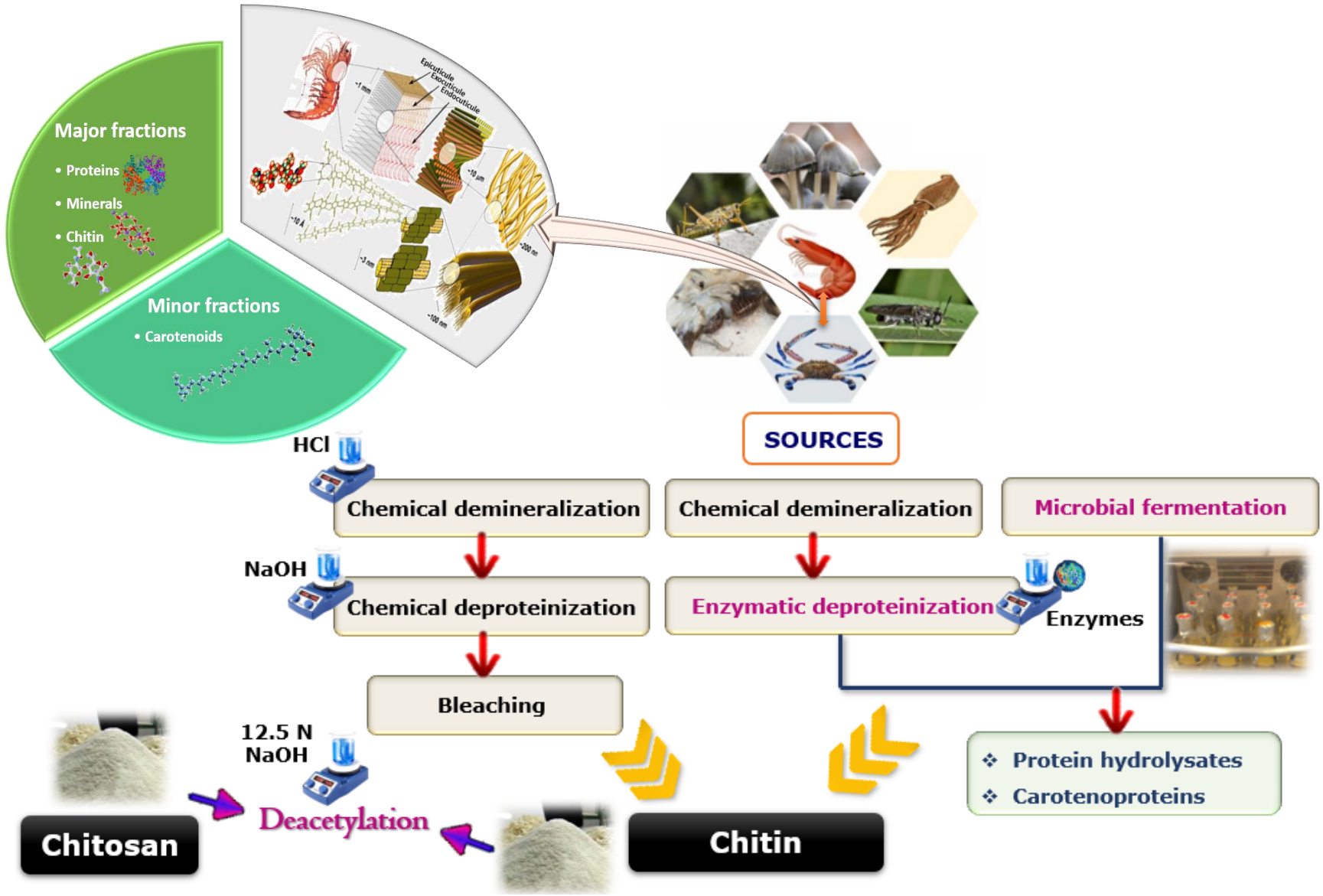

Major fractions
• Proteins
• Minerals
• Chitin
Minor fractions
• Carotenoids
SOURCES
HCl
Chemical demineralization
Chemical demineralization
Microbial fermentation
NaOH
Chemical deproteinization
Enzymatic deproteinization
Enzymes
Bleaching
12.5 N NaOH
Deacetylation
Chitosan
Chitin
Protein hydrolysates
Carotenoproteins

Fig. 4

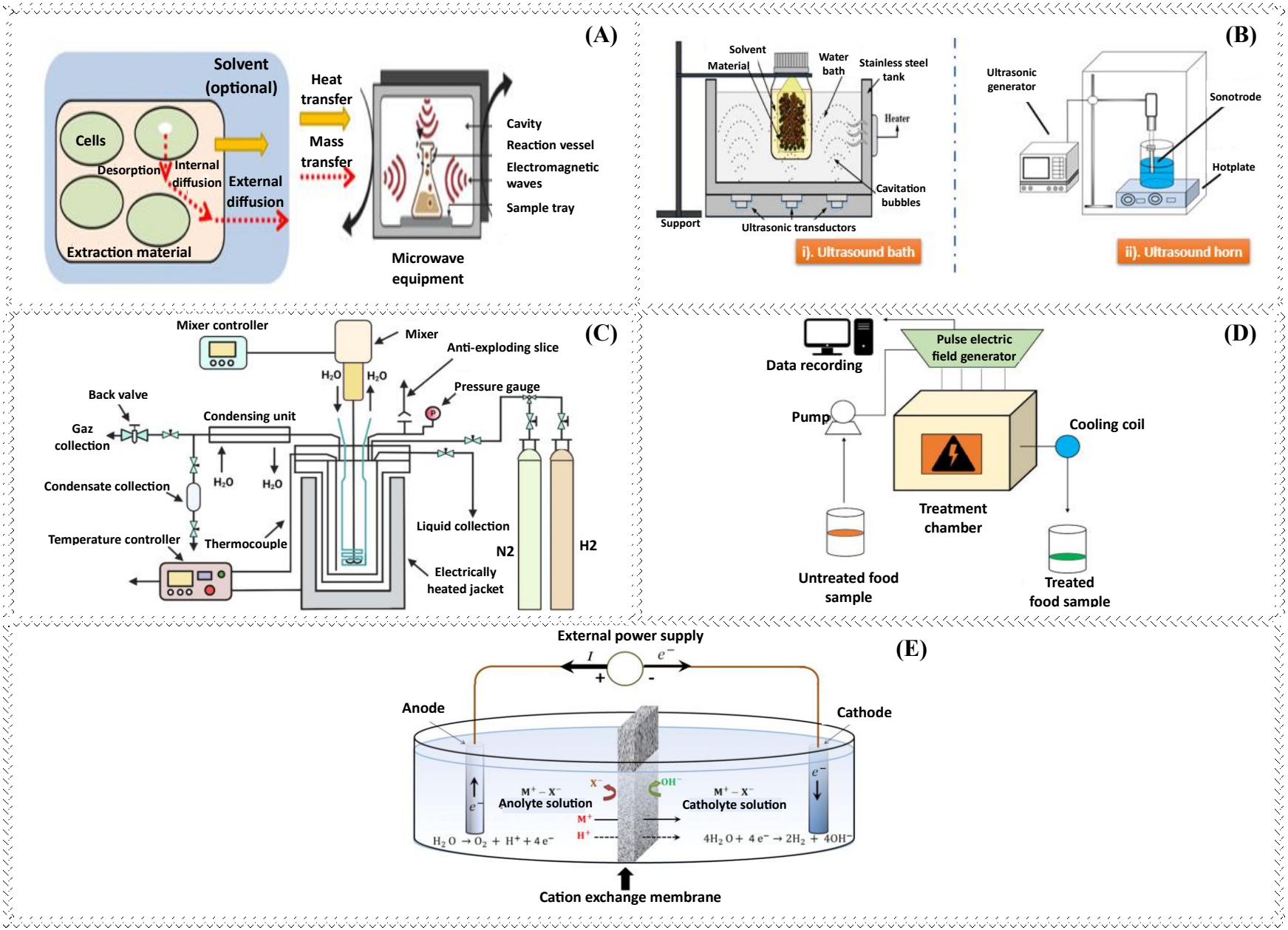
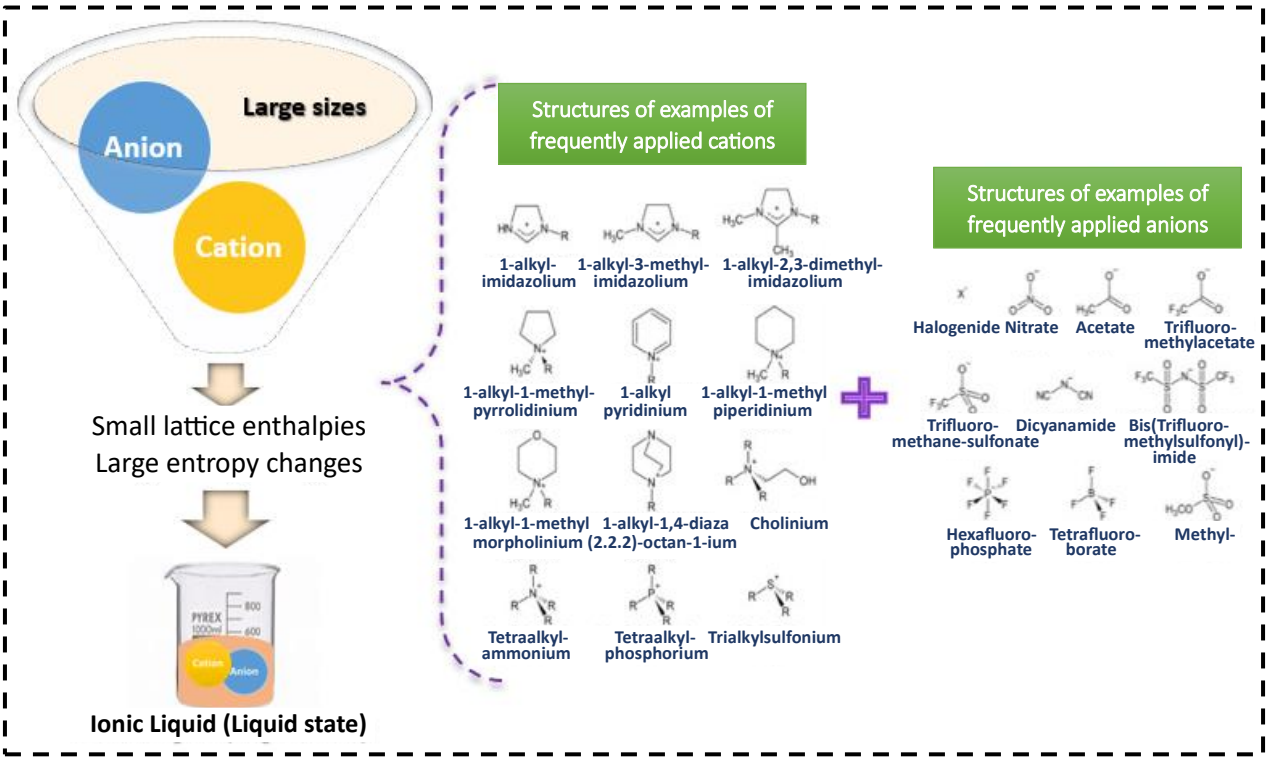
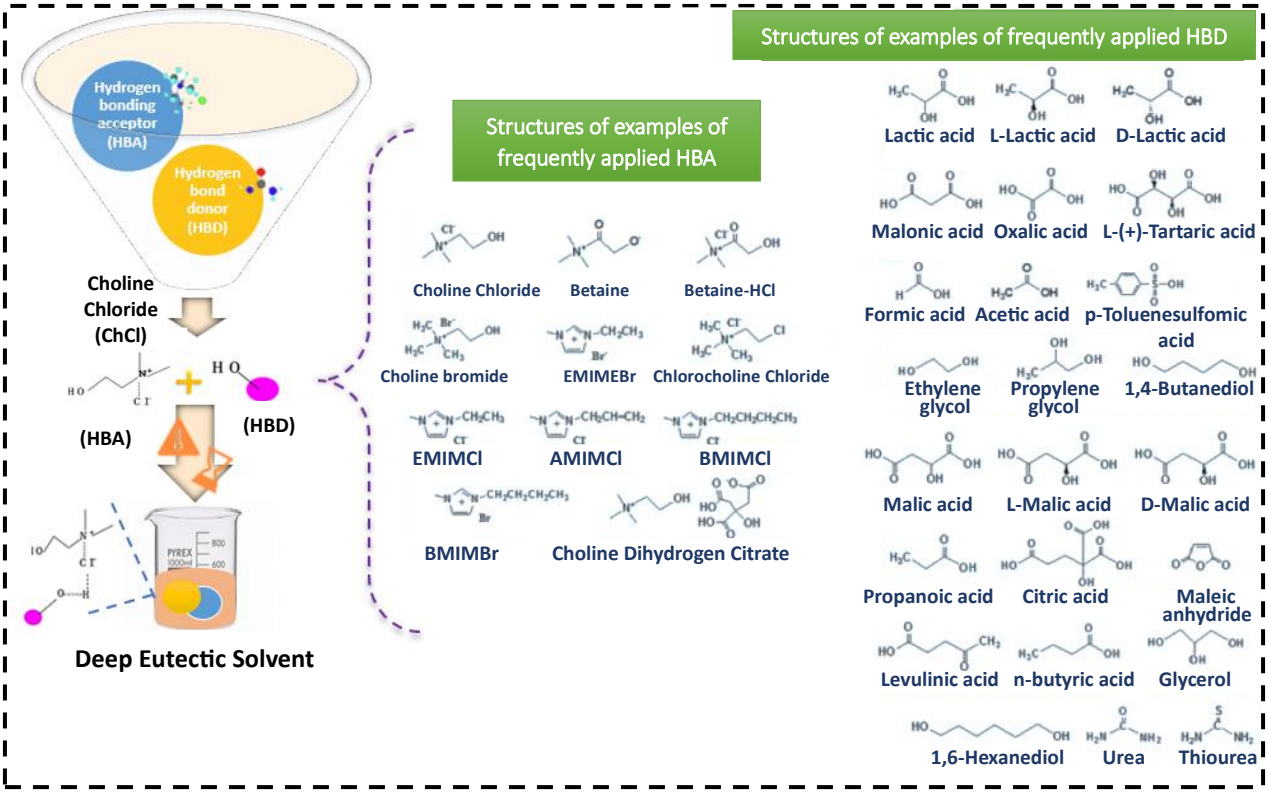

(A)
Solvent (optional)
Cells
Desorption
Internal diffusion
External diffusion
Extraction material
Heat transfer
Mass transfer
Cavity
Reaction vessel
Electromagnetic waves
Sample tray
Microwave equipment
(B)
Solvent
Material
Water bath
Stainless steel tank
Heater
Cavitation bubbles
Support
Ultrasonic transductors
i). Ultrasound bath
Ultrasonic generator
Sonotrode
Hotplate
ii). Ultrasound horn
(C)
Mixer controller
Mixer
Anti-exploding slice
Pressure gauge
Back valve
Condensing unit
Gaz collection
Condensate collection
Temperature controller
Thermocouple
Liquid collection
N2
H2
Electrically heated jacket
(D)
Data recording
Pulse electric field generator
Pump
Cooling coil
Treatment chamber
Untreated food sample
Treated food sample
(E)
External power supply
Anode
Cathode
Anolyte solution
Catholyte solution
Cation exchange membrane

Fig. 5

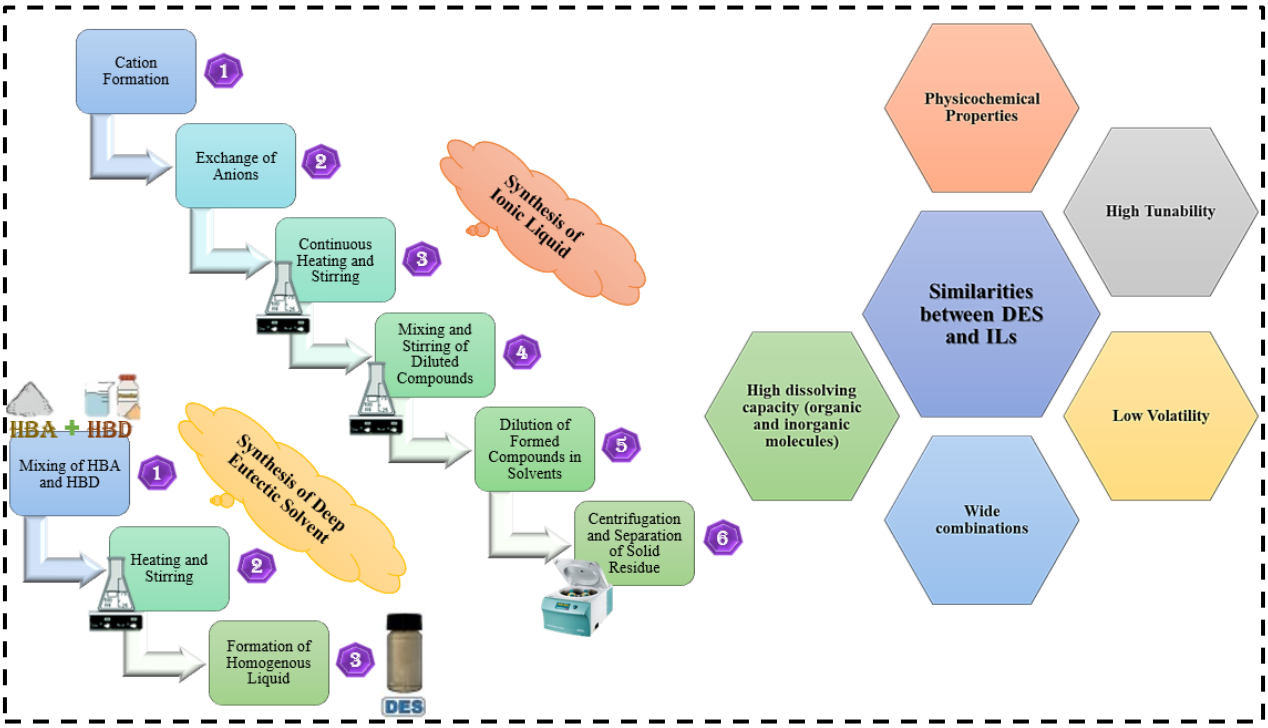
(A)


Large sizes
Anion
Cation
Small lattice enthalpies
Large entropy changes
Ionic Liquid (Liquid state)
Structures of examples of frequently applied cations
1-alkyl-imidazolium
1-alkyl-3-methyl-imidazolium
1-alkyl-2,3-dimethyl-imidazolium
1-alkyl-1-methyl-pyrrolidinium
1-alkyl pyridinium
1-alkyl-1-methyl piperidinium
1-alkyl-1-methyl morpholinium
1-alkyl-1,4-diaza (2.2.2)-octan-1-ium
Cholinium
Tetraalkyl-ammonium
Tetraalkyl-phosphorium
Trialkylsulfonium
Structures of examples of frequently applied anions
Halogenide
Nitrate
Acetate
Trifluoro-methylacetate
Trifluoro-methane-sulfonate
Dicyanamide
Bis(Trifluoro-methylsulfonyl)-imide
Hexafluoro-phosphate
Tetrafluoro-borate
Methyl-

(B)


Hydrogen bonding acceptor (HBA)
Hydrogen bond donor (HBD)
Choline Chloride (ChCl)
(HBA)
(HBD)
Deep Eutectic Solvent
Structures of examples of frequently applied HBA
Choline Chloride
Betaine
Betaine-HCl
Choline bromide
EMIMEBr
Chlorocholine Chloride
EMIMCl
AMIMCl
BMIMCl
BMIMBr
Choline Dihydrogen Citrate
Structures of examples of frequently applied HBD
Lactic acid
L-Lactic acid
D-Lactic acid
Malonic acid
Oxalic acid
L-(+)-Tartaric acid
Formic acid
Acetic acid
p-Toluenesulfomic acid
Ethylene glycol
Propylene glycol
1,4-Butanediol
Malic acid
L-Malic acid
D-Malic acid
Propanoic acid
Citric acid
Maleic anhydride
Levulinic acid
n-butyric acid
Glycerol
1,6-Hexanediol
Urea
Thiourea

(C)


Cation Formation
1
Exchange of Anions
2
Continuous Heating and Stirring
3
Mixing and Stirring of Diluted Compounds
4
Dilution of Formed Compounds in Solvents
5
Centrifugation and Separation of Solid Residue
6
Synthesis of Ionic Liquid
HBA + HBD
Mixing of HBA and HBD
1
Heating and Stirring
2
Formation of Homogenous Liquid
3
DES
Synthesis of Deep Eutectic Solvent
Physicochemical Properties
High Tunability
Similarities between DES and ILs
High dissolving capacity (organic and inorganic molecules)
Low Volatility
Wide combinations

(D)


4
Recycling
1
DES
OR
ILs
Chitin source powders
Residue
CHITIN
5
(NaOH)
Deacetylation
CHITOSAN
2
Demineralization
$CaCO_3$
CO_2
+
Deproteinization
Proteins
Carotenoids
Recovery / Separation:
* Washing
* Filtration
* Drying
3
Circular economy approach:
Ecological,
high yield,
less acid/base

Fig. 6

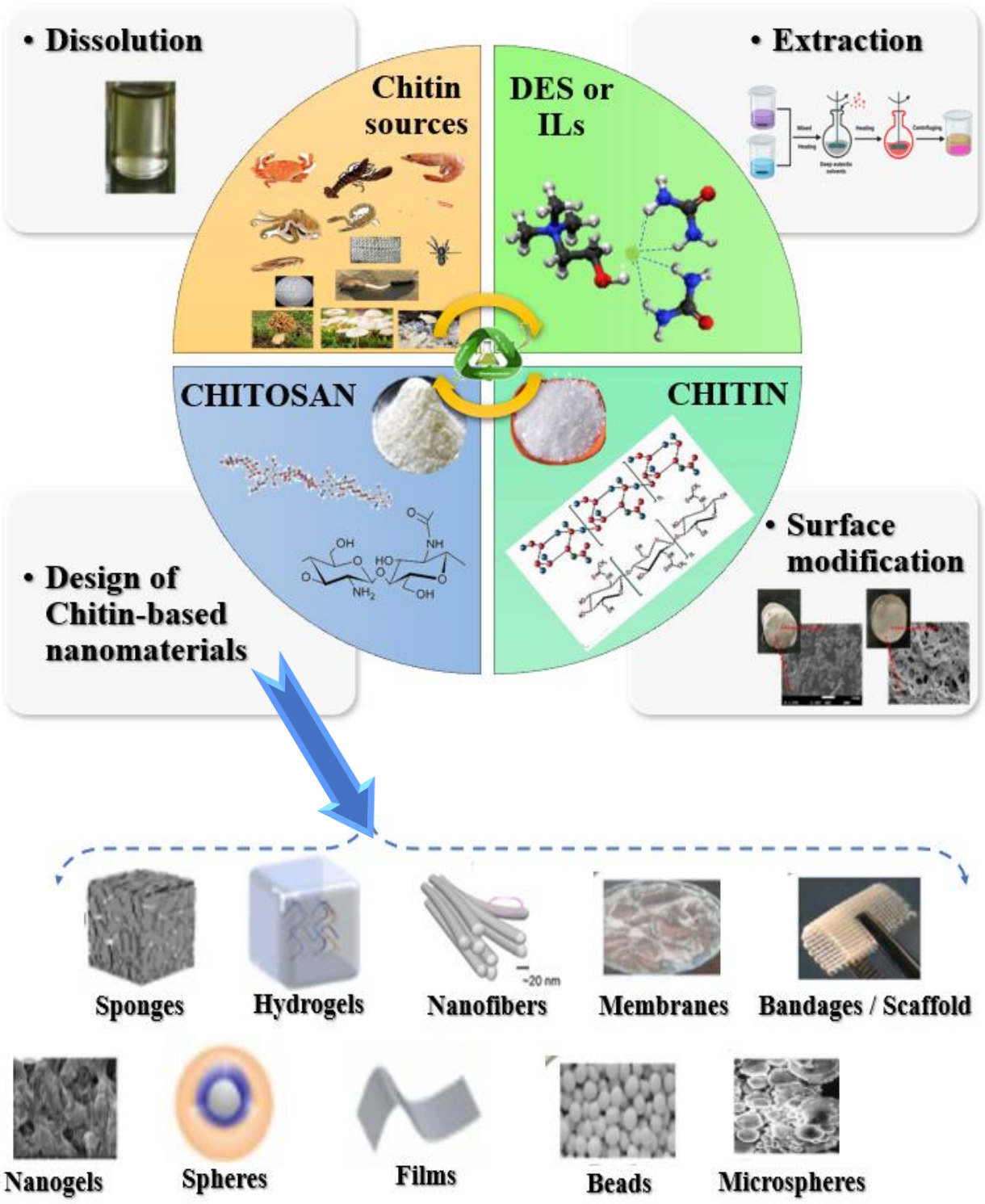

• Dissolution
• Extraction
Chitin sources
DES or ILs
CHITOSAN
CHITIN
• Design of Chitin-based nanomaterials
• Surface modification
Sponges
Hydrogels
Nanofibers
~20 nm
Membranes
Bandages / Scaffold
Nanogels
Spheres
Films
Beads
Microspheres

Fig. 7

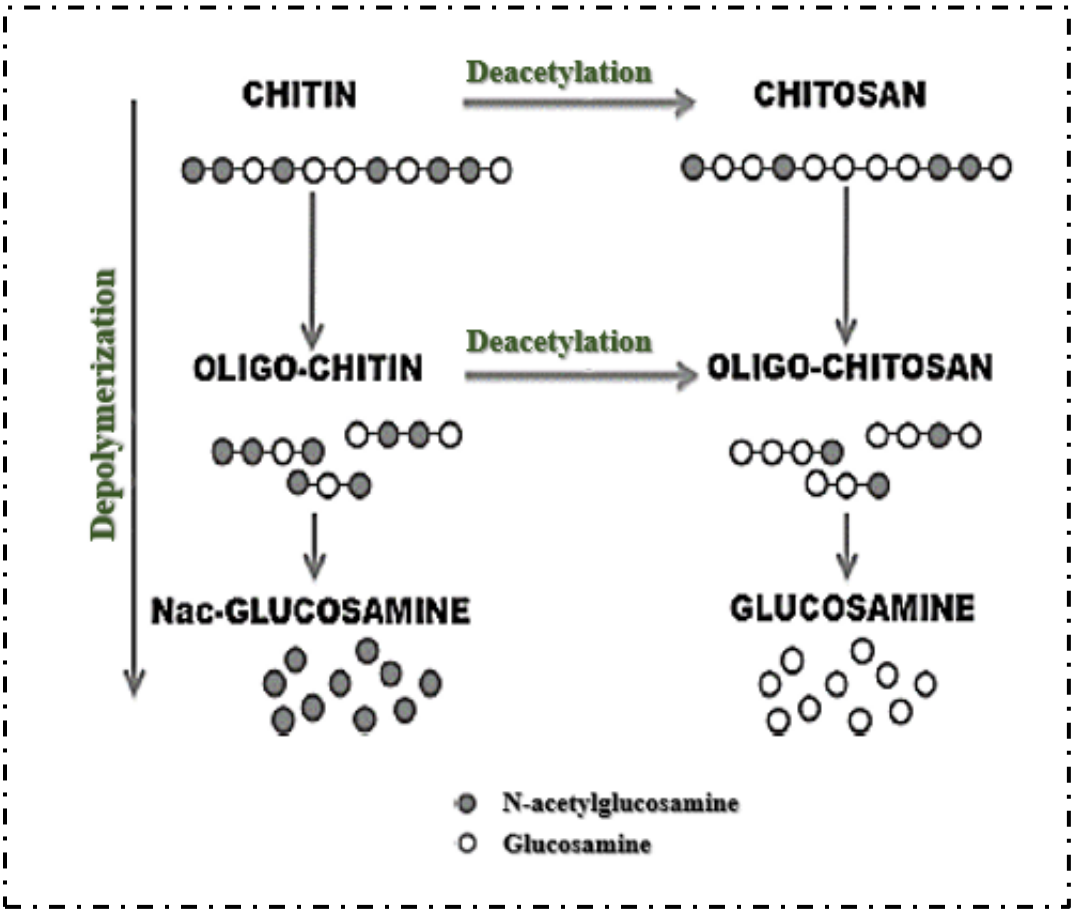

CHITIN
Deacetylation
CHITOSAN
Depolymerization
OLIGO-CHITIN
Deacetylation
OLIGO-CHITOSAN
Nac-GLUCOSAMINE
GLUCOSAMINE
N-acetylglucosamine
Glucosamine

Fig. 8

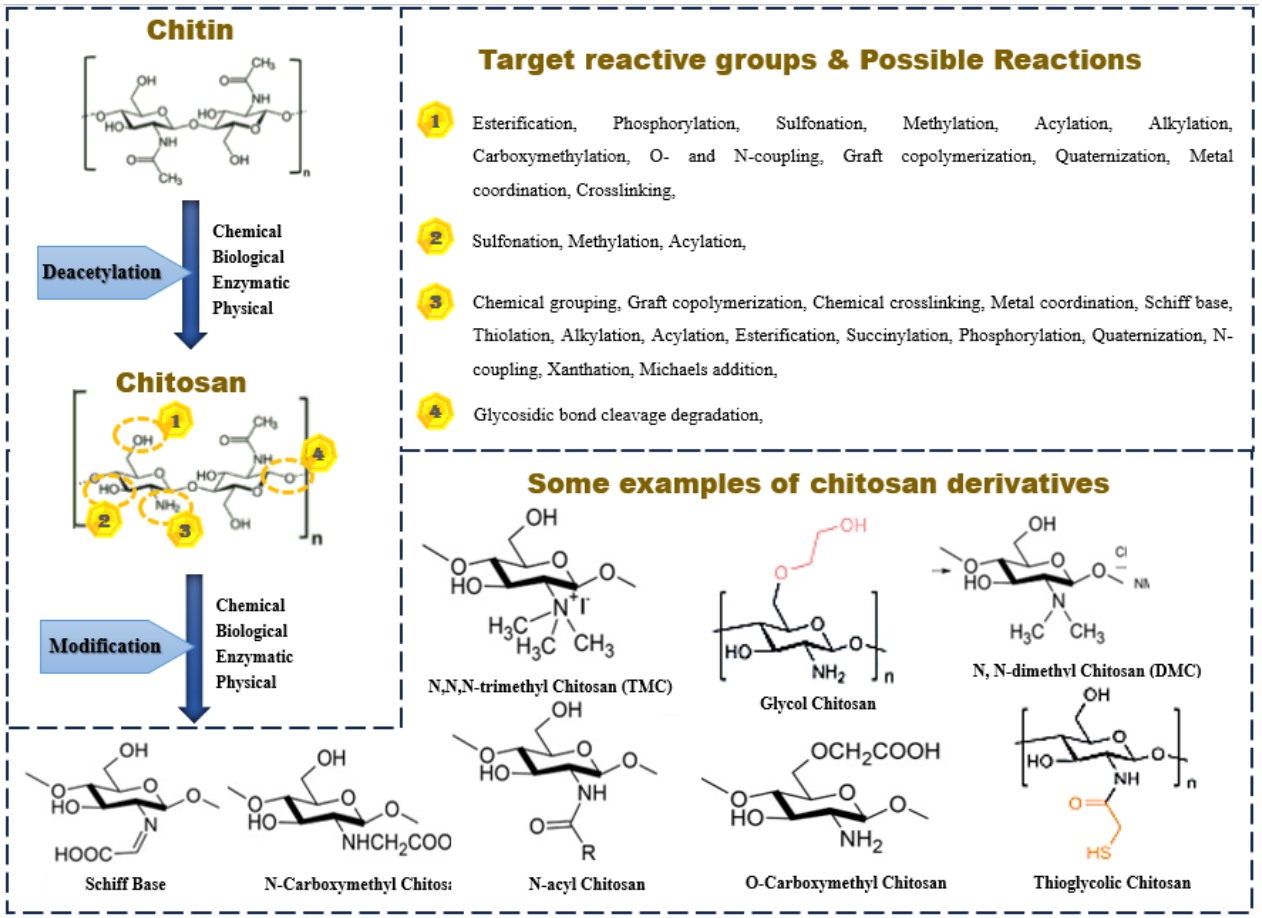
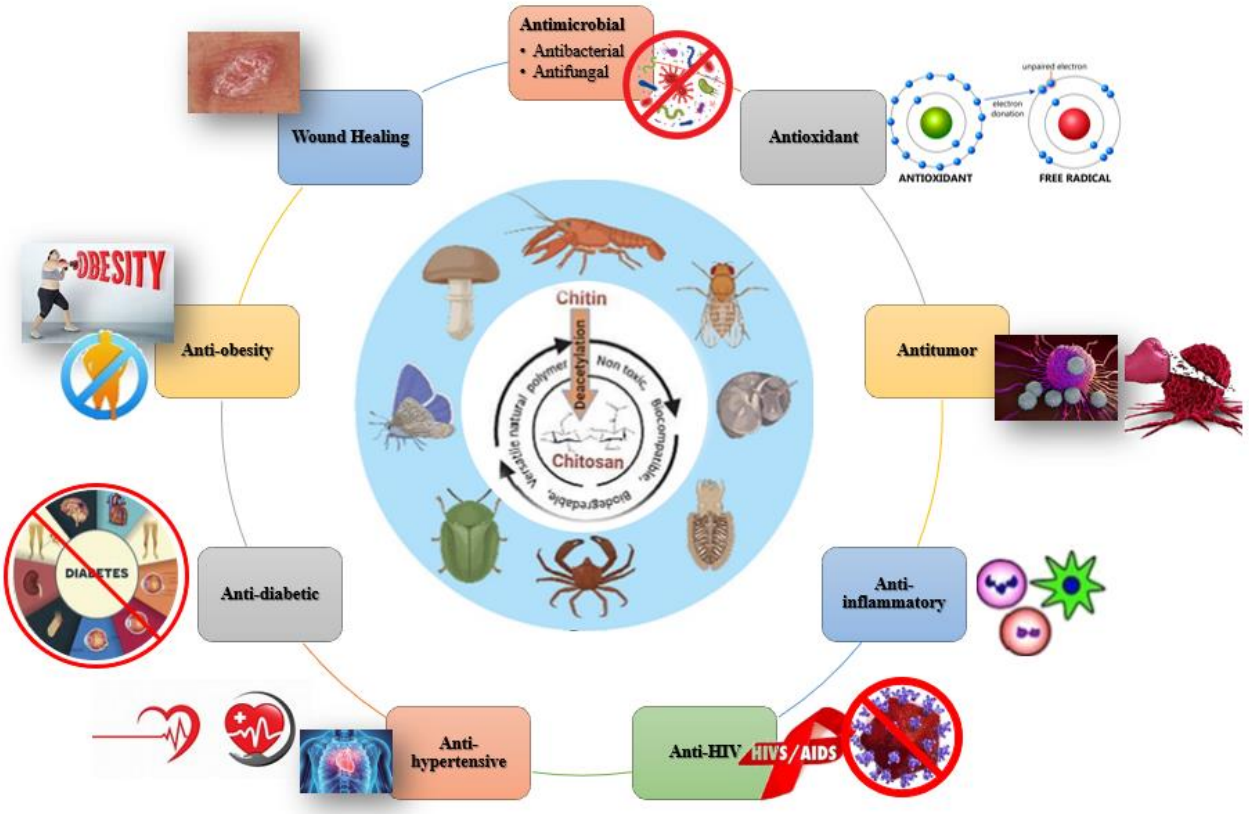

Chitin
Deacetylation
Chemical
Biological
Enzymatic
Physical
Chitosan
Modification
Chemical
Biological
Enzymatic
Physical
Target reactive groups & Possible Reactions
1 Esterification, Phosphorylation, Sulfonation, Methylation, Acylation, Alkylation, Carboxymethylation, O- and N-coupling, Graft copolymerization, Quaternization, Metal coordination, Crosslinking,
2 Sulfonation, Methylation, Acylation,
3 Chemical grouping, Graft copolymerization, Chemical crosslinking, Metal coordination, Schiff base, Thiolation, Alkylation, Acylation, Esterification, Succinylation, Phosphorylation, Quaternization, N-coupling, Xanthation, Michaels addition,
4 Glycosidic bond cleavage degradation,
Some examples of chitosan derivatives
N,N,N-trimethyl Chitosan (TMC)
Glycol Chitosan
N, N-dimethyl Chitosan (DMC)
Schiff Base
N-Carboxymethyl Chitos
N-acyl Chitosan
O-Carboxymethyl Chitosan
Thioglycolic Chitosan

Fig. 9

(A)


Antimicrobial
• Antibacterial
• Antifungal
Wound Healing
Antioxidant
ANTIOXIDANT
FREE RADICAL
Chitin
Deacetylation
Chitosan
Anti-obesity
Antitumor
Anti-diabetic
Anti-inflammatory
Anti-hypertensive
Anti-HIV
HIVS/AIDS

(B)

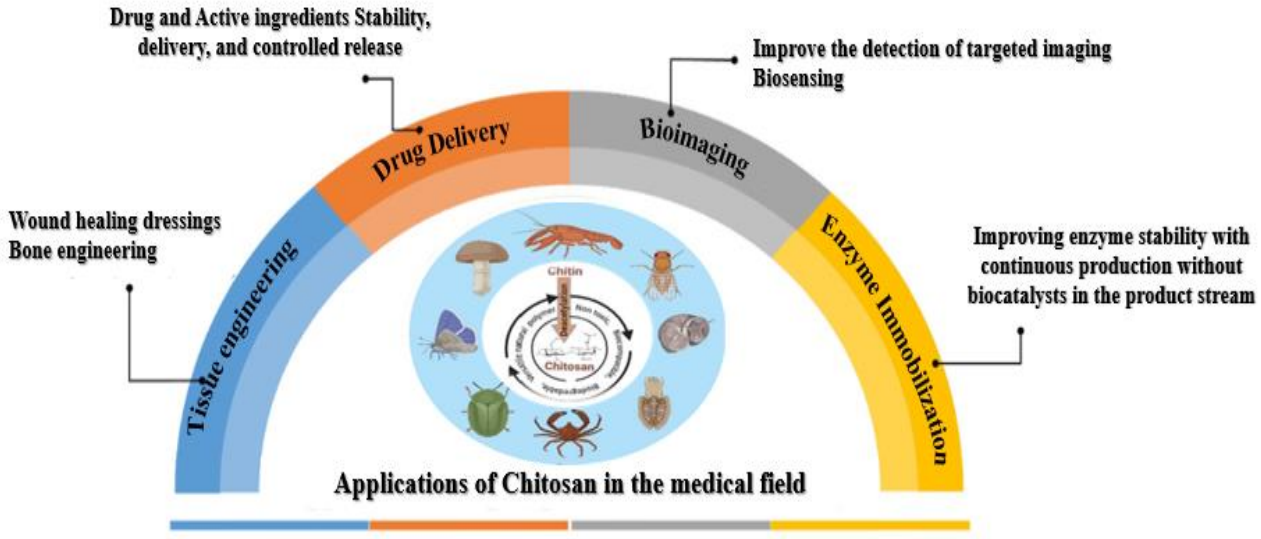

Drug and Active ingredients Stability, delivery, and controlled release
Improve the detection of targeted imaging Biosensing
Drug Delivery
Bioimaging
Wound healing dressings Bone engineering
Improving enzyme stability with continuous production without biocatalysts in the product stream
Tissue engineering
Enzyme Immobilization
Chitin
Chitosan
Applications of Chitosan in the medical field

Fig. 10

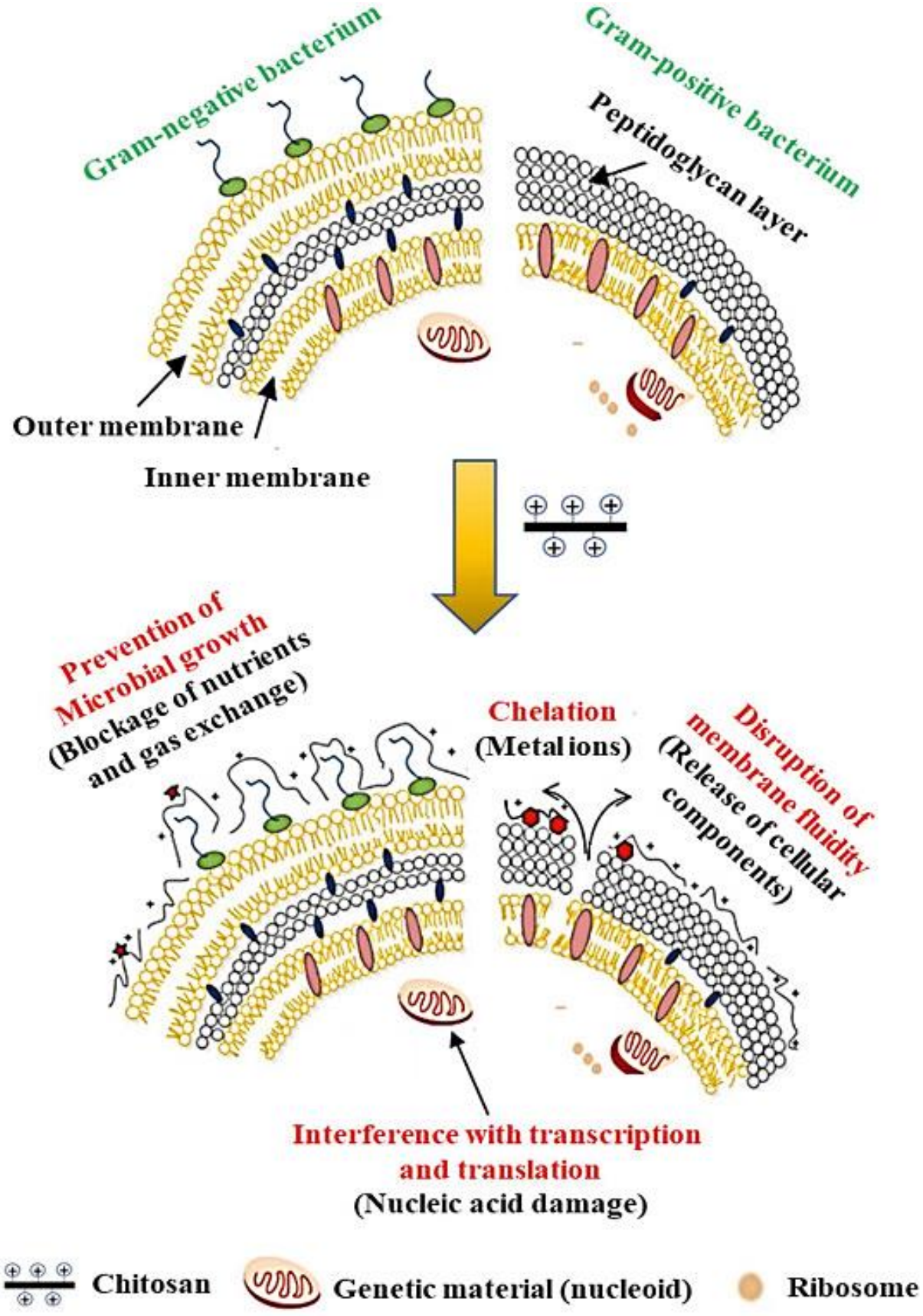

Gram-negative bacterium
Gram-positive bacterium
Peptidoglycan layer
Outer membrane
Inner membrane
Prevention of Microbial growth
(Blockage of nutrients and gas exchange)
Chelation
(Metal ions)
Disruption of membrane fluidity
(Release of cellular components)
Interference with transcription and translation
(Nucleic acid damage)
Chitosan
Genetic material (nucleoid)
Ribosome

Fig. 11

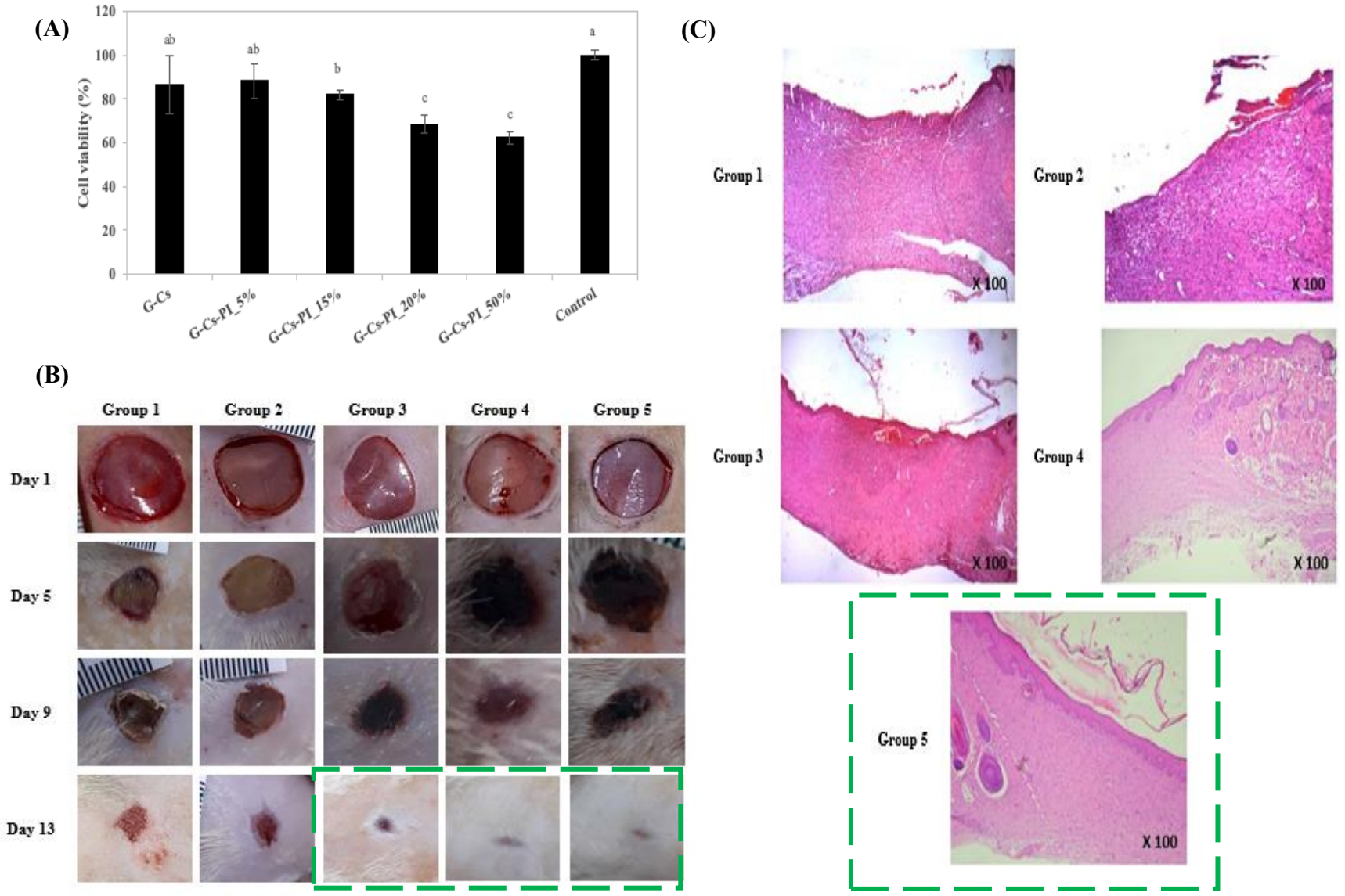

(A)
Cell viability (%)
120
100
80
60
40
20
0
ab
ab
b
c
c
a
G-Cs
G-Cs-PI_5%
G-Cs-PI_15%
G-Cs-PI_20%
G-Cs-PI_50%
Control
(B)
Group 1
Group 2
Group 3
Group 4
Group 5
Day 1
Day 5
Day 9
Day 13
(C)
Group 1
Group 2
Group 3
Group 4
Group 5
X 100

Fig. 12

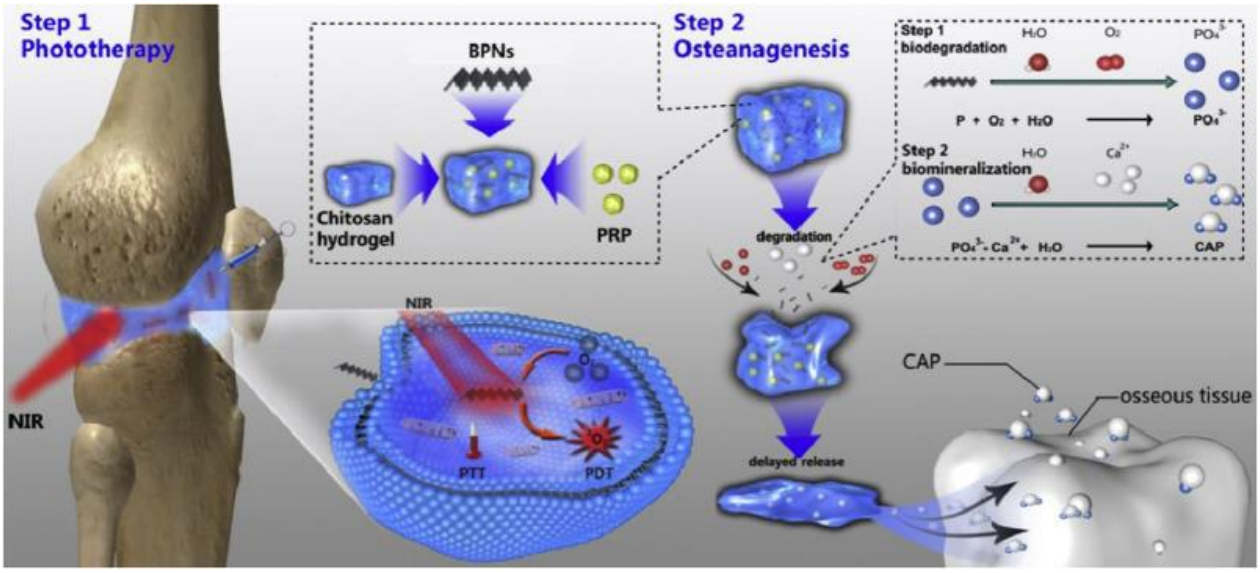

Step 1
Phototherapy
BPNs
Chitosan
hydrogel
PRP
Step 2
Osteanagenesis
Step 1
biodegradation
P + O2 + H2O
Step 2
biomineralization
PO4 3- + Ca 2+ + H2O
CAP
degradation
NIR
PTT
PDT
delayed release
CAP
osseous tissue
NIR

Fig. 13

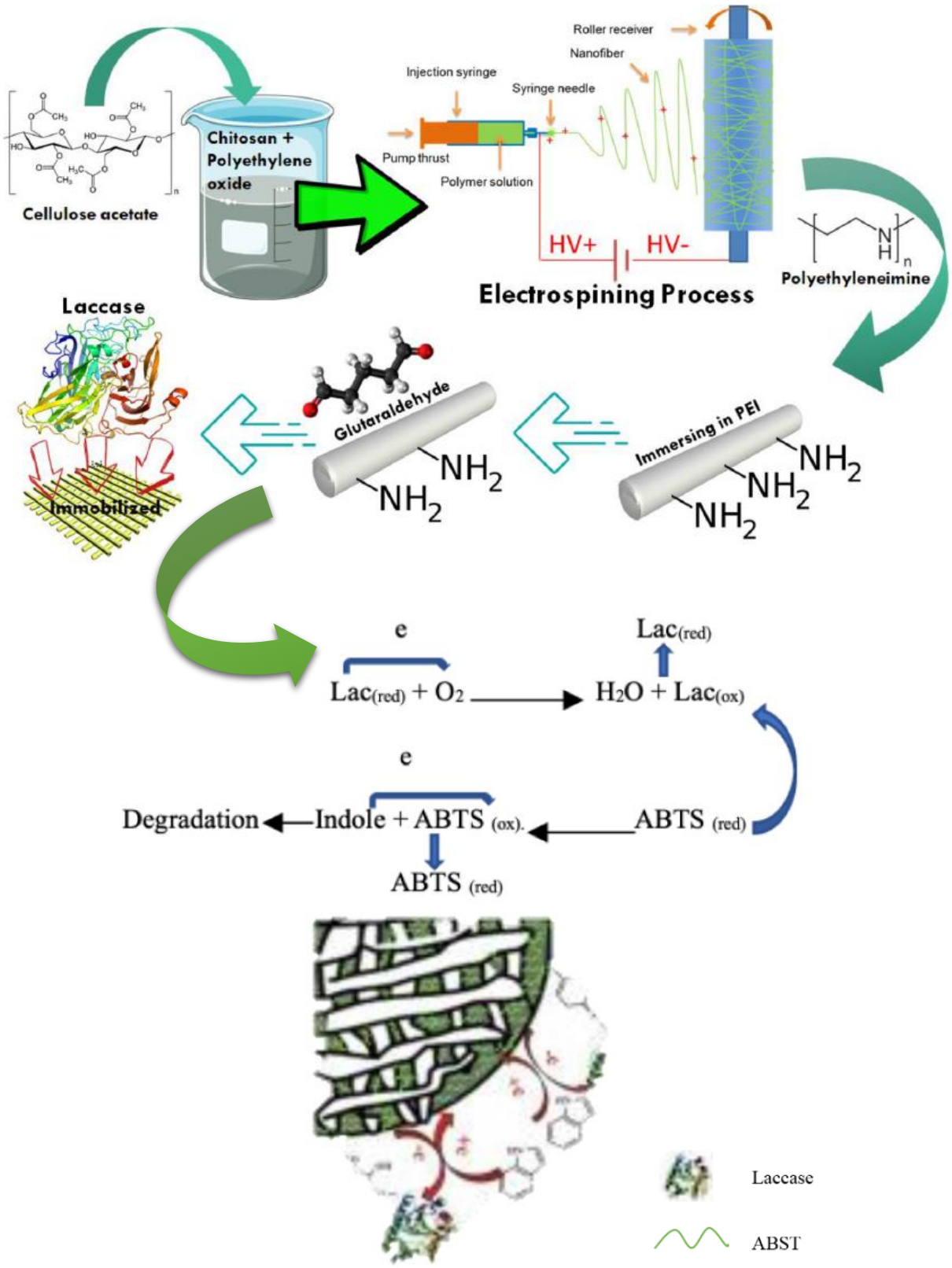

Cellulose acetate
Chitosan + Polyethylene oxide
Roller receiver
Nanofiber
Injection syringe
Syringe needle
Pump thrust
Polymer solution
HV+
HV-
Electrospining Process
Polyethyleneimine
Laccase
Immobilized
Glutaraldehyde
Immersing in PEI
e
Lac(red) + O2
H2O + Lac(ox)
Lac(red)
e
Degradation
Indole + ABTS (ox).
ABTS (red)
ABTS (red)
Laccase
ABST

Table 1. Advantages and Disadvantages associated with techniques applied for the extraction of chitin and its derivative chitosan.

Benefits	Limitations
 **Conventional Extraction Processes** 	
Chemical preparation	
* More efficient at the industrial scale (Commercial methods) * Possibility to scale-up * Easily applicable * Cost-effective * Simple equipment * Short-time processing	* Used as harsh chemicals (acids, alkali, etc.) * Consumption of a large amount of energy, solvent, and water (for washing steps) * Extended extraction period * High environmental pollution (Non-eco-friendly) * Uneconomical * Sufficiently damaged proteins and minerals
Biological preparation	
* Environment friendly * Safer * Possibility of recovery of additional high-added value compounds following the deproteinization step * Decreased consumption of acids and alkalis * Low amount of consumed energy	* Enzymes high price * Reduced yield * Higher treatment time * Not sufficient in the full removal of residual proteins and minerals * More complicated to industrially scale up
 **Recent Green Extraction Processes** 	
Deep Eutectic Solvent-based technology	
* Green solvents (eco-friendly) * Recyclable and Reused * Nontoxic (safe)	* Some display toxicity at high concentrations * Long time processing * Not in commercial method

* Alternative solvents and ease of synthesis * Biodegradable * Low cost * High recovery efficacy and selectivity * Fast phase separation * One-time removal of proteins and minerals * Reduced energy consumption	
Ionic Liquids-based technology	
* Green solvents * High extraction efficacy * Fast phase separation	* High cost * Complex synthesis processing * Low biodegradability * Long time processing * Not in commercial method
Microwave irradiation coupling	
* Reduced treatment period * High extraction efficacy * Low energy consumption * Low cost	* Final product structure alterations
Ultrasound vibration coupling	
* Adapted to industrial requirements * Reduced solvent consumption * Reduced processing time and energy-saving	* Structure modifications at high-vibration reaction periods * Higher chitin degradation
Pulsed Electric Field-assisted extraction	
* Ecofriendly * Short treatment time * Less energy consumption * Microorganisms' inactivation	* High cost of initial investment * Safety concern

Subcritical Water-assisted extraction	
* Eco-innovative * Non-toxic * Non-flammable extraction method * No residual solvent after the extraction * Shortened reaction time	* High cost of industrial infrastructure * Optimization of reaction conditions is complex and extremely required
Electrochemical extraction	
* Long lifetime of water metastable states * Higher profitability of chitin recovery due to * Accurate processing of raw material * Possibility of recovering all valuable components * Low cost *Short time * Reduced ecological risk	* Entails a constant electricity reserve that can result in an augmentation of the processing costs and the amount of the consumed energy. * Limitedly studied for the extraction of chitin
Mechanochemical extraction	
* Solvents omission * Considerable diminution of the amount of required chemicals * Reduced water consumption * Energy saving * Possibility of chitin oligomers or small derivatives recovery * Low cost	* Limitedly studied for the extraction of chitin

Table 2. Examples of chitin and chitosan extracted from different nature-derived resources using routine and innovative extraction technologies.

Biopolymer and source	Extraction technique	Yield (%)	Reference
Insect (*Bombyx mori*)	Deproteinization: NaOH (1.0 mol/L) for 24 h at 80 °C Demineralization: HCl (1.0 mol /L), 20 min, 100 °C Decoloration: 0.4% Na2CO3 Deacetylation: 40 % wt NaOH and NaBH4	Chitin: 2.59 Chitosan: 88.40	(Mohan et al., 2020)
Insect (*Tenebrio molitor*)	Deproteinization: 500 mL 5% NaOH at 95 °C, 3 h Demineralization: 3 h in 1500 mL 2 N HCl, 20 °C Deacetylation: 500 mL of NaOH at 95 or 105 °C for 3 h or 5 h	Chitin: 17.32 Chitosan: 14.48	
Aquatic bug (*Ranatra linearis*)	Deproteinization: 100 mL of 1 M NaOH at 110 °C for 18 h Demineralization: 100 mL of 1 M HCl at 90 °C for 1 h Decoloration: Chloroform, methanol, and water (1:2:4)	Chitin: 15-16 Chitosan: 70	
Chitin from *Cicada orni* sloughs	Demineralization: 1 M HCl; 1:15 (m/v); 30 °C for 2 h Deproteinization: 1 M NaOH; 1:15 (m/v); 90 °C; 2 h	42.6	(Ben Aoun et al., 2024)
Chitin and Chitosan from Fish Scales (*Labeo rohita*)	Demineralization: 1% HCl; 36 h Deproteinization: 0.5 N NaOH; 18 h Deacetylation: 50% NaOH; (w/v); 80◦C; 2 h	n. d	

Chitin from Shrimp shells (*Litopenaeus vannamei*)	Successive two-step fermentation: Lactobacillus rhamnoides: inoculum level 4%; pH 6.5; 37 °C; 5% glucose; 48 h *Bacillus amyloliquefaciens*: inoculum level 6%, pH 6.5; 37 °C; 4% glucose; 84 h.	19	
Chitin from shrimp shell powder	Microbial fermentation using *Paenibacillus jamilae* BAT1	24.5	
Chitin from *Litopenaeus vannamei* shell	Microbial fermentation using *Alcaligenes faecalis* S3 and *Bacillus coagulans* L2	25.4	
Chitin from Shrimp head waste	Microbial fermentation using *Paenibacillus mucilaginosus TKU032*	20.67	
Chitin from Shrimp shell (*Litopenaeus vannamei*)	Enzyme-assisted extraction using Chitinase and protease A and B: pH: 6.0, 45 °C, enzyme: substrate-7.5–100:25–200 U/g, time: 1–7 h	88.9	(Mohan et al., 2022)
Chitin from Mealworm's cuticles (*Tenebrio molitor*)	Enzyme-assisted extraction using Alcalase (from *Bacillus licheniformis*): pH: 8.5; time: 10 min	31.9	
Chitin from Shrimp shell (*L. vannamei*)	Enzyme-assisted extraction using Trypsin and ficin: pH: 7.7 and 7.5, temp: 45 °C, time: 4 h	32.12	
Chitin from Blue crab (*Portunus segnis*) shell powder	Enzyme-assisted extraction: successive and separate addition of blue crab alkaline digestive enzymes at 5 U/mg, 50 °C, pH 8.0, 3 h. After stopping the reaction and filtration, 5 U/mg *Bacillus safensis* proteolytic enzymes were added at 45 °C, pH 10.5, 3 h.	19.06	(Hamdi et al., 2018)

Chitin from Mealworm's (*Tenebrio molitor*) cuticles	Enzyme-assisted extraction: 2% Alcalase (w/w; enzyme/ substrate); pH 8; 50 °C; 10 min	70	
Chitin from Crayfish shell waste (*Procambarus clarkii*)	Combined extraction: Fermentation and Proteolytic enzymes: Demineralization: *Bacillus coagulans* LA204; 5 g crayfish shell powder; 5 g glucose; 3% (v/v) seed culture medium; 37 °C; 6 days; 1 mol /L HCl; 1:10 (w/v); 3 h Deproteinization: Enzymatic hydrolysis and fermentation:50 °C, 150 rpm; 5% (w/v) 0.5–2.0 mm shell powder, 5% (w/v) glucose, proteinase K of 1000 U/g crayfish shell	94	(Ben Aoun et al., 2024)
Chitin and Chitosan from Shrimp shells	Microwave irradiation-assisted extraction: Demineralization: 3% HCl; 3–8 min; 160–500 W Deproteinization: 10% NaOH; 5 min; 160 W Deacetylation: 50% NaOH; 8 min; 350 W	82.7	
Chitin from Spider molt cuticle (*Caribena versicolor*)	Microwave irradiation-assisted extraction: Removal of lipids and waxes: Chloroform: ethanol; (v/v 2:1); 1 min; 750 W; 2450 MHz Deproteinization: 2.5 M NaOH; 3 min; 750 W; 2450 MHz; 95 °C Depigmentation: 30% H2O2; Time 2 min; 750 W; 2450 MHz	19	

Chitin from Shrimp waste	Microwave-assisted extraction: 1400 W, 10 min	36.43	(Mohan et al., 2022)
Chitin from Spider molt cuticle (*Caribena versicolor*)	Microwave-assisted extraction: 2450 MHz, 750 W, 1 min	19	
Chitosan from Fungal biomass (*Rhizopus oryzae* NRRL1526)	Microwave-assisted extraction: 300 W, 22 min	13.43	
Chitosan from Squid pens (*Doryteuthis* spp.)	Ultrasounds-assisted extraction: 24 kHz for 50 min in NaOH at 60 °C	88	(Mohan et al., 2022)
Chitin from Squid pen (*Loligo formosana*)	Ultrasounds-assisted extraction: 20 kHz, 41 min, Solid: Liquid ratio of 1:20 (w/v), 30 to 40 °C	34	
Chitosan from Shrimp shell waste (*Parapenaeus Longirostris*)	Ultrasounds-assisted extraction: 50 kHz, 3 h, 15 M NaOH (1/20, w/v), 60 °C	17	
Chitin from North Atlantic Shrimps (*Pandalus borealis*) shells	Ultrasounds-assisted extraction: Demineralization in 0.25 M HCl (1:40, w/v) at 40 °C, sonication for 4 h. Deproteinization in 0.25 M NaOH (1:15, w/v) at 40 °C, sonication for 4 h	12.8	(de Aguiar Saldanha Pinheiro et al., 2021)
Chitosan from Pacific white shrimp	Ultrasounds-assisted extraction: Deacetylation: NaOH (35%–65%, w/w), using 1:15 (w chitin/v), 80° C, 360 min., 37 kHz and 300 W	n. d	

Chitin from Shrimp cephalothorax	Subcritical water extraction: 260 °C, 30 min	82.2	(Mohan et al., 2022)
Chitosan from Swimming crab (*Portunus trituberculatus*)	Subcritical water extraction: 170 °C, 30 min	12.2	
Chitin-glucan complexes from (*Aspergillus niger*)	Subcritical water extraction: 300 °C, 2 to 50 s	50.8	
Chitin from Shrimp shell biomass waste	DES-assisted extraction: HBA (Choline Chloride) – HBD (Lactic acid, Malonic acid, Urea, Citric acid)	90	(Mohan et al., 2022)
Chitin from Lobster shells	DES-assisted extraction: HBA (Choline Chloride) – HBD (Thiourea)	20.63	
Chitin from Shrimp shell (*Marsupenaeus japonicus*)	DES-assisted extraction: HBA (Choline Chloride) – HBD (Malonic Acid)	19.41	
Chitin from Shrimp shell	DES-assisted extraction: HBA (Betaine HCl) – HBD (Urea)	23.6	
Chitin from *Pandalus borealis*	DES-assisted extraction: HBA (Choline Chloride) – HBD (Lactic Acid); solid-to-liquid ratio 1/25 (w/w), 6 h, 60 °C	14.21	(Li et al., 2022)
Chitin from *Solenocera crassicornis*	DES-assisted extraction: HBA (Choline Chloride) – HBD (Malic Acid); solid-to-liquid ratio 1/20 (w/w), 3 h, 130 °C	19.2	

Chitin from *Agaricus bisporus*	DES-assisted extraction: HBA (Choline Chloride) – HBD (Urea); solid-to-liquid ratio 1/20 (w/w), Ultrasonication 60 min, room temperature	29.40	
Chitin from Shrimp shell	DES-assisted extraction: HBA (Choline Chloride) – HBD (Urea); solid-to-liquid ratio 1/20 (w/w), Microwave irradiation 9 min	25.1	
Chitin nanocrystals from Crab shells	DES-assisted extraction: HBA (Choline Chloride) – HBD (Oxalic Acid, Lactic Acid, Malonic Acid, Citric Acid, Malic Acid)	79.5-87.5	
O-acylated Chitin from Shrimp shells	DES-assisted extraction: HBA (Choline Chloride) – HBD (Malic Acid)	56.60	
Chitin powder from Shrimp shells (*Marsupenaeus japonicas*)	DES-assisted extraction: HBA (Choline Chloride) – HBD (Malonic Acid)	19.41	(Khajavian et al., 2022)
Chitin nanofibers from Commercial chitin	DES-assisted extraction: HBA (Choline Chloride) – HBD (Thiourea)	84	
Chitin from White mushroom (*Agaricus bisporus*)	DES-assisted extraction: HBA (Choline Chloride) – HBD (Urea)	29.8	
α-chitin from Crab shells	ILs-assisted extraction: 1-butyl-3 methylimidazolium acetate (BminAc) 1.0 wt% of ionic liquid, heating to 100–150 °C in an oil bath for 2-5 h, cooled down to room temperature, and washing using ethanol	n. d	(Sulthan et al., 2023)

β-chitin from Squid Pen	ILs-assisted extraction: 1-butyl-3 methylimidazolium acetate (BminAc) 1.0 wt% of ionic liquid, heating to 100–150 °C in oil bath for 2-5hr, cooled down to room temperature, and washing using ethanol	n. d	
α-chitin from Crab Shells	ILs-assisted extraction: 1-allyl-3-methylimidazolium bromide Dissolved in ionic liquid by heating at 100 °C for 48 h	n. d	
Chitin from Shrimp shells	ILs-assisted extraction: 1 g/10 g 1-Ethyl-3-methyl-imidazolium acetate [C2mim] [OAc] Microwave irradiation at 100 °C for 19 h	94	(Ben Aoun et al., 2024)
Chitin from Shrimp shells	ILs-assisted extraction: 1 g/49 ml 1-ethyl-3-methylimidazolium acetate [C2mim] [OAc] Pulse-heated in the household microwave for 2.5 min	4	
Chitin from Green Crab Shells	Mechanochemistry and Aging: Vibrational Milling (29.5 Hz, 30 min) with Malic Acid (470 mg)	16.1	(Hajiali et al., 2022)

n. d.: Not described

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for *[Journal name]* and was not involved in the editorial review or the decision to publish this article.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Chitosan and its Derivatives as Potential Biomaterials for Biomedical And Pharmaceutical Applications: A Comprehensive Review on Green Extraction Approaches, Recent Progresses, and Perspectives

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Abstract

***Background*:** Nowadays, the search for new renewable and broad-spectrum natural biopolymers for biotechnological and medical applications has become an absolute necessity. **Chitin and its deacetylated derivative, chitosan,** are considered interesting and auspicious biopolymers being potentially applied in a wide range of biotechnological sectors, including medicine, food beverages, agriculture, and cosmetics, owing to their enormous ability to undergo changes in structure and mechanical properties to generate new functions (used as a matrix in beads, membranes, gels, etc.) and applications.

Scope and Approach: The current review provides a comprehensive report summarizing research on the routine chemical and greener non-conventional extraction methodologies of chitin and chitosan and focuses on the progress in their application over the past two decades, in terms of challenges, opportunities, and future perspectives.

Key Findings and Conclusions: **Chitosan** is an effective material with enormous potential for biotechnology and medicine owing to its biocompatible, biodegradable, and non-toxic traits, besides its antimicrobial potential and low immunogenic potency. To standardize applications in the industrial field considering cost-effectiveness and biocompatibility, the search for innovative recovery and production methods for chitin/chitosan-based materials industrialization is required. Conventional chemical chitin extraction approaches present drawbacks and induce numerous environmental issues. Greener extraction technologies have recently perceived considerable advancement in the polymer chemistry field. This review can serve as a guideline for exploring nature-originated biopolymers as innovative feedstocks for several technologies that show highly appealing potential for application in countless fields.

Keywords: Chitin; Chitosan; Extraction; Green Chemistry; Bioactivity; Biomedical applications.

45 **Summary**

69

1. Introduction

Chitosan is a semi-crystalline unbranched polysaccharide consisting of two recurrent patterns, D-glucosamine and N-acetyl-D-glucosamine connected by glycosidic β-(1-4) bonds. It is obtained by fractional or total deacetylation of chitin, reported to be the second most abundant natural polysaccharide after cellulose (**Fig. 1**). Chitin is a foremost constituent of the extracellular matrix of fungi and the exoskeleton of shellfish and insects [1-3]. The chitin/chitosan nomination depends on the proportion of acetamide units or acetylation degree (AD). For an AD < 50%, the polymeric product is commonly quoted as chitosan, while the term chitin reflects an AD > 50% [4]. Contingent on the origin and method of production, the molecular weight of chitin can sweep from 300 to more than 1000 kDa. Chitin occurs in three different crystal structures: α-chitin (the most stable and abundant form with macromolecules arranged antiparallelly), β-chitin (parallel alignment), and γ-chitin (both parallel and antiparallel forms) [5-6]. Compared to cellulose, chitin is recognized as much easier to undergo changes *via* chemical reactions, owing to the presence of the acetamide group among the structured units of chitin (**Fig. 1**). It has been reported that although a minimum of 10^{11} t of chitin are synthesized and degraded each year, only 150,000 t are made available for commercial use [7]. A stumbling block of the appropriate biotechnological utilization of chitin is its non-solubility in almost all common solvents.

Chitosan, as the simplest and most commonly studied derivative of chitin, is the only naturally occurring cationic polysaccharide, soluble in dilute acids (pH < 6.0) *via* cationization of the $–NH_2$ group at the C-2 site of the repeating units of D-glucosamine, as a function of the AD. Thus, under the crystalline arrangement, chitosan cannot be solubilized in neutral to alkaline water-based media (pH > 7.0) [8]. With the presence of bioactive functional -OH and $-NH_2$ groups, chitosan has excellent physicochemical and biological properties and has proven to be of remarkable interest for versatile fields and applications [9-14]. Currently, several

industrial areas such as food [15], environment (wastewater treatment), agriculture (seeds, packaging), textiles [16], and cosmetics are concerned, with particular attention to their relevancy in the pharmacy-based and biomedical sectors [17,18], as summarized in **Fig. 2**. In fact, chitosan is considered as one of the most promising natural substances with fascinating biofunctional properties for tissue engineering [19], drug delivery [20], wound healing [21], even in gene delivery as a promising non-viral vector [22-24]. Chitosan can be readily transformed into different forms of materials, such as gels, membranes, nanoparticles, and so on [23,25-27]. Tissue engineering researchers have recently focused on designing nanomaterials that will provide long-term benefits to humans in the event of a medical emergency, such as bone fracture, cartilage tissue damage, etc. [28].

2. Chitin Recovery and Production Processes and its Conversion to Chitosan

2.1. Routine/Traditional Recovery Approaches

Chitin is mainly derived from the crustaceans' outer skeleton, such as crabs and shrimp. Chitin is present in shells in the form of chitin-protein-mineral complexes (mainly calcium carbonate) **(Fig. 3)**. Traditionally, to produce chitin from crustacean shells, outdated chemical pathways that involve steps of demineralization and deproteinization are applied using large volumes of hazardous chemicals (acids and bases, respectively) **(Fig. 3)**. The use of these chemicals can affect the quality and functional properties of the final product (partial deacetylation and depolymerization of chitin), as well as they are hazardous to the environment [29,30]. To overcome these problems, various biotechnological processes (fermentation and enzymatic) are studied and developed [31-33]. Biological chitin recovery processes (extraction under mild conditions) preserve the quality of chitin and promote the valorization of other compounds in crustacean co-products, such as peptides and pigments [34-36]. Chemical and enzymatic processes have been widely studied for chitin production. In contrast, the order of

the demineralization and deproteinization steps has not been investigated except in a few studies [30,37]. Indeed, it was established that, although in the case of enzymatic deproteinization handling, minerals associated with the chitinous matrix can hinder the accessibility of proteases to the linked proteins and thus affect the deproteinization efficiency, the order of the proteins and associated minerals removal steps can be reversed without affecting the quality and yield of chitin extraction and chemical treatments [30].

Chemical and biological extraction methods for chitin have advantages and disadvantages or challenges **(Table 1)**. **"Green"** products and techniques evolve into one of the cutting-edge fields. In this aspect, **"green chemistry"**, which is interpreted as the utilization of chemistry technologies and approaches that diminish or omit the usage and inception of feedstocks, products, by-products, solvents, and reagents that are precarious to human health or the environment, can be deemed as one of the sectors with utmost importance at industry and educational levels for the hands-on fulfillment of the **"environmentally friendly or eco-friendly"** motif [6,31]. Accordingly, toward a greener future, the development of new methods using greener and more environmentally safe extractants such as Ionic Liquids (ILs) and Deep Eutectic Solvents (DES) is thereby necessary for the production of high-quality chitin with a desired AD of up to 100%, without severe degradation of the molecular chain. Scientists have explored a widespread assortment of technologies **(Table 2)**, such as enzyme, electrochemistry, photochemistry, sonochemistry, ultrasound, and microwave or radiofrequency-using extraction approaches, for the efficient and environmentally friendly extraction of chitin [11,37,38].

One of the main difficulties in valorizing chitin is its insolubility. Thus, various chitin derivatives have been prepared, including chitosan which is the simplest and most studied derivative recovered by fractional or total deacetylation of chitin employing either chemical or enzyme-using processes [38]. During the synthesis progression, numerous parameters and conditions can affect the physicochemical properties of chitosan as well as extraction yields,

primarily alkalescent solution concentration, treatment period, chitin/alkaline solution proportion, atmosphere (air/nitrogen), heating, chitin origin (shrimp, crab, etc.), granulometry of the raw powder, and the usage of one- or multi-step acetyl groups removal procedures [39-41]. In terms of availability, chitosan production has reached more than 10^{13} kg per year [7].

Marketed chitosan typically has an AD of 5-30 % and a molecular weight (MW) of 10^4-10^6 g mol^{-1} [42-44]. The determination of AD is extremely crucial in the evaluation of the efficiency of the acetyl group removal from the chitin polymeric chain and the final bio-functional performance of the biopolymer, hence the definition of its future biotechnological application [45-49].

2.2. Emerging/Alternative Recovery Approaches

2.2.1. Physical Approaches

Microwave heating technology has been broadly implemented in numerous food and chemical industries as it not only reduces chemical reaction time but also improves the recovery yield and the quality of the final product (chitin, chitosan, and so on) in terms of purity and technofunctional features in comparison with conventional methods **(Table 1)**, besides the advantages of green status, energy effectiveness, convenience, low application cost, selectivity, and simple handling of the specific procedure [50,51]. The thermal effects and high efficacy of the heating by microwave irradiation are attributed to the reversed and quick heat transmission between the biomass substrate and the catalyst, the consistent irradiation field inside the sample, and the discriminating radiation assimilation by polar materials involving two major phenomena: ① dipolar polarization and ② ionic conduction, as shown in **Fig. 4** [52]. Considering the aforementioned advantages, to minimize the utilization of non-ecofriendly chemicals, the application of microwave irradiation has been monitored in the recovery of various polysaccharides, such as chitin/chitosan [31] and cellulose [53], among others.

Nonetheless, to achieve the uppermost yields of chitin or chitosan extraction, considering the reduction of the process cost, it is crucial to optimize several microwave irradiation process operational parameters, mainly the heating period, the extractant concentration (water content of the extraction solvent), and the biomass to solvent ratio, which exert a strong influence on the AD and MW of the final product, chitin or chitosan [32].

Ultrasound-assisted extraction, using ultrasonic waves in the range of 20 Hz to 20 kHz is another innovative eco-friendly technology applied to bolster the extraction of chitin, through the cavitation effect that allows the intensification of chitin-linked proteins solubilization, involving mainly ① macromolecules depolymerization, ② polymer-based covalent bonds, and ③ conformational modifications in terms of aggregates scattering [36,54]. Ultrasound-assisted extraction can be performed *via* either indirect sonication using an ultrasound horn or direct sonication in an ultrasound bath **(Fig. 4)**. Accordingly, enhanced removal rates of proteins (deproteinization) and minerals (demineralization) associated with chitin can be accomplished by coupling ultrasounds and chemical processes [39]. Ultrasonication treatment is attracting attention as a preferable non-thermal approach for the improvement of chitin and chitosan recovery from different resources, due to its several advantages such as faster energy and mass transfer, reduced temperature and time, high process control, extraction selectivity, and faster start-up/easy to install **(Table 1)**. The effects of ultrasonication treatment on the AD, MW, and particle size of the biopolymer products were explored, and results reveal that the rise in the ultrasound treatment time led to more effective associated protein removal, along with an increase in the crystallinity, reduction of the particle size, and a surface erosion to variable extents of the resulting biopolymers (chitin and chitosan). Moreover, lower sonication periods led to the recovery of chitosan with higher AD and MW, compared to higher ultrasonic treatment periods (recovered polymeric materials with medium and lower MW and AD) [55].

Subcritical water treatment is based on the usage of subcritical water as the reaction medium with no need for enzymes or chemicals. It is an environment-friendly and energy-efficient technology that has recently attracted attention for a wide range of high-added-value products recovered from waste biomass, including chitin and chitosan [56]. Subcritical water could act as an acid or base catalyst and is defined as liquid water under heating treatment in the range of 100 °C and 374 °C and a pressure < 22 MPa, underneath the critical point of water [57] **(Fig. 4)**. Subcritical water extraction is an extraordinary eco-innovative, non-toxic, non-flammable extraction method, with no residual solvent after the extraction and considerably shortened reaction time **(Table 1)**. This technology allows changes in chitin structure for improved enzyme-based proteolysis, as well as associated protein elimination and mineral removal [58]. However, the major challenges of this technology are the high cost of industrial infrastructure for the subcritical water systems installation, and the optimization of reaction temperature, pressure, treatment time, solid-water ratio, particle size, and pH, along with the solute characteristics and flow rate, is extremely required [31].

Pulsed electric fields are an additional novel and non-thermal favorable technology that has been developed, during the last ten years, for enhancing the release of food-derived biopolymer compounds, and thereby improving the extraction yield. This technology uses high-intensity brief pulsations of electric fields (strength in the range of 10 and 80 kV/cm) surpassing the critical value in a short period (μs or ms) on a biomass material positioned between two electrodes [59,60] **(Fig. 4)**. This treatment induces the electroporation mechanism (temporary or permanent), commonly defined as pores creation phenomenon on the cell membrane, hence an intensification of cells transmembrane capability in the biomass product [61]. This process has been employed as a tool to expand the extraction of a plethora of high-added values materials from diverse natural resources [62,63]. The profits of such eco-friendly methodology are mainly a quick handling period, a reduced amount of consumed energy, additionally to

microorganisms' deactivation **(Table 1)**. However, the high cost of the initial investment along with safety concerns (problem of electrochemical reactions at the commonly used stainless steel electrodes) has made the development of this technology a challenging task [64].

2.2.2. Mechano- and Electrochemistry-based Approaches

As an alternative to the conventional approaches of chitin and chitosan extraction, **mechanical energy** has been integrated into the recovery processes of shellfish-derived chitin and its byproducts, defining the **mechanochemical technology**, which is referred to as the field of chemistry related to the chemical and physicochemical transformation of biomass through the effect of mechanical energy (solid-state reactions), as defined by Heinicke [65,66]. Such technology has shown a plethora of advantages **(Table 1)**, including ① Solvents deletion throughout the conversion reactions of the biomass waste for the recovery of chitin and its polymeric derivatives, with much lower consumption of water that is required only for the filtration stages and chemicals such as acids and bases, due to a lower required matter-chemical mass-molar ratio, as compared to the routine approaches [67]; ② Considerable saving of energy consummation during the extraction (a 1/9 part, approximately), since the majority of the transformation procedure is monitored only in a single unit operation (the mill) with no heat treatment for a briefer period [68]; ③ Appealing possibility of chitin/chitosan oligomers development and recovery, with better homogeneity of acetylation and polymerization degrees, especially when biomass matter is milled at elevated frequencies with the increase of the mechanical processing period [66].

The electrochemical approach, including accurate pigments, lipids, proteins, and minerals removal steps, has been additionally introduced in the processes of matter transformation for high MW chitin and chitosan, allowing a significant growth of the cost-effectiveness of the chitin-producing process, with precise biomass waste treatment and the opportunity to recover all its valued products [31]. The principle of the technology is the

biomass waste smooth disintegration due to the effect of in-situ generated alkaline and acidic extraction media (redox reactions) inside an electrolytic cell, in the presence of a diluted aquatic sodium chloride solution and under mild circumstances [69]. Herein, an anode and a cathode are immersed in a particular electrolyzer (electrochemical cell) containing electrolytes (aqueous salt solution), creating two chambers: the anolyte (anode cell) and the catholyte (cathode cell) parceled by an ion exchange membrane **(Fig. 4)**. The catholyte compartment produces the catholyte solution (H_2 and OH^-), inducing the occurrence of water electrolysis, while in the anolyte compartment, oxidation of the Cl^- ions of sodium chloride induces the production of Cl_2, subsequently electrolyzed on the anolyte surface, leading to the production of the anolyte solution (H^+ and HClO) [70]. Such technology, based on the electrochemical activation of aquatic media, is considered more advantageous as compared to other activation systems such as sonication or microwave irradiation **(Table 1)**, due to the consistency in the process physicochemical conditions, such as redox potential, pH, surface tension, electrical conductivity, dielectric constant, among others, since the duration of water metastable status is rather extended [31,68,69].

2.2.3. Introduction of Green Solvents

Traditional extraction processes have been widely utilized to extract bioactive compounds from various biomass waste resources over the past decades. However, such traditional extraction methods present a major disadvantage, *i.e.* the use of large amounts of organic solvents, which are not sustainable as they are flammable, hazardous, and have limited disposal and recycling possibilities [71]. Other drawbacks of applying the conventional chemical chitin-producing methods include time-consuming, labor-intensive, and lack of automation, resulting in low selectivity and low extraction rates [72]. To achieve sustainable extraction, there is a growing need for safe and sustainable green extraction technologies. In this field, "***Green Chemistry***" is defined as the use of chemical techniques and methods that diminish or omit the

usage and generation of unprocessed matters, side components, solvents, chemicals, and testing agents known to be destructive to human healthiness or the natural ecosystem equilibrium [73]. **Green extraction** is the latest goal of scientific and industrial research and development as it aims to reduce or eliminate the usage of toxic chemical solvents/reagents, reduce energy consumption, minimize environmental impact, and further provide significant benefits to human health and well-being [74].

Alternatives to conventional solvents are **ionic liquids (ILs)**, recognized as organic salts, with a low temperature at which it melts (inferior to 100 °C), involving a bulky organic cation with a minor organic or inorganic anion **(Fig. 5)**, allowing thereby several conceivable cations and anions groupings, were first used as green solvents [75]. Such features permit the adjustment of the ILs' intrinsic parameters, including thickness, ions conduction, hardness, polarity, solvating capacity, and hydrophilic and hydrophobic behaviors for the extraction of a high number of molecules with different polarity indexes, such as chitin from marine sources [76]. The advantages of ILs over conventional solvents are low vapor pressure, facility, and security of monitoring, wide range of miscibility and solubility, good thermal properties, and good recyclability. Additionally, ILs have been utilized not only in the extraction of chitin and its derivatives but likewise for chitin and chitosan solubilization and soft materialization to design a plethora of biomaterials, among which hydrogels, membranes, and spheres at the nano- and microscale, fibers, etc. **(Fig. 6)**, primarily for medicine and pharmacy-related uses [77]. The 1-ethyl-3-methylimidazolium acetate, 1-butyl-3-methylimidazolium chloride, and 1-allyl-3-methylimidazolium acetate have been conveyed as the furthermost frequently applied ILs for efficient evocation and separation of chitin from marine sources [31]. However, in addition to low toxicity and biodegradability **(Table 1)**, ILs have some disadvantages such as high viscosity, limited solute solubility, corrosiveness, and high production cost [78]. The observed

toxicity of ILs is found to be mainly related to cationic molecules, side chain length, and anions. Compared to conventional solvents, ILs are more toxic and harmful to bacteria [79,80].

The use of **deep eutectic solvents (DES)** has received increasing attention as an **eco-friendly green solvent** and an alternative to routine solvents and ILs for the derivation and solubilization of chitin and its derivatives. DESs are prepared by mixing two or more nonionic compounds that form eutectic mixtures through hydrogen bonding with melting points lower than those of the constituents. Salts and molecular compounds are typically used, where one constituent performs as a hydrogen bond donor (HBD) and the other as a hydrogen bond acceptor (HBA), at a defined molar ratio **(Fig. 5)**. In general, the composition of DES is expressed as Cat+X-zY, where Cat+ is an ammonium, phosphonium, or sulfonium cation, X is a Lewis base, and Y is a Lewis acid. The complex is formed between X and Y, where z is the number of Y particles interacting with the anionic molecules [73,81]. The use of DES is a relatively young field of research, with the first studies published in 2001. In the last two decades, extensive research on DES has been performed, with more than 2000 papers published in 2022 [76]. The DES presents the advantages of low volatility, non-toxicity, biodegradability, non-flammability, chemical stability, ease of preparation, and relatively low cost, compared to traditional industrial solvents and ILs **(Table 1)**. The application of DES in the extraction of bioactive compounds, such as chitin and chitosan from natural resources, and the synthesis of different classes of DES have been extensively studied [6]. Nowadays, DES is being effectively utilized as an efficient medium to synthesize and solubilize chitosan and its derivatives, owing to their robust intermolecular hydrogen bonds that permit the intensification of the opportunity to disrupt and break down the solid intrinsic hydrogen bonds network inside the chitin polymeric chains [82].

Recent trends in the green approaches for sustainable valorization of renewables have led to the design and conceptualization of **natural deep eutectic solvents (NADES)**, consisting of

primary plant-based metabolites (sugars, carboxylic acids, amino acids), known to exhibit a key function in cellular processes [83]. Interestingly, using NADES as an extractant medium, chitin can be recovered in a single-step process, for a faster and more eco-friendly manner, hence minimizing the amount of consumed water and toxic reagents in the proteins and minerals removal steps [84]. Several DES or NADES systems have been formulated to extract chitin from marine biomass wastes with extraction yields of around 20%, such as choline chloride (HBA)-Lactic acid (HBD) (1/1; w/w) [85], choline chloride (HBA)-Malonic acid (HBD) (1/2; w/w) [86], choline chloride (HBA)-Glycerol (HBD) (1/2; w/w) [87]. More advantageously, DES and NADES have been efficiently applied to better dissolve and chemically functionalize chitin and its derivatives for the development of innovative chitin or chitosan-based nano-materials, such as films, membranes, hydrogels, particles, fibers, as shown in **Fig. 6** [6,68,71,76,82,88].

2.3. Chitin/Chitosan Oligomers Production

In recent years, a plethora of scientific and methodological schemes have been implemented to concoct chitooligosaccharides or chitosan oligomeric derivatives and products **(Fig. 7)**, including acidic digestion [89], enzyme-using methodologies [90], ultrasounds-based breakdown processes [91], subcritical water hydrolysis [92], and oxidative degradation [93]. Among the methods of preparation of chitosan oligomers widely used on an industrial scale, acid hydrolysis is most commonly used. Nonetheless, a major part of compounds resulting from acidic degradation show short rates of polymeric disintegration, with unsatisfactory manufacturing effectiveness rates [94]. Subsequently, tremendous attention has been allocated to enzymatic production methods owing to their capability of minimizing undesirable molecular alteration phenomena and indorsing bio-functions. Sundry non-specific enzymes, especially cellulase [95], and chitosanase [96], have been widely exploited and utilized in the research and production of chitosan oligomers.

Chitosan oligomeric derivatives have been recognized as having keen importance, mainly in the medicine-related industries, thanks to their peculiar attributes counting dissolvability in aqueous media, low MW, low thickness/hardness, and terse polymeric sequences. These features allow them to upsurge their bio-functional activities inside *in vivo* structures and schemes [68,97].

3. Bio-functionalities of Chitosan and its derivatives

3.1. Chemical-Physical Characteristics of Chitosan

Unlike other polysaccharides in nature, the occurrence of hydroxyl and amine functional/reactive sites in the chitosan molecular chain affords a favorable foundation for interconnections with further polymeric and biologic-based compounds, such as lipids with opposite negative charge, proteins, cholesterol, macromolecules, and metal ions [98]. Otherwise, the interesting chemical properties of chitosan are ascribed to the occurrence and availability of reactive amine and hydroxyl groups, its linear polyamine structure, and its ability to chelate many transition metals [44]. The configuration and dimensions of chitosan polymeric matrix sequences diversify contingent on their source and the method of chitin extraction and existing acetyl groups removal. Chitosan is generally provided in the form of a semi-crystalline powder with a white or slightly yellow appearance [99].

Chitosan cannot be dissolved in aqueous media, caustic base media, or organic solvents. Contrarywise, it is highly dissolved in weakly acidic aqueous media with a pH below 6.0 [100,101]. The utmost characteristic attribute of chitosan is AD, which strongly has an impact on and prompts its aspects, attributes, and ultimately biotechnological relevance. The commonly monitored technologies for the precise estimation of chitosan AD are infrared spectroscopy (IR) and nuclear magnetic resonance (NMR), besides potentiometric titration [102]. Chitosan exhibited a widespread array of viscosities in weakly acidic aqueous media as

365 a function of the final MW [99]. Commercial chitosan readily supplied in the market unveiled
366 a significantly large MW, meanwhile, for agrifood-processing and pharmacy-related, low MW
367 chitosan is demanded and provided [103]. It is reported that the biochemical structure and
368 composition of chitosan offer countless opportunities for complex intra-molecular and ionic
369 amendments, which allows for a thorough adaptation and regulation of the bio-techno-
370 functional attributes of resulting biomedical tools constructed utilizing chitosan and its
371 derivatives [98,104].

372 Owing to the occurrence of hydroxyl -OH, acetamide, and amine -NH_2 sites in the
373 chitosan polymeric matrix, the desired physicochemical properties of chitosan can be
374 incorporated into the structure of chitosan by chemical, physical, and enzymatic modifications,
375 as summarized in **Fig. 8** [105]. Among the various techniques in vogue, chemical modification
376 is widely used, allowing the synthesis of derivatives with controlled solubility, ionic
377 characteristics, and hydrophilic character. Native chitosan is hydrophilic with low degrees of
378 order and flexibility. To improve its hydrophobicity, N-acylation with various fatty acid
379 chlorides (C6-C16) is usually performed [21,106], modifying the polymeric structure of
380 chitosan, thus making it an interesting excipient in controlled drug delivery systems.

381 In another aspect of chitosan modification, radiation treatment (gamma rays, electron
382 beams, UV rays, sonication, microwave, etc.) is an interesting evolving domain of scientific
383 investigations that aims to synthesize derivatives with enhanced properties. These approaches
384 are cost-effective and environmentally friendly alternatives that exhibit several advantages,
385 such as limited sample preparation, shorter preparation time, no catalysts, and no need for
386 temperature changes. Crosslinking, degradation, and free radical formation are among the
387 structural changes resulting from irradiation [107,108].

388 In addition, the use of enzymes in the synthesis or functionalization of chitosan has many
389 advantages. The enzymatic modification of chitosan leads to homogeneous deacetylation,

generating derivatives of low MW, compared to heterogeneous deacetylation. In addition, enzymes have the capability of catalyzing cellular processes with fast-moving operating and strong distinction and no irreversible and constant structural change [109].

Interestingly, chitosan can be easily transformed into plentiful functional materials, such as nanoparticles [2,110], beads [111,112], microparticles [113], nanofibers [114,115], membranes [116,117], hydrogels, and nano-gels [118,119].

3.2. Biological Properties of Chitosan

Chitosan's unique chemical characteristics allow it to benefit from a multitude of captivating biological and functional properties **(Fig. 9)** [4,72], including biocompatibility with body components, non-toxicity, antioxidant activity, antimicrobial potential, and biodegradability [8,24,120,121]. Chitosan can, similarly, bind to mammalian cells, accelerating the establishment of osteoblasts at the helm for bone construction, hence its restorative and healing influences. Chitosan has been furthermore described to be endowed with central nervous system depressants, hemostatic, fungistatic, spermicidal, antitumoral, immunoadjuvant, etc., among others [122].

The antioxidizing peculiarities of chitosan and its derivatives detained considerable prospectives for the handling of oxidative-based illnesses [123,124]. Chitosan's ability to be absorbed by cells and the intestine besides its bio-safety allows it to be a highly encouraging product to be applied as a nature-based antioxidant. Chitosan regulates the activities of antioxidant enzymes and reduces lipid peroxidation. Chitosan can elevate the activity of key antioxidative enzymes, counting superoxide dismutase (SOD), catalase (CAT), and phospholipid hydroperoxides glutathione peroxidase (GSH-PX) [125]. The precise mode of action of radical scavenging by chitosan and its derivatives is still not copiously elucidated. It is assumed that non-stabilized free radicals interact with the amine and hydroxyl groups at

positions C-2, C-3, and C-6 of the six-membered cyclic glucosamine nucleus for the creation of stabilized macromolecular groups [4].

The microbial growth inhibition potency of chitosan and its derivatives has been detected against a diverse set of microbial organisms, such as fungi, viruses, and bacteria [126-128]. This microbial growth inhibition attribute, which has boosted the relevance of chitosan and its derivatives in food-products conversation and biomedical domains, is, nevertheless, handled and swayed by the AD and notch of polymerization of the biopolymer, the host, and the surrounding circumstances [127]. A crucial and key attribute for understanding the mechanism of action is the positively charged groups that characterize chitosan polymeric backbone in weakly acid aqueous solutions (pH 5.5), due to the cationization of the amine group current in the repeating units of glucosamine which facilitates its dissolution in a hydrophilic water-based environment, hence its biocidal properties **(Fig. 10)** [129-131]. Younes et al. [40] found that chitosan with 2≤ AD≤ 24% exhibits the highest bactericidal potential, particularly with AD of 2 and 12%, against Gram- strains than Gram+ strains. Chang et al. [132] examined the combined effects of chitosan molecular weight, reaction temperature, and pH on bacterial growth. The authors found that the pH of the chitosan solution could explain the relationship between bacterial growth inhibition potential and the MW of chitosan. Under acidic pH conditions, chitosan antibacterial potential augmented with the increase of MW, while at neutral pH, bacterial growth inhibition levels boosted with the decline of MW. At pH in the range of 5.0-6.0, chitosan displayed better dissolution rates in aqueous media along with a diminution in the zeta charge with the MW, whilst at neutral pH, the dissolution and zeta charge diminished with higher MW, most probably explaining the reduction of the resulting chitosan abilities in inhibiting bacterial growth at neutral pH.

In recent decades, several studies have explored and projected the potential usage of chitosan as an additive to preclude the assimilation of consumed fats, thereby monitoring body

mass [133]. Indeed, chitosan is reported to be able to dissolve in the acidic environment of the abdomen through the formation of a homogenous mixture with oil. Then, with the augmentation of the pH of the duodenum, chitosan sediments, and the apprehended oils became unable to be assimilated by crossing the intestine epithelium [134,135]. Egan et al. [136] reported that chitosan exhibited anti-obesity activity in livestock, owing to its capability of adjusting feeding comportment and regulating hungriness. Notwithstanding numerous existing studies, the accurate behavior patterns of chitosan are still a major debate [137]. A plethora of research works have further shown that chitosan displayed the potential to curtail blood fat concentration in animals and humans [138]. Park et al. [139] disclosed that chitosan is effective in reducing total cholesterol (TC) and low-density cholesterol (LDL) levels and increasing the amount of high-density cholesterol (HDL) in rats. Rizzo et al. [140] described noteworthy advantageous actions of chitosan on fats and plasma lipoproteins, where TC levels were slashed by around 9% and triglycerides by around 20%. In this research work, none of the 28 patients with hypercholesterolemia received any other treatment with lipid regulators.

More recently, the efficacy of chitosan and its derivatives in inhibiting bacterial biofilm formation and removing preformed biofilms has been studied for the first time [48]. Blue crab chitosan was found to be more effective in removing preformed films from all bacterial strains tested, with the lowest ED50 values (concentration halving microbial adhesion) and the highest adhesion inhibition values. The efficacy of blue crab chitosan in post-treatment can be a result of its dissemination and assimilation at the juncture between the solid surface and adhering bacterial organisms establishing a biofilm, thus promoting the removal of bacterial biofilms.

4. Expanded Horizons in Chitosan Biomedical and Pharmaceutical Applications

Considering the keywords chitin and/or chitosan, the significant and irrefutable position of these polymeric materials is obvious in the worldwide scientific research field and market, as reported in the scientific literature volume market reports, and commercial products [141],

with USD 10.88 billion of chitosan market part in 2022 and 20.1 % of compound annual growth rate for the period of 2023-2030 [68]. Waste-water treatment, pharmacy, cosmetics, medicine-related, food and beverage, and agriculture, among others, are the most demanding fields [142], implying the potential utilization and relevance of chitin, chitosan, and their polymeric derivatives in practically the bulk most crucial worldwide economy areas. Chitosan is currently highly sought after for its potential application in the medicine-related domain **(Fig. 9)**. Investigations in this sector have flourished rapidly and remain a speedily evolving scope, describing the journey of chitin and chitosan from biomaterials to advanced bio-functional materials.

4.1. Application in Drug and Active Ingredients Delivery and Controlled Release

Currently, drug administration and controlled release is a very interesting topic. Targeted drug delivery aims to deliver pharmaceuticals to the patient to improve the concentration of the drug at certain sites compared to others, and to cause extensive, localized, targeted, and protected interactions with the diseased tissue(s) [143-145]. Drug release has been recognized to occur from delivery systems through different mechanisms, including diffusion, swelling, erosion, and stimuli-based pathways [146,147].

Polymers, such as chitosan, have been broadly applied to effectively develop pharmaceuticals and active ingredient deliverance structures due to the ability of the polymer matrix to control the drug release rate from these systems. Researchers in the domain of gene delivery techniques are widely using biopolymers-based materials as promising non-viral vectors [23,56].

Due to its ability to be metabolized by certain human enzymes, particularly lysozymes, hence its biodegradability, chitosan is considered efficacious for the establishment of pharmaceuticals and active ingredient deliverance matrices. In this context, it is worth mentioning that for such kind of assignments, it seems paramount that chitosan be water-soluble

with a positively charged feature to be capable of reacting with biological molecules or polyanions with opposite charges in a watery-based hydrophilic medium [148]. In fact, among the most beneficial properties for drug transport, chitosan with a net positive charge is efficient in interacting with mucins and opening fitted intersections between epithelium cells. Thus, deliverance structures and matrices built using chitosan have revealed prodigious wherewithal potentialities to carry anti-cancer, antibacterial, antifungal, anti-inflammatory, vaccine, nucleic acids, peptides and therapeutic proteins, DNA, and genes, among others [44,149].

It is widely recognized that hydrogels, biodegradable delivery systems, polyelectrolyte complexes, and drug conjugates are the primary transport platforms for drug delivery using chitosan derivatives [137]. Chitosan-based hydrogels have been reported to exhibit significant benefits for the establishment of pharmaceuticals and active ingredient deliverance by allowing for a particular site and/or monitored administration in the time of small or large drugs. They similarly offer many benefits, such as improved biosafety and medicament effectiveness. Chitosan-based hydrogels can enable activity/site-specific deliverance and heightened steadiness of pharmaceutical compounds against chemical/enzymatic decomposition [150]. Therefore, chitosan-derived hydrogels have been studied for the effective deliverance and liberation of proteins/peptides, growth hormones, anti-inflammatories, and antimicrobial therapeutics, besides nucleic acids in gene therapy [151-152]. It has been pointed out that compared to other polymeric materials, chitosan can display a gel-like comportment thanks to its 3D assembly that can captivate and hold large volumes of water, permitting its swelling with no need to dissolve entirely, thus retaining its 3D structure [23].

Chitosan hydrogels loaded with osteogenesis promoter proteins have been shown to be efficient in enhancing the restoration of cartilage damage [13,153] and increase the production of chondroitin sulfate to enhance cartilage formation *in vivo* [154]. In addition, parenteral and mucosal administration of antigen vaccines was performed using chitosan-based

micro/nanogels [155,156]. More recently, insulin-laden chitosan nanogels have been able to improve nasal absorption [157] and have provided insulin activity without painful injections. In another report [158], paclitaxel was effectively administered using chitosan nano-gels grafted with salicylic acid. Endothelial growth factors with a short therapeutic half-life necessitate recurrent delivery and management to uphold constructive and proficient quantity, but chitosan-albumin hydrogels have been described to promote the deliverance of growth factor from endothelial cells for more than 3 weeks after subcutaneous implantation *in vivo* in rats with an increase in vascularization [159].

On the other hand, Tan et al. [160] synthesized chitosan carboxymethyl glycol β-cyclodextrin to deliver different hydrophobic anticancer drugs (5-fluorouracil, doxorubicin, and vinblastine). The obtained results showed that the three hydrophobic anticancer agents can be successfully loaded and covalently bound in the cavities of carboxymethyl chitosan dextrins. The resulting system has revealed a promising potential for the efficient administration of anti-cancer therapeutics in tumor therapy. The capability of environmentally friendly nanocapsules built by using chitosan has likewise been expansively examined and considered for the release of diverse compounds, such as anti-cancer drugs [161-163]. In this context, Liu et al. [164] manufactured hollow chitosan nano-bead-like particles by solid-liquid phase separation with particle size between 500 and 1000 nm and openings between 300 and 500 nm. These nanospheres were characterized and evaluated for the delivery of curcumin, a natural anti-cancer drug, and showed a maximum curcumin loading capacity of 63.9%.

4.2. Application in the Preparation of Wound Healing Dressings

Wound healing is based on repairing the integrity of the injured tissue by preventing dysregulated homeostasis. An ideal dressing should preserve moisture at the wound interface, permit gas interchange, behave as a hindrance against pathogens, and eliminate additional exudations. The dressing is further required to guarantee the dissolution of growth factors

and/or microbicide therapeutics and sustain fibroblast proliferation and differentiation, be non-allergenic, non-adherent, non-toxic, and disposed of without trauma [155,165]. The minor behavior to adhere to the wound surface and the aptitude to allow oxygen interchange further promote curative/restorative effects.

Chitosan-based skin tissue restoration bandages have unique kind of features and attributes, including hemostasis, biodegradability, and antibacterial properties [4]. Chitosan exhibits bacterial growth inhibition activity heretofore detected at reduced quantities against various pathogenic microorganisms and can be employed in numerous kinds of formulations including gels, films, or nanoparticles, hence its wide use in the medicine and animal care-related fields as a promoter of skin tissue restoration [2,145].

Chitosan has been proven to be engaged in all phases of skin tissue restoration, causing few adverse effects with little or no fibrous encapsulation and providing protection against bacterial infections [166]. Chitosan is additionally capable of accelerating skin tissue restoration when applied as powders, nanoparticles and microparticles, granules, sponges, or in the form of complexes with additional constituents [167-169]. During the initial phases of wound healing, chitosan promotes the infiltration and migration of neutrophils and macrophages, subsequently, the cleaning of wounds of foreign agents and the formation of granulation tissue, allowing the construction and remodeling of fibrous tissues. For such an eventuality of hypertrophic scar establishment, triggered by disproportionate collagenous structures formation during the metamorphosis segment, chitosan is capable of reducing scar tissue, thus permitting worthy tissue reconstruction and restoration [170]. Chitosan has been correspondingly reported to regulate the synthesis of drivers of growth involved in the skin tissue restoration process and collagen production at the beginning of the after-wound stage (3rd day), thus smoothing skin characteristic patterns regeneration. At the end of the after-

563 wound segment (7^{th} day), chitosan permitted the shrinkage of the synthesis of growth factors,
564 thus promoting scar formation [171,172].

565 Chitosan can be suitably applied as a viscous liquid that is being gelled when applied to
566 the wound surface. To provoke gel formation *in situ*, circumstances and factors counting ion
567 load, acidity/alkalinity, and heating must be adjusted and controlled [3]. In addition, chitosan
568 hydrogels are described as being able to act as matrices for the topical deliverance of proteolytic
569 compounds such as growth promoters, which can help guide skin tissue regeneration
570 responsiveness. The characteristically rapid release of growth activators (within some minutes)
571 is delayed by ionic or hydrophobic physical interactions of the proteolytic groups with the
572 hydrogel matrix [173,174].

573 Alemdaroglu et al. [175] established a chitosan-based hydrogel for the controlled
574 deliverance of EGF (epidermal growth factor) during 2^{nd}-degree burn-based wound therapy in
575 rats. EGF was released entirely from the gel in less than 24 hours and led to quicker skin tissue
576 regeneration times in comparison with injuries managed with chitosan-based hydrogel without
577 the addition of EGF. In another study [176], for the mimicking of the extracellular matrix
578 (ECM) of an injury location, the effect of PDGF (platelet-derived growth factor) formulation
579 in a collagen chitosan hydrogel-based matricidal composite on an ablation-based injury during
580 the wound healing process was evaluated. The degree of wound shrinkage and the quantities of
581 growth activators in the collagenous proliferative fibroblasts in mice were determined. In *vivo,*
582 outcomes disclosed that the addition of PDFG permits amplified relocation and dissemination
583 of fibroblasts. More recently, composite sponge-like wound healing bandages built by using
584 chitosan glutamate and sodium hyaluronate have been explored for the administration of
585 platelet lysate, which is a proficient origin of various growth modulators needed for skin tissue
586 regeneration, in chronic wounds. *In vitro* studies against human fibroblasts have revealed that
587 this formulation can accelerate cell proliferation despite its fragility upon elongation greater

588 than 30-40% of its original length, supporting the fact that chitosan-based gel-like materials
589 behave as a protecting injury microenvironment with advantageous effects on skin wound
590 remodeling therapy [177]. In another study, Hamdi et al. [178] developed a blue crab-derived
591 chitosan and protein isolate 15% (w/w chitosan) composite hydrogel, for carotenoids-controlled
592 delivery and *in vivo* wound healing. The resulting miscellaneous hydrogels portrayed a very
593 high biocompatibility performance towards MG-63 osteosarcoma cells. *In vitro*, the charged
594 carotenoids' release patterns showed that the synthetized gel-forming materials can be utilized
595 as pH-sensitive smart vehicles, for drug-regulated deliverance and liberation, with stimulating
596 antioxidative aptitudes. Moreover, topical application of chitosan composite hydrogel-based
597 patches in a rat model permitted the quickening of the skin tissue remodeling cascades and
598 ultimately the whole curative, for composite hydrogel supplemented with carotenoproteins
599 extracts **(Fig. 11)**.

600 Chitosan can be suitably applied to wounds as dried sponges, films, or powders that
601 exhibit the ability to moisturize speedily upon absorbing exudate to create a chitosan gel-like
602 material on the wound surface [179]. Hydrogels are suitable architectured biomaterials for skin
603 tissue regeneration owing to their simplicity of deliverance and liberation processes,
604 preservation, moisture-holding capacity, and oxygen penetrability [166]. As a result, chitosan
605 and its derivatives, such as carboxymethyl chitosan, can be used as exceptional materials for
606 wound healing applications [180].

607 **4.3. Application in the Sector of Tissue Engineering**

608 Tissue engineering is a burgeoning field of scientific investigations whose ultimate target
609 is indeed to repair/regenerate/replace tissues and organs in the body that have been damaged
610 far beyond their function. Tissue engineering is partitioned into numerous sections according
611 to the targeted tissue/organ nature [1,44]. Tissue engineering is becoming a momentous domain
612 of polymeric-based materials investigations since the requirement for a completely viable and

healthy network has been rapidly substituting traditional replenishment therapeutic strategies, showing some shortcomings in the utilization of non-degradable and non-biocompatible artificial-based scaffolds, etc. The current peer group of tissue engineering focuses on transplanting cells inside spongy bio-decomposable polymeric matrices. Indeed, the foremost apprehension involved the availableness and accessibility of bio-decomposable matters that can be used as temporary matrices [181].

Of late, chitosan and its derivatives have been conveyed as outstanding competitive alternatives for tissue-engineered scaffolds, thanks to their ability to degrade as tissues are built with minimal immune system response or noxious decomposition products. In addition, the positively charged attribute of chitosan is contributive to the creation of non-covalent chemical bonding with anionic glycosaminoglycans, proteoglycans, and other products with opposite charges [8]. Otherwise, chitosan and its polymeric subsidiary products have been broadly utilized as effective alternatives in bone tissue engineering for the promotion of cell proliferation cascades and mineralized matrices accumulation by cultured osteoblast cells. Animal model data demonstrated that such designs allowed a controlled delivery of the methotrexate after the intra-articular injection of injectable hydrogel into mice's arthritic joints **(Fig. 12)**, ultimately promoting a respite of the inflammation for an effective treatment of the rheumatoid arthritis [13].

In this context, the feasibility of hydroxyapatite-sheathed carboxymethyl chitosan platforms for the simulation of bone remodeling has been demonstrated by experimentation [182]. In more recent work, Serra et al. [183] manufactured bio-resorbable implants by lyophilization employing diverse preparations based on chitosan, gelatin, and tricalcium β-phosphate (β-TCP) for bone tissue regeneration. Mechanical improvement was observed with chitosan scaffolds combined with gelatin and β-TCP, up to 70% compared to pure chitosan. The prepared 3D scaffolds were bioactive and biocompatible, allowing excellent cell adhesion

with better effectiveness in the stimulation of osteoblast cellular activity hence internalization and distinctive osteoblastic morphologic features. Thus, these three-dimensional chitosan-based structures have shown great potential in bone regeneration, promoting cell adhesion and proliferation, further preventing the formation of biofilm on their surface, the foremost origin of implanted scaffold failure. Numerous studies on the creation of implantable scaffold-based bone substitutes exploiting chitosan and its polymeric subsidiary products have been described in the literature and excellent results are available [184-186].

4.4. Application in Bioimaging

Bioimaging is based on the real-time, non-invasive visualization of biological processes often in a three-dimensional (3D) structure using various imaging sources such as light, fluorescence, electrons, X-rays, ultrasound, positrons, and magnetic resonance [2,50,145].

Chitosan is a nature-derived amino-polysaccharide that occurs with interesting physicochemical and biofunctional features. The amine and hydroxyl functional sites existing on the chitosan backbone make available a pathway for reactions with functional biological compounds [24]. In addition, chitosan is extensively employed as a bioactive organic matrix material in the sector of bioimaging due to its exceptional biodegradability and minimal toxicity [187-189]. The possibility of introducing imaging agents into the chitosan matrix has favored its use for bioimaging. Lee et al. [190] reported that the incorporation of an imaging agent, Fe_3O_4, plays an effective contribution in scanning with a nuclear magnetic resonance field and that the prefabricated self-arranged nanobeads improve the revealing and recognition of specified depiction on hepatocytes. Chitosan-reduced gold nanoparticles have been used as a photothermal converter and photodynamic support in photodynamic therapeutic strategies, which have a role in the application of bioimaging and are used to destroy breast cancer cells [191]. Moreover, gold-coated Fe_3O_4 nanoparticles with a typical diameter of 9.8 nm were further synthesized by chemical glucose-reducing reactions by chemical coprecipitation and

balanced with chitosan amide the assembly with formaldehyde as a cross-linking reagent. The developed nanomaterial has been reported as a potential candidate material for biodetection and bioimaging applications [192].

4.5. Application in Enzyme Immobilization

For biobased economy and biotechnology implementation and relevance of enzymes, immobilized enzymes are more beneficial and gainful over unfettered enzymes, facilitating their recycling (reusability), improving enzyme stability, and allowing continuous production with no biocatalysts in the product stream [81,193,194]. Analytically, immobilized enzymes are primarily used in biosensors (sensors used for biological systems) and in diagnostic test strips [195]. Biosensors have been built employing the integration of biomaterials-based sensing platforms such as enzymes with transducers, which convert the reaction into a measurable response. Otherwise, biosensors have been recognized to be based on biological materials that can recognize explicit and precise biochemical fragments and indicate their occurrence, and quantities, alongside their functionality through biochemical ways. The foremost favors and conveniences of biomaterials-based sensors are transportability, great specificity, flexibility, fundamental discrimination, and ease of practice in multifaceted situations owing to their rapid responsiveness. Within this framework, most research focuses on nanomaterials for the reason that they are endowed with unique electric, photosensitive, and catalysis attributes [196].

Bio-decomposable polymeric materials either synthesized or recovered from nature-based resources like chitosan and its polymeric subsidiary products have been considered among the supreme appropriate platforms to immobilize enzymes due to their non-toxicity, bio-safety, and large specific reactive sites and locations for charging a greater quantity of enzymes, due to their NH_2 and OH reactive units that are capable of interreacting with dynamic components [44,197]. Such fascinating characteristics make chitosan the furthermost widely

688 used organic-based polymeric material to manufacture advanced hybrid materials for
689 biosensors' engineering and industrialization [198]. Thus, chitosan-catechol films are used as
690 detectors for active bacterial metabolites with redox activity [199]. More recently, Han et al.
691 [200] designed an innovative tyrosinase immobilization biosensor by using chitosan
692 nanocomposites for the recognition and distinction of phenolics. Similarly, the properties of
693 Laccase enzyme following the immobilization process on cellulose
694 acetate/chitosan/poly(ethylene oxide) electrospun nanofiber were explored **(Fig. 13),** and
695 results revealed that the immobilization of Laccase on the cellulose
696 acetate/chitosan/poly(ethylene oxide) nanofibers with fine diameters boosted the loading of the
697 enzyme, suggesting its potential as an ideal candidate for industrial application instead of free
698 enzymes [198]**.** The developed nanocomposite films permitted considerably satisfactory
699 transmissivity and bio-safe surrounding conditions for the immobilized enzyme with a high
700 affinity for substrates. In addition, a chitosan-carbon nanotube system was used for
701 electrochemistry-based detection by using dehydrogenase enzymes [201]. Furthermore, one-
702 use biomaterials-based sensors employing enzyme-immobilized indium tin oxide electrodes
703 modified by gold and chitosan have been developed, with amperometric analysis by flow
704 injection [202]. The use of chitosan-based polymeric platforms to immobilize enzymes for bio-
705 sensitivity has been further conveyed [203].

706 **4.6. Applications in the Cosmetics Sector**

707 Currently, in the cosmetics industry, the integration of natural substances in both products
708 and formulations is considered, given the increasingly demanding regulations concerning
709 public health and the environment, in addition to consumers' awareness. Among nature-derived
710 microbial growth-inhibiting compounds of aquatic derivation, chitosan has been broadly
711 utilized in cosmetic products [204,205]. In this context, chitosan has been proposed as an
712 antimicrobial polymeric matrix with hydroxyapatite ceramics in a multifunctional sunscreen

713 [206]. The prepared gel exhibits optical absorption of ultraviolet light at 254 nm, and
714 antibacterial activity against *S. aureus*, *Klebsiella pneumoniae*, and *P. aeruginosa*, in addition
715 to a significant effect on the growth of multidrug-resistant bacteria. In another study, Wongkom
716 and Jimtaisong [207] prepared carboxymethyl cellulose-based biocomposites from the peels of
717 *Ananas comosus* and carboxymethyl chitosan cross-linked with ferulic acid, for use as novel
718 matrices for hydrophilic sunscreens, following concerns about the effect of ultraviolet radiation
719 on skin cancers, sunburn and photo-aging. Hydrophilic TiO_2 and phenylbenzimidazole sulfonic
720 acid have been applied as sunblock and protecting agents at a proportion of 2:1 (w/w). The
721 prepared biocomposites have proven their ability as good matrices for anti-UV agents whose
722 concentration can be modified to fix the required protection factor.

723 Chitosan was further operated for the encapsulation and stabilization of bio-functional
724 constituents in beauty and personal care commodities. Indeed, uniform and well-balanced
725 microcapsules involving a fluid aquaphobic nucleus containing linoleic acid, encircled by an
726 envelope of chitosan and lactoionic acid, by the simple association in water, were prepared, and
727 their ability to encapsulate phenyl ethyl resorcinol, a hydrophilic skin brightening or
728 depigmenting agent, was evaluated. The prepared microparticles were revealed as suitable for
729 encapsulating phenylethyl resorcinol as a skin lightener [208]. Otherwise, Libio et al. [209]
730 prepared chitosan films with or without glycerol to determine the composition best suited to
731 physical integrity and skin biocompatibility for makeup removal applications. After evaluation
732 of their physicochemical properties, glycerol-free chitosan films were selected to perform the
733 release experiments using a pigskin model. Although hyaluronic acid interacts with chitosan
734 reducing the moisturizing effect of the film, a noteworthy intensification in the degree of
735 hydration of the skin was distinguished.

736 Recently, some studies on the encapsulation of fragrance compounds in chitosan-based
737 nanoparticles have been established and reported to be capable of avoiding the loss of highly

volatile fragrance ingredients. Xiao et al. [210] developed a tuberose fragrance compound encapsulated in chitosan nanoparticles for cosmetic applications. The microbial growth inhibition activity of nanoparticles against *S. aureus*, *E. coli,* and *Bacillus subtilis* has been explored, in addition to their sustained-release property of nanoparticles, indicating a promising application of these nanoparticles as controlled-release vectors, not only for fragrance but also for antimicrobials [11,211].

5. Conclusions and Perspectives

Chitosan is a naturally occurring, bio-decomposable, bio-safe, biologically compatible hydrophilic polymeric material, derived from chitin, which is among Earth's most abundant and sustainable natural materials. Chitosan and its derivatives propound very interesting innovative matters and platforms with a broad spectrum of encouraging biotechnological applicability and relevance. In recent decades, the marketing of chitosan as a nutritional additive product has enlarged primarily due to its advantageous antioxidative potential, anti-lipidemic action, and ability to enable body mass loss. Overall, such kind of bio-functionalities have prompted the utilization and implementation of chitosan-based materials for the anticipation and handling of long-lasting sicknesses. There are significant obstacles to the commercial exploitation of chitosan, as it is difficult to prepare uniformly reproducible chitosan products in large quantities from a variety of aquatic-based resources all over the world. The adaptation and modification of chitosan additionally complement the global net value and conceivable distinctions in the homogeneousness of the characteristics. Although a range of specimens of chitosan subsidiary polymeric products are being evaluated for uses in pharmacy and medicine-related fields, just a limited number of them, counting carboxymethyl chitosan, trimethyl chitosan, and PEGylated chitosan, have attained a deep-documented and adequately acknowledged implementation profile. Overall, there is still tremendous research work to be

762 performed and completed for the entire exploitation of the interest and convenience of chitosan
763 and its derivatives in pharmacy and medicine-related domains.

Acknowledgments

The funding of this research by the Qingdao University of Science and Technology through a postdoctoral project, Qingdao City through a 2024 postdoctoral funds-3 project grant, and the Science and technology department of Qingdao Municipality (project number: 24-1-4-xxgg-11-nsh) is extremely acknowledged.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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Chitosan and its Derivatives as Potential Biomaterials for Biomedical And Pharmaceutical Applications: A Comprehensive Review on Green Extraction Approaches, Recent Progresses, and Perspectives

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Abstract

***Background*:** Nowadays, the search for new renewable and broad-spectrum natural biopolymers for biotechnological and medical applications has become an absolute necessity. **Chitin and its deacetylated derivative, chitosan,** are considered interesting and auspicious biopolymers being potentially applied in a wide range of biotechnological sectors, including medicine, food beverages, agriculture, and cosmetics, owing to their enormous ability to undergo changes in structure and mechanical properties to generate new functions (used as a matrix in beads, membranes, gels, etc.) and applications.

Scope and Approach: The current review provides a comprehensive report summarizing research on the routine chemical and greener non-conventional extraction methodologies of chitin and chitosan and focuses on the progress in their application over the past two decades, in terms of challenges, opportunities, and future perspectives.

Key Findings and Conclusions: **Chitosan** is an effective material with enormous potential for biotechnology and medicine owing to its biocompatible, biodegradable, and non-toxic traits, besides its antimicrobial potential and low immunogenic potency. To standardize applications in the industrial field considering cost-effectiveness and biocompatibility, the search for innovative recovery and production methods for chitin/chitosan-based materials industrialization is required. Conventional chemical chitin extraction approaches present drawbacks and induce numerous environmental issues. Greener extraction technologies have recently perceived considerable advancement in the polymer chemistry field. This review can serve as a guideline for exploring nature-originated biopolymers as innovative feedstocks for several technologies that show highly appealing potential for application in countless fields.

Keywords: Chitin; Chitosan; Extraction; Green Chemistry; Bioactivity; Biomedical applications.

45 **Summary**

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1. Introduction

Chitosan is a semi-crystalline unbranched polysaccharide consisting of two recurrent patterns, D-glucosamine and N-acetyl-D-glucosamine connected by glycosidic β-(1-4) bonds. It is obtained by fractional or total deacetylation of chitin, reported to be the second most abundant natural polysaccharide after cellulose (**Fig. 1**). Chitin is a foremost constituent of the extracellular matrix of fungi and the exoskeleton of shellfish and insects [1-3]. The chitin/chitosan nomination depends on the proportion of acetamide units or acetylation degree (AD). For an AD < 50%, the polymeric product is commonly quoted as chitosan, while the term chitin reflects an AD > 50% [4]. Contingent on the origin and method of production, the molecular weight of chitin can sweep from 300 to more than 1000 kDa. Chitin occurs in three different crystal structures: α-chitin (the most stable and abundant form with macromolecules arranged antiparallelly), β-chitin (parallel alignment), and γ-chitin (both parallel and antiparallel forms) [5-6]. Compared to cellulose, chitin is recognized as much easier to undergo changes *via* chemical reactions, owing to the presence of the acetamide group among the structured units of chitin (**Fig. 1**). It has been reported that although a minimum of 10^{11} t of chitin are synthesized and degraded each year, only 150,000 t are made available for commercial use [7]. A stumbling block of the appropriate biotechnological utilization of chitin is its non-solubility in almost all common solvents.

Chitosan, as the simplest and most commonly studied derivative of chitin, is the only naturally occurring cationic polysaccharide, soluble in dilute acids (pH < 6.0) *via* cationization of the $–NH_2$ group at the C-2 site of the repeating units of D-glucosamine, as a function of the AD. Thus, under the crystalline arrangement, chitosan cannot be solubilized in neutral to alkaline water-based media (pH > 7.0) [8]. With the presence of bioactive functional -OH and $-NH_2$ groups, chitosan has excellent physicochemical and biological properties and has proven to be of remarkable interest for versatile fields and applications [9-14]. Currently, several

industrial areas such as food [15], environment (wastewater treatment), agriculture (seeds, packaging), textiles [16], and cosmetics are concerned, with particular attention to their relevancy in the pharmacy-based and biomedical sectors [17,18], as summarized in **Fig. 2**. In fact, chitosan is considered as one of the most promising natural substances with fascinating biofunctional properties for tissue engineering [19], drug delivery [20], wound healing [21], even in gene delivery as a promising non-viral vector [22-24]. Chitosan can be readily transformed into different forms of materials, such as gels, membranes, nanoparticles, and so on [23,25-27]. Tissue engineering researchers have recently focused on designing nanomaterials that will provide long-term benefits to humans in the event of a medical emergency, such as bone fracture, cartilage tissue damage, etc. [28].

2. Chitin Recovery and Production Processes and its Conversion to Chitosan

2.1. Routine/Traditional Recovery Approaches

Chitin is mainly derived from the crustaceans' outer skeleton, such as crabs and shrimp. Chitin is present in shells in the form of chitin-protein-mineral complexes (mainly calcium carbonate) **(Fig. 3)**. Traditionally, to produce chitin from crustacean shells, outdated chemical pathways that involve steps of demineralization and deproteinization are applied using large volumes of hazardous chemicals (acids and bases, respectively) **(Fig. 3)**. The use of these chemicals can affect the quality and functional properties of the final product (partial deacetylation and depolymerization of chitin), as well as they are hazardous to the environment [29,30]. To overcome these problems, various biotechnological processes (fermentation and enzymatic) are studied and developed [31-33]. Biological chitin recovery processes (extraction under mild conditions) preserve the quality of chitin and promote the valorization of other compounds in crustacean co-products, such as peptides and pigments [34-36]. Chemical and enzymatic processes have been widely studied for chitin production. In contrast, the order of

the demineralization and deproteinization steps has not been investigated except in a few studies [30,37]. Indeed, it was established that, although in the case of enzymatic deproteinization handling, minerals associated with the chitinous matrix can hinder the accessibility of proteases to the linked proteins and thus affect the deproteinization efficiency, the order of the proteins and associated minerals removal steps can be reversed without affecting the quality and yield of chitin extraction and chemical treatments [30].

Chemical and biological extraction methods for chitin have advantages and disadvantages or challenges **(Table 1)**. **"Green"** products and techniques evolve into one of the cutting-edge fields. In this aspect, **"green chemistry"**, which is interpreted as the utilization of chemistry technologies and approaches that diminish or omit the usage and inception of feedstocks, products, by-products, solvents, and reagents that are precarious to human health or the environment, can be deemed as one of the sectors with utmost importance at industry and educational levels for the hands-on fulfillment of the **"environmentally friendly or eco-friendly"** motif [6,31]. Accordingly, toward a greener future, the development of new methods using greener and more environmentally safe extractants such as Ionic Liquids (ILs) and Deep Eutectic Solvents (DES) is thereby necessary for the production of high-quality chitin with a desired AD of up to 100%, without severe degradation of the molecular chain. Scientists have explored a widespread assortment of technologies **(Table 2)**, such as enzyme, electrochemistry, photochemistry, sonochemistry, ultrasound, and microwave or radiofrequency-using extraction approaches, for the efficient and environmentally friendly extraction of chitin [11,37,38].

One of the main difficulties in valorizing chitin is its insolubility. Thus, various chitin derivatives have been prepared, including chitosan which is the simplest and most studied derivative recovered by fractional or total deacetylation of chitin employing either chemical or enzyme-using processes [38]. During the synthesis progression, numerous parameters and conditions can affect the physicochemical properties of chitosan as well as extraction yields,

primarily alkalescent solution concentration, treatment period, chitin/alkaline solution proportion, atmosphere (air/nitrogen), heating, chitin origin (shrimp, crab, etc.), granulometry of the raw powder, and the usage of one- or multi-step acetyl groups removal procedures [39-41]. In terms of availability, chitosan production has reached more than 10^{13} kg per year [7].

Marketed chitosan typically has an AD of 5-30 % and a molecular weight (MW) of 10^4-10^6 g mol^{-1} [42-44]. The determination of AD is extremely crucial in the evaluation of the efficiency of the acetyl group removal from the chitin polymeric chain and the final bio-functional performance of the biopolymer, hence the definition of its future biotechnological application [45-49].

2.2. Emerging/Alternative Recovery Approaches

2.2.1. Physical Approaches

Microwave heating technology has been broadly implemented in numerous food and chemical industries as it not only reduces chemical reaction time but also improves the recovery yield and the quality of the final product (chitin, chitosan, and so on) in terms of purity and technofunctional features in comparison with conventional methods **(Table 1)**, besides the advantages of green status, energy effectiveness, convenience, low application cost, selectivity, and simple handling of the specific procedure [50,51]. The thermal effects and high efficacy of the heating by microwave irradiation are attributed to the reversed and quick heat transmission between the biomass substrate and the catalyst, the consistent irradiation field inside the sample, and the discriminating radiation assimilation by polar materials involving two major phenomena: ① dipolar polarization and ② ionic conduction, as shown in **Fig. 4** [52]. Considering the aforementioned advantages, to minimize the utilization of non-ecofriendly chemicals, the application of microwave irradiation has been monitored in the recovery of various polysaccharides, such as chitin/chitosan [31] and cellulose [53], among others.

Nonetheless, to achieve the uppermost yields of chitin or chitosan extraction, considering the reduction of the process cost, it is crucial to optimize several microwave irradiation process operational parameters, mainly the heating period, the extractant concentration (water content of the extraction solvent), and the biomass to solvent ratio, which exert a strong influence on the AD and MW of the final product, chitin or chitosan [32].

Ultrasound-assisted extraction, using ultrasonic waves in the range of 20 Hz to 20 kHz is another innovative eco-friendly technology applied to bolster the extraction of chitin, through the cavitation effect that allows the intensification of chitin-linked proteins solubilization, involving mainly ① macromolecules depolymerization, ② polymer-based covalent bonds, and ③ conformational modifications in terms of aggregates scattering [36,54]. Ultrasound-assisted extraction can be performed *via* either indirect sonication using an ultrasound horn or direct sonication in an ultrasound bath **(Fig. 4)**. Accordingly, enhanced removal rates of proteins (deproteinization) and minerals (demineralization) associated with chitin can be accomplished by coupling ultrasounds and chemical processes [39]. Ultrasonication treatment is attracting attention as a preferable non-thermal approach for the improvement of chitin and chitosan recovery from different resources, due to its several advantages such as faster energy and mass transfer, reduced temperature and time, high process control, extraction selectivity, and faster start-up/easy to install **(Table 1)**. The effects of ultrasonication treatment on the AD, MW, and particle size of the biopolymer products were explored, and results reveal that the rise in the ultrasound treatment time led to more effective associated protein removal, along with an increase in the crystallinity, reduction of the particle size, and a surface erosion to variable extents of the resulting biopolymers (chitin and chitosan). Moreover, lower sonication periods led to the recovery of chitosan with higher AD and MW, compared to higher ultrasonic treatment periods (recovered polymeric materials with medium and lower MW and AD) [55].

Subcritical water treatment is based on the usage of subcritical water as the reaction medium with no need for enzymes or chemicals. It is an environment-friendly and energy-efficient technology that has recently attracted attention for a wide range of high-added-value products recovered from waste biomass, including chitin and chitosan [56]. Subcritical water could act as an acid or base catalyst and is defined as liquid water under heating treatment in the range of 100 °C and 374 °C and a pressure < 22 MPa, underneath the critical point of water [57] **(Fig. 4)**. Subcritical water extraction is an extraordinary eco-innovative, non-toxic, non-flammable extraction method, with no residual solvent after the extraction and considerably shortened reaction time **(Table 1)**. This technology allows changes in chitin structure for improved enzyme-based proteolysis, as well as associated protein elimination and mineral removal [58]. However, the major challenges of this technology are the high cost of industrial infrastructure for the subcritical water systems installation, and the optimization of reaction temperature, pressure, treatment time, solid-water ratio, particle size, and pH, along with the solute characteristics and flow rate, is extremely required [31].

Pulsed electric fields are an additional novel and non-thermal favorable technology that has been developed, during the last ten years, for enhancing the release of food-derived biopolymer compounds, and thereby improving the extraction yield. This technology uses high-intensity brief pulsations of electric fields (strength in the range of 10 and 80 kV/cm) surpassing the critical value in a short period (μs or ms) on a biomass material positioned between two electrodes [59,60] **(Fig. 4)**. This treatment induces the electroporation mechanism (temporary or permanent), commonly defined as pores creation phenomenon on the cell membrane, hence an intensification of cells transmembrane capability in the biomass product [61]. This process has been employed as a tool to expand the extraction of a plethora of high-added values materials from diverse natural resources [62,63]. The profits of such eco-friendly methodology are mainly a quick handling period, a reduced amount of consumed energy, additionally to

microorganisms' deactivation **(Table 1)**. However, the high cost of the initial investment along with safety concerns (problem of electrochemical reactions at the commonly used stainless steel electrodes) has made the development of this technology a challenging task [64].

2.2.2. Mechano- and Electrochemistry-based Approaches

As an alternative to the conventional approaches of chitin and chitosan extraction, **mechanical energy** has been integrated into the recovery processes of shellfish-derived chitin and its byproducts, defining the **mechanochemical technology**, which is referred to as the field of chemistry related to the chemical and physicochemical transformation of biomass through the effect of mechanical energy (solid-state reactions), as defined by Heinicke [65,66]. Such technology has shown a plethora of advantages **(Table 1)**, including ① Solvents deletion throughout the conversion reactions of the biomass waste for the recovery of chitin and its polymeric derivatives, with much lower consumption of water that is required only for the filtration stages and chemicals such as acids and bases, due to a lower required matter-chemical mass-molar ratio, as compared to the routine approaches [67]; ② Considerable saving of energy consummation during the extraction (a 1/9 part, approximately), since the majority of the transformation procedure is monitored only in a single unit operation (the mill) with no heat treatment for a briefer period [68]; ③ Appealing possibility of chitin/chitosan oligomers development and recovery, with better homogeneity of acetylation and polymerization degrees, especially when biomass matter is milled at elevated frequencies with the increase of the mechanical processing period [66].

The electrochemical approach, including accurate pigments, lipids, proteins, and minerals removal steps, has been additionally introduced in the processes of matter transformation for high MW chitin and chitosan, allowing a significant growth of the cost-effectiveness of the chitin-producing process, with precise biomass waste treatment and the opportunity to recover all its valued products [31]. The principle of the technology is the

biomass waste smooth disintegration due to the effect of in-situ generated alkaline and acidic extraction media (redox reactions) inside an electrolytic cell, in the presence of a diluted aquatic sodium chloride solution and under mild circumstances [69]. Herein, an anode and a cathode are immersed in a particular electrolyzer (electrochemical cell) containing electrolytes (aqueous salt solution), creating two chambers: the anolyte (anode cell) and the catholyte (cathode cell) parceled by an ion exchange membrane **(Fig. 4)**. The catholyte compartment produces the catholyte solution (H_2 and OH^-), inducing the occurrence of water electrolysis, while in the anolyte compartment, oxidation of the Cl^- ions of sodium chloride induces the production of Cl_2, subsequently electrolyzed on the anolyte surface, leading to the production of the anolyte solution (H^+ and HClO) [70]. Such technology, based on the electrochemical activation of aquatic media, is considered more advantageous as compared to other activation systems such as sonication or microwave irradiation **(Table 1)**, due to the consistency in the process physicochemical conditions, such as redox potential, pH, surface tension, electrical conductivity, dielectric constant, among others, since the duration of water metastable status is rather extended [31,68,69].

2.2.3. Introduction of Green Solvents

Traditional extraction processes have been widely utilized to extract bioactive compounds from various biomass waste resources over the past decades. However, such traditional extraction methods present a major disadvantage, *i.e.* the use of large amounts of organic solvents, which are not sustainable as they are flammable, hazardous, and have limited disposal and recycling possibilities [71]. Other drawbacks of applying the conventional chemical chitin-producing methods include time-consuming, labor-intensive, and lack of automation, resulting in low selectivity and low extraction rates [72]. To achieve sustainable extraction, there is a growing need for safe and sustainable green extraction technologies. In this field, "***Green Chemistry***" is defined as the use of chemical techniques and methods that diminish or omit the

usage and generation of unprocessed matters, side components, solvents, chemicals, and testing agents known to be destructive to human healthiness or the natural ecosystem equilibrium [73]. **Green extraction** is the latest goal of scientific and industrial research and development as it aims to reduce or eliminate the usage of toxic chemical solvents/reagents, reduce energy consumption, minimize environmental impact, and further provide significant benefits to human health and well-being [74].

Alternatives to conventional solvents are **ionic liquids (ILs)**, recognized as organic salts, with a low temperature at which it melts (inferior to 100 °C), involving a bulky organic cation with a minor organic or inorganic anion **(Fig. 5)**, allowing thereby several conceivable cations and anions groupings, were first used as green solvents [75]. Such features permit the adjustment of the ILs' intrinsic parameters, including thickness, ions conduction, hardness, polarity, solvating capacity, and hydrophilic and hydrophobic behaviors for the extraction of a high number of molecules with different polarity indexes, such as chitin from marine sources [76]. The advantages of ILs over conventional solvents are low vapor pressure, facility, and security of monitoring, wide range of miscibility and solubility, good thermal properties, and good recyclability. Additionally, ILs have been utilized not only in the extraction of chitin and its derivatives but likewise for chitin and chitosan solubilization and soft materialization to design a plethora of biomaterials, among which hydrogels, membranes, and spheres at the nano- and microscale, fibers, etc. **(Fig. 6)**, primarily for medicine and pharmacy-related uses [77]. The 1-ethyl-3-methylimidazolium acetate, 1-butyl-3-methylimidazolium chloride, and 1-allyl-3-methylimidazolium acetate have been conveyed as the furthermost frequently applied ILs for efficient evocation and separation of chitin from marine sources [31]. However, in addition to low toxicity and biodegradability **(Table 1)**, ILs have some disadvantages such as high viscosity, limited solute solubility, corrosiveness, and high production cost [78]. The observed

toxicity of ILs is found to be mainly related to cationic molecules, side chain length, and anions. Compared to conventional solvents, ILs are more toxic and harmful to bacteria [79,80].

The use of **deep eutectic solvents (DES)** has received increasing attention as an **eco-friendly green solvent** and an alternative to routine solvents and ILs for the derivation and solubilization of chitin and its derivatives. DESs are prepared by mixing two or more nonionic compounds that form eutectic mixtures through hydrogen bonding with melting points lower than those of the constituents. Salts and molecular compounds are typically used, where one constituent performs as a hydrogen bond donor (HBD) and the other as a hydrogen bond acceptor (HBA), at a defined molar ratio **(Fig. 5)**. In general, the composition of DES is expressed as Cat+X-zY, where Cat+ is an ammonium, phosphonium, or sulfonium cation, X is a Lewis base, and Y is a Lewis acid. The complex is formed between X and Y, where z is the number of Y particles interacting with the anionic molecules [73,81]. The use of DES is a relatively young field of research, with the first studies published in 2001. In the last two decades, extensive research on DES has been performed, with more than 2000 papers published in 2022 [76]. The DES presents the advantages of low volatility, non-toxicity, biodegradability, non-flammability, chemical stability, ease of preparation, and relatively low cost, compared to traditional industrial solvents and ILs **(Table 1)**. The application of DES in the extraction of bioactive compounds, such as chitin and chitosan from natural resources, and the synthesis of different classes of DES have been extensively studied [6]. Nowadays, DES is being effectively utilized as an efficient medium to synthesize and solubilize chitosan and its derivatives, owing to their robust intermolecular hydrogen bonds that permit the intensification of the opportunity to disrupt and break down the solid intrinsic hydrogen bonds network inside the chitin polymeric chains [82].

Recent trends in the green approaches for sustainable valorization of renewables have led to the design and conceptualization of **natural deep eutectic solvents (NADES)**, consisting of

primary plant-based metabolites (sugars, carboxylic acids, amino acids), known to exhibit a key function in cellular processes [83]. Interestingly, using NADES as an extractant medium, chitin can be recovered in a single-step process, for a faster and more eco-friendly manner, hence minimizing the amount of consumed water and toxic reagents in the proteins and minerals removal steps [84]. Several DES or NADES systems have been formulated to extract chitin from marine biomass wastes with extraction yields of around 20%, such as choline chloride (HBA)-Lactic acid (HBD) (1/1; w/w) [85], choline chloride (HBA)-Malonic acid (HBD) (1/2; w/w) [86], choline chloride (HBA)-Glycerol (HBD) (1/2; w/w) [87]. More advantageously, DES and NADES have been efficiently applied to better dissolve and chemically functionalize chitin and its derivatives for the development of innovative chitin or chitosan-based nano-materials, such as films, membranes, hydrogels, particles, fibers, as shown in **Fig. 6** [6,68,71,76,82,88].

2.3. Chitin/Chitosan Oligomers Production

In recent years, a plethora of scientific and methodological schemes have been implemented to concoct chitooligosaccharides or chitosan oligomeric derivatives and products **(Fig. 7)**, including acidic digestion [89], enzyme-using methodologies [90], ultrasounds-based breakdown processes [91], subcritical water hydrolysis [92], and oxidative degradation [93]. Among the methods of preparation of chitosan oligomers widely used on an industrial scale, acid hydrolysis is most commonly used. Nonetheless, a major part of compounds resulting from acidic degradation show short rates of polymeric disintegration, with unsatisfactory manufacturing effectiveness rates [94]. Subsequently, tremendous attention has been allocated to enzymatic production methods owing to their capability of minimizing undesirable molecular alteration phenomena and indorsing bio-functions. Sundry non-specific enzymes, especially cellulase [95], and chitosanase [96], have been widely exploited and utilized in the research and production of chitosan oligomers.

Chitosan oligomeric derivatives have been recognized as having keen importance, mainly in the medicine-related industries, thanks to their peculiar attributes counting dissolvability in aqueous media, low MW, low thickness/hardness, and terse polymeric sequences. These features allow them to upsurge their bio-functional activities inside *in vivo* structures and schemes [68,97].

3. Bio-functionalities of Chitosan and its derivatives

3.1. Chemical-Physical Characteristics of Chitosan

Unlike other polysaccharides in nature, the occurrence of hydroxyl and amine functional/reactive sites in the chitosan molecular chain affords a favorable foundation for interconnections with further polymeric and biologic-based compounds, such as lipids with opposite negative charge, proteins, cholesterol, macromolecules, and metal ions [98]. Otherwise, the interesting chemical properties of chitosan are ascribed to the occurrence and availability of reactive amine and hydroxyl groups, its linear polyamine structure, and its ability to chelate many transition metals [44]. The configuration and dimensions of chitosan polymeric matrix sequences diversify contingent on their source and the method of chitin extraction and existing acetyl groups removal. Chitosan is generally provided in the form of a semi-crystalline powder with a white or slightly yellow appearance [99].

Chitosan cannot be dissolved in aqueous media, caustic base media, or organic solvents. Contrarywise, it is highly dissolved in weakly acidic aqueous media with a pH below 6.0 [100,101]. The utmost characteristic attribute of chitosan is AD, which strongly has an impact on and prompts its aspects, attributes, and ultimately biotechnological relevance. The commonly monitored technologies for the precise estimation of chitosan AD are infrared spectroscopy (IR) and nuclear magnetic resonance (NMR), besides potentiometric titration [102]. Chitosan exhibited a widespread array of viscosities in weakly acidic aqueous media as

365 a function of the final MW [99]. Commercial chitosan readily supplied in the market unveiled
366 a significantly large MW, meanwhile, for agrifood-processing and pharmacy-related, low MW
367 chitosan is demanded and provided [103]. It is reported that the biochemical structure and
368 composition of chitosan offer countless opportunities for complex intra-molecular and ionic
369 amendments, which allows for a thorough adaptation and regulation of the bio-techno-
370 functional attributes of resulting biomedical tools constructed utilizing chitosan and its
371 derivatives [98,104].

372 Owing to the occurrence of hydroxyl -OH, acetamide, and amine -NH_2 sites in the
373 chitosan polymeric matrix, the desired physicochemical properties of chitosan can be
374 incorporated into the structure of chitosan by chemical, physical, and enzymatic modifications,
375 as summarized in **Fig. 8** [105]. Among the various techniques in vogue, chemical modification
376 is widely used, allowing the synthesis of derivatives with controlled solubility, ionic
377 characteristics, and hydrophilic character. Native chitosan is hydrophilic with low degrees of
378 order and flexibility. To improve its hydrophobicity, N-acylation with various fatty acid
379 chlorides (C6-C16) is usually performed [21,106], modifying the polymeric structure of
380 chitosan, thus making it an interesting excipient in controlled drug delivery systems.

381 In another aspect of chitosan modification, radiation treatment (gamma rays, electron
382 beams, UV rays, sonication, microwave, etc.) is an interesting evolving domain of scientific
383 investigations that aims to synthesize derivatives with enhanced properties. These approaches
384 are cost-effective and environmentally friendly alternatives that exhibit several advantages,
385 such as limited sample preparation, shorter preparation time, no catalysts, and no need for
386 temperature changes. Crosslinking, degradation, and free radical formation are among the
387 structural changes resulting from irradiation [107,108].

388 In addition, the use of enzymes in the synthesis or functionalization of chitosan has many
389 advantages. The enzymatic modification of chitosan leads to homogeneous deacetylation,

generating derivatives of low MW, compared to heterogeneous deacetylation. In addition, enzymes have the capability of catalyzing cellular processes with fast-moving operating and strong distinction and no irreversible and constant structural change [109].

Interestingly, chitosan can be easily transformed into plentiful functional materials, such as nanoparticles [2,110], beads [111,112], microparticles [113], nanofibers [114,115], membranes [116,117], hydrogels, and nano-gels [118,119].

3.2. Biological Properties of Chitosan

Chitosan's unique chemical characteristics allow it to benefit from a multitude of captivating biological and functional properties **(Fig. 9)** [4,72], including biocompatibility with body components, non-toxicity, antioxidant activity, antimicrobial potential, and biodegradability [8,24,120,121]. Chitosan can, similarly, bind to mammalian cells, accelerating the establishment of osteoblasts at the helm for bone construction, hence its restorative and healing influences. Chitosan has been furthermore described to be endowed with central nervous system depressants, hemostatic, fungistatic, spermicidal, antitumoral, immunoadjuvant, etc., among others [122].

The antioxidizing peculiarities of chitosan and its derivatives detained considerable prospectives for the handling of oxidative-based illnesses [123,124]. Chitosan's ability to be absorbed by cells and the intestine besides its bio-safety allows it to be a highly encouraging product to be applied as a nature-based antioxidant. Chitosan regulates the activities of antioxidant enzymes and reduces lipid peroxidation. Chitosan can elevate the activity of key antioxidative enzymes, counting superoxide dismutase (SOD), catalase (CAT), and phospholipid hydroperoxides glutathione peroxidase (GSH-PX) [125]. The precise mode of action of radical scavenging by chitosan and its derivatives is still not copiously elucidated. It is assumed that non-stabilized free radicals interact with the amine and hydroxyl groups at

positions C-2, C-3, and C-6 of the six-membered cyclic glucosamine nucleus for the creation of stabilized macromolecular groups [4].

The microbial growth inhibition potency of chitosan and its derivatives has been detected against a diverse set of microbial organisms, such as fungi, viruses, and bacteria [126-128]. This microbial growth inhibition attribute, which has boosted the relevance of chitosan and its derivatives in food-products conversation and biomedical domains, is, nevertheless, handled and swayed by the AD and notch of polymerization of the biopolymer, the host, and the surrounding circumstances [127]. A crucial and key attribute for understanding the mechanism of action is the positively charged groups that characterize chitosan polymeric backbone in weakly acid aqueous solutions (pH 5.5), due to the cationization of the amine group current in the repeating units of glucosamine which facilitates its dissolution in a hydrophilic water-based environment, hence its biocidal properties **(Fig. 10)** [129-131]. Younes et al. [40] found that chitosan with 2≤ AD≤ 24% exhibits the highest bactericidal potential, particularly with AD of 2 and 12%, against Gram- strains than Gram+ strains. Chang et al. [132] examined the combined effects of chitosan molecular weight, reaction temperature, and pH on bacterial growth. The authors found that the pH of the chitosan solution could explain the relationship between bacterial growth inhibition potential and the MW of chitosan. Under acidic pH conditions, chitosan antibacterial potential augmented with the increase of MW, while at neutral pH, bacterial growth inhibition levels boosted with the decline of MW. At pH in the range of 5.0-6.0, chitosan displayed better dissolution rates in aqueous media along with a diminution in the zeta charge with the MW, whilst at neutral pH, the dissolution and zeta charge diminished with higher MW, most probably explaining the reduction of the resulting chitosan abilities in inhibiting bacterial growth at neutral pH.

In recent decades, several studies have explored and projected the potential usage of chitosan as an additive to preclude the assimilation of consumed fats, thereby monitoring body

mass [133]. Indeed, chitosan is reported to be able to dissolve in the acidic environment of the abdomen through the formation of a homogenous mixture with oil. Then, with the augmentation of the pH of the duodenum, chitosan sediments, and the apprehended oils became unable to be assimilated by crossing the intestine epithelium [134,135]. Egan et al. [136] reported that chitosan exhibited anti-obesity activity in livestock, owing to its capability of adjusting feeding comportment and regulating hungriness. Notwithstanding numerous existing studies, the accurate behavior patterns of chitosan are still a major debate [137]. A plethora of research works have further shown that chitosan displayed the potential to curtail blood fat concentration in animals and humans [138]. Park et al. [139] disclosed that chitosan is effective in reducing total cholesterol (TC) and low-density cholesterol (LDL) levels and increasing the amount of high-density cholesterol (HDL) in rats. Rizzo et al. [140] described noteworthy advantageous actions of chitosan on fats and plasma lipoproteins, where TC levels were slashed by around 9% and triglycerides by around 20%. In this research work, none of the 28 patients with hypercholesterolemia received any other treatment with lipid regulators.

More recently, the efficacy of chitosan and its derivatives in inhibiting bacterial biofilm formation and removing preformed biofilms has been studied for the first time [48]. Blue crab chitosan was found to be more effective in removing preformed films from all bacterial strains tested, with the lowest ED50 values (concentration halving microbial adhesion) and the highest adhesion inhibition values. The efficacy of blue crab chitosan in post-treatment can be a result of its dissemination and assimilation at the juncture between the solid surface and adhering bacterial organisms establishing a biofilm, thus promoting the removal of bacterial biofilms.

4. Expanded Horizons in Chitosan Biomedical and Pharmaceutical Applications

Considering the keywords chitin and/or chitosan, the significant and irrefutable position of these polymeric materials is obvious in the worldwide scientific research field and market, as reported in the scientific literature volume market reports, and commercial products [141],

with USD 10.88 billion of chitosan market part in 2022 and 20.1 % of compound annual growth rate for the period of 2023-2030 [68]. Waste-water treatment, pharmacy, cosmetics, medicine-related, food and beverage, and agriculture, among others, are the most demanding fields [142], implying the potential utilization and relevance of chitin, chitosan, and their polymeric derivatives in practically the bulk most crucial worldwide economy areas. Chitosan is currently highly sought after for its potential application in the medicine-related domain **(Fig. 9)**. Investigations in this sector have flourished rapidly and remain a speedily evolving scope, describing the journey of chitin and chitosan from biomaterials to advanced bio-functional materials.

4.1. Application in Drug and Active Ingredients Delivery and Controlled Release

Currently, drug administration and controlled release is a very interesting topic. Targeted drug delivery aims to deliver pharmaceuticals to the patient to improve the concentration of the drug at certain sites compared to others, and to cause extensive, localized, targeted, and protected interactions with the diseased tissue(s) [143-145]. Drug release has been recognized to occur from delivery systems through different mechanisms, including diffusion, swelling, erosion, and stimuli-based pathways [146,147].

Polymers, such as chitosan, have been broadly applied to effectively develop pharmaceuticals and active ingredient deliverance structures due to the ability of the polymer matrix to control the drug release rate from these systems. Researchers in the domain of gene delivery techniques are widely using biopolymers-based materials as promising non-viral vectors [23,56].

Due to its ability to be metabolized by certain human enzymes, particularly lysozymes, hence its biodegradability, chitosan is considered efficacious for the establishment of pharmaceuticals and active ingredient deliverance matrices. In this context, it is worth mentioning that for such kind of assignments, it seems paramount that chitosan be water-soluble

with a positively charged feature to be capable of reacting with biological molecules or polyanions with opposite charges in a watery-based hydrophilic medium [148]. In fact, among the most beneficial properties for drug transport, chitosan with a net positive charge is efficient in interacting with mucins and opening fitted intersections between epithelium cells. Thus, deliverance structures and matrices built using chitosan have revealed prodigious wherewithal potentialities to carry anti-cancer, antibacterial, antifungal, anti-inflammatory, vaccine, nucleic acids, peptides and therapeutic proteins, DNA, and genes, among others [44,149].

It is widely recognized that hydrogels, biodegradable delivery systems, polyelectrolyte complexes, and drug conjugates are the primary transport platforms for drug delivery using chitosan derivatives [137]. Chitosan-based hydrogels have been reported to exhibit significant benefits for the establishment of pharmaceuticals and active ingredient deliverance by allowing for a particular site and/or monitored administration in the time of small or large drugs. They similarly offer many benefits, such as improved biosafety and medicament effectiveness. Chitosan-based hydrogels can enable activity/site-specific deliverance and heightened steadiness of pharmaceutical compounds against chemical/enzymatic decomposition [150]. Therefore, chitosan-derived hydrogels have been studied for the effective deliverance and liberation of proteins/peptides, growth hormones, anti-inflammatories, and antimicrobial therapeutics, besides nucleic acids in gene therapy [151-152]. It has been pointed out that compared to other polymeric materials, chitosan can display a gel-like comportment thanks to its 3D assembly that can captivate and hold large volumes of water, permitting its swelling with no need to dissolve entirely, thus retaining its 3D structure [23].

Chitosan hydrogels loaded with osteogenesis promoter proteins have been shown to be efficient in enhancing the restoration of cartilage damage [13,153] and increase the production of chondroitin sulfate to enhance cartilage formation *in vivo* [154]. In addition, parenteral and mucosal administration of antigen vaccines was performed using chitosan-based

514 micro/nanogels [155,156]. More recently, insulin-laden chitosan nanogels have been able to
515 improve nasal absorption [157] and have provided insulin activity without painful injections.
516 In another report [158], paclitaxel was effectively administered using chitosan nano-gels grafted
517 with salicylic acid. Endothelial growth factors with a short therapeutic half-life necessitate
518 recurrent delivery and management to uphold constructive and proficient quantity, but chitosan-
519 albumin hydrogels have been described to promote the deliverance of growth factor from
520 endothelial cells for more than 3 weeks after subcutaneous implantation *in vivo* in rats with an
521 increase in vascularization [159].

522 On the other hand, Tan et al. [160] synthesized chitosan carboxymethyl glycol β-
523 cyclodextrin to deliver different hydrophobic anticancer drugs (5-fluorouracil, doxorubicin, and
524 vinblastine). The obtained results showed that the three hydrophobic anticancer agents can be
525 successfully loaded and covalently bound in the cavities of carboxymethyl chitosan dextrins.
526 The resulting system has revealed a promising potential for the efficient administration of anti-
527 cancer therapeutics in tumor therapy. The capability of environmentally friendly nanocapsules
528 built by using chitosan has likewise been expansively examined and considered for the release
529 of diverse compounds, such as anti-cancer drugs [161-163]. In this context, Liu et al. [164]
530 manufactured hollow chitosan nano-bead-like particles by solid-liquid phase separation with
531 particle size between 500 and 1000 nm and openings between 300 and 500 nm. These
532 nanospheres were characterized and evaluated for the delivery of curcumin, a natural anti-
533 cancer drug, and showed a maximum curcumin loading capacity of 63.9%.

534 **4.2. Application in the Preparation of Wound Healing Dressings**

535 Wound healing is based on repairing the integrity of the injured tissue by preventing
536 dysregulated homeostasis. An ideal dressing should preserve moisture at the wound interface,
537 permit gas interchange, behave as a hindrance against pathogens, and eliminate additional
538 exudations. The dressing is further required to guarantee the dissolution of growth factors

539 and/or microbicide therapeutics and sustain fibroblast proliferation and differentiation, be non-
540 allergenic, non-adherent, non-toxic, and disposed of without trauma [155,165]. The minor
541 behavior to adhere to the wound surface and the aptitude to allow oxygen interchange further
542 promote curative/restorative effects.

543 Chitosan-based skin tissue restoration bandages have unique kind of features and
544 attributes, including hemostasis, biodegradability, and antibacterial properties [4]. Chitosan
545 exhibits bacterial growth inhibition activity heretofore detected at reduced quantities against
546 various pathogenic microorganisms and can be employed in numerous kinds of formulations
547 including gels, films, or nanoparticles, hence its wide use in the medicine and animal care-
548 related fields as a promoter of skin tissue restoration [2,145].

549 Chitosan has been proven to be engaged in all phases of skin tissue restoration, causing
550 few adverse effects with little or no fibrous encapsulation and providing protection against
551 bacterial infections [166]. Chitosan is additionally capable of accelerating skin tissue
552 restoration when applied as powders, nanoparticles and microparticles, granules, sponges, or in
553 the form of complexes with additional constituents [167-169]. During the initial phases of
554 wound healing, chitosan promotes the infiltration and migration of neutrophils and
555 macrophages, subsequently, the cleaning of wounds of foreign agents and the formation of
556 granulation tissue, allowing the construction and remodeling of fibrous tissues. For such an
557 eventuality of hypertrophic scar establishment, triggered by disproportionate collagenous
558 structures formation during the metamorphosis segment, chitosan is capable of reducing scar
559 tissue, thus permitting worthy tissue reconstruction and restoration [170]. Chitosan has been
560 correspondingly reported to regulate the synthesis of drivers of growth involved in the skin
561 tissue restoration process and collagen production at the beginning of the after-wound stage
562 (3rd day), thus smoothing skin characteristic patterns regeneration. At the end of the after-

563 wound segment (7th day), chitosan permitted the shrinkage of the synthesis of growth factors,
564 thus promoting scar formation [171,172].

565 Chitosan can be suitably applied as a viscous liquid that is being gelled when applied to
566 the wound surface. To provoke gel formation *in situ*, circumstances and factors counting ion
567 load, acidity/alkalinity, and heating must be adjusted and controlled [3]. In addition, chitosan
568 hydrogels are described as being able to act as matrices for the topical deliverance of proteolytic
569 compounds such as growth promoters, which can help guide skin tissue regeneration
570 responsiveness. The characteristically rapid release of growth activators (within some minutes)
571 is delayed by ionic or hydrophobic physical interactions of the proteolytic groups with the
572 hydrogel matrix [173,174].

573 Alemdaroglu et al. [175] established a chitosan-based hydrogel for the controlled
574 deliverance of EGF (epidermal growth factor) during 2nd-degree burn-based wound therapy in
575 rats. EGF was released entirely from the gel in less than 24 hours and led to quicker skin tissue
576 regeneration times in comparison with injuries managed with chitosan-based hydrogel without
577 the addition of EGF. In another study [176], for the mimicking of the extracellular matrix
578 (ECM) of an injury location, the effect of PDGF (platelet-derived growth factor) formulation
579 in a collagen chitosan hydrogel-based matricidal composite on an ablation-based injury during
580 the wound healing process was evaluated. The degree of wound shrinkage and the quantities of
581 growth activators in the collagenous proliferative fibroblasts in mice were determined. In *vivo,*
582 outcomes disclosed that the addition of PDFG permits amplified relocation and dissemination
583 of fibroblasts. More recently, composite sponge-like wound healing bandages built by using
584 chitosan glutamate and sodium hyaluronate have been explored for the administration of
585 platelet lysate, which is a proficient origin of various growth modulators needed for skin tissue
586 regeneration, in chronic wounds. *In vitro* studies against human fibroblasts have revealed that
587 this formulation can accelerate cell proliferation despite its fragility upon elongation greater

than 30-40% of its original length, supporting the fact that chitosan-based gel-like materials behave as a protecting injury microenvironment with advantageous effects on skin wound remodeling therapy [177]. In another study, Hamdi et al. [178] developed a blue crab-derived chitosan and protein isolate 15% (w/w chitosan) composite hydrogel, for carotenoids-controlled delivery and *in vivo* wound healing. The resulting miscellaneous hydrogels portrayed a very high biocompatibility performance towards MG-63 osteosarcoma cells. *In vitro*, the charged carotenoids' release patterns showed that the synthetized gel-forming materials can be utilized as pH-sensitive smart vehicles, for drug-regulated deliverance and liberation, with stimulating antioxidative aptitudes. Moreover, topical application of chitosan composite hydrogel-based patches in a rat model permitted the quickening of the skin tissue remodeling cascades and ultimately the whole curative, for composite hydrogel supplemented with carotenoproteins extracts **(Fig. 11)**.

Chitosan can be suitably applied to wounds as dried sponges, films, or powders that exhibit the ability to moisturize speedily upon absorbing exudate to create a chitosan gel-like material on the wound surface [179]. Hydrogels are suitable architectured biomaterials for skin tissue regeneration owing to their simplicity of deliverance and liberation processes, preservation, moisture-holding capacity, and oxygen penetrability [166]. As a result, chitosan and its derivatives, such as carboxymethyl chitosan, can be used as exceptional materials for wound healing applications [180].

4.3. Application in the Sector of Tissue Engineering

Tissue engineering is a burgeoning field of scientific investigations whose ultimate target is indeed to repair/regenerate/replace tissues and organs in the body that have been damaged far beyond their function. Tissue engineering is partitioned into numerous sections according to the targeted tissue/organ nature [1,44]. Tissue engineering is becoming a momentous domain of polymeric-based materials investigations since the requirement for a completely viable and

healthy network has been rapidly substituting traditional replenishment therapeutic strategies, showing some shortcomings in the utilization of non-degradable and non-biocompatible artificial-based scaffolds, etc. The current peer group of tissue engineering focuses on transplanting cells inside spongy bio-decomposable polymeric matrices. Indeed, the foremost apprehension involved the availableness and accessibility of bio-decomposable matters that can be used as temporary matrices [181].

Of late, chitosan and its derivatives have been conveyed as outstanding competitive alternatives for tissue-engineered scaffolds, thanks to their ability to degrade as tissues are built with minimal immune system response or noxious decomposition products. In addition, the positively charged attribute of chitosan is contributive to the creation of non-covalent chemical bonding with anionic glycosaminoglycans, proteoglycans, and other products with opposite charges [8]. Otherwise, chitosan and its polymeric subsidiary products have been broadly utilized as effective alternatives in bone tissue engineering for the promotion of cell proliferation cascades and mineralized matrices accumulation by cultured osteoblast cells. Animal model data demonstrated that such designs allowed a controlled delivery of the methotrexate after the intra-articular injection of injectable hydrogel into mice's arthritic joints **(Fig. 12)**, ultimately promoting a respite of the inflammation for an effective treatment of the rheumatoid arthritis [13].

In this context, the feasibility of hydroxyapatite-sheathed carboxymethyl chitosan platforms for the simulation of bone remodeling has been demonstrated by experimentation [182]. In more recent work, Serra et al. [183] manufactured bio-resorbable implants by lyophilization employing diverse preparations based on chitosan, gelatin, and tricalcium β-phosphate (β-TCP) for bone tissue regeneration. Mechanical improvement was observed with chitosan scaffolds combined with gelatin and β-TCP, up to 70% compared to pure chitosan. The prepared 3D scaffolds were bioactive and biocompatible, allowing excellent cell adhesion

with better effectiveness in the stimulation of osteoblast cellular activity hence internalization and distinctive osteoblastic morphologic features. Thus, these three-dimensional chitosan-based structures have shown great potential in bone regeneration, promoting cell adhesion and proliferation, further preventing the formation of biofilm on their surface, the foremost origin of implanted scaffold failure. Numerous studies on the creation of implantable scaffold-based bone substitutes exploiting chitosan and its polymeric subsidiary products have been described in the literature and excellent results are available [184-186].

4.4. Application in Bioimaging

Bioimaging is based on the real-time, non-invasive visualization of biological processes often in a three-dimensional (3D) structure using various imaging sources such as light, fluorescence, electrons, X-rays, ultrasound, positrons, and magnetic resonance [2,50,145].

Chitosan is a nature-derived amino-polysaccharide that occurs with interesting physicochemical and biofunctional features. The amine and hydroxyl functional sites existing on the chitosan backbone make available a pathway for reactions with functional biological compounds [24]. In addition, chitosan is extensively employed as a bioactive organic matrix material in the sector of bioimaging due to its exceptional biodegradability and minimal toxicity [187-189]. The possibility of introducing imaging agents into the chitosan matrix has favored its use for bioimaging. Lee et al. [190] reported that the incorporation of an imaging agent, Fe_3O_4, plays an effective contribution in scanning with a nuclear magnetic resonance field and that the prefabricated self-arranged nanobeads improve the revealing and recognition of specified depiction on hepatocytes. Chitosan-reduced gold nanoparticles have been used as a photothermal converter and photodynamic support in photodynamic therapeutic strategies, which have a role in the application of bioimaging and are used to destroy breast cancer cells [191]. Moreover, gold-coated Fe_3O_4 nanoparticles with a typical diameter of 9.8 nm were further synthesized by chemical glucose-reducing reactions by chemical coprecipitation and

663 balanced with chitosan amide the assembly with formaldehyde as a cross-linking reagent. The
664 developed nanomaterial has been reported as a potential candidate material for biodetection and
665 bioimaging applications [192].

666 **4.5. Application in Enzyme Immobilization**

667 For biobased economy and biotechnology implementation and relevance of enzymes,
668 immobilized enzymes are more beneficial and gainful over unfettered enzymes, facilitating
669 their recycling (reusability), improving enzyme stability, and allowing continuous production
670 with no biocatalysts in the product stream [81,193,194]. Analytically, immobilized enzymes
671 are primarily used in biosensors (sensors used for biological systems) and in diagnostic test
672 strips [195]. Biosensors have been built employing the integration of biomaterials-based
673 sensing platforms such as enzymes with transducers, which convert the reaction into a
674 measurable response. Otherwise, biosensors have been recognized to be based on biological
675 materials that can recognize explicit and precise biochemical fragments and indicate their
676 occurrence, and quantities, alongside their functionality through biochemical ways. The
677 foremost favors and conveniences of biomaterials-based sensors are transportability, great
678 specificity, flexibility, fundamental discrimination, and ease of practice in multifaceted
679 situations owing to their rapid responsiveness. Within this framework, most research focuses
680 on nanomaterials for the reason that they are endowed with unique electric, photosensitive, and
681 catalysis attributes [196].

682 Bio-decomposable polymeric materials either synthesized or recovered from nature-
683 based resources like chitosan and its polymeric subsidiary products have been considered
684 among the supreme appropriate platforms to immobilize enzymes due to their non-toxicity, bio-
685 safety, and large specific reactive sites and locations for charging a greater quantity of enzymes,
686 due to their NH_2 and OH reactive units that are capable of interreacting with dynamic
687 components [44,197]. Such fascinating characteristics make chitosan the furthermost widely

used organic-based polymeric material to manufacture advanced hybrid materials for biosensors' engineering and industrialization [198]. Thus, chitosan-catechol films are used as detectors for active bacterial metabolites with redox activity [199]. More recently, Han et al. [200] designed an innovative tyrosinase immobilization biosensor by using chitosan nanocomposites for the recognition and distinction of phenolics. Similarly, the properties of Laccase enzyme following the immobilization process on cellulose acetate/chitosan/poly(ethylene oxide) electrospun nanofiber were explored **(Fig. 13),** and results revealed that the immobilization of Laccase on the cellulose acetate/chitosan/poly(ethylene oxide) nanofibers with fine diameters boosted the loading of the enzyme, suggesting its potential as an ideal candidate for industrial application instead of free enzymes [198]**.** The developed nanocomposite films permitted considerably satisfactory transmissivity and bio-safe surrounding conditions for the immobilized enzyme with a high affinity for substrates. In addition, a chitosan-carbon nanotube system was used for electrochemistry-based detection by using dehydrogenase enzymes [201]. Furthermore, one-use biomaterials-based sensors employing enzyme-immobilized indium tin oxide electrodes modified by gold and chitosan have been developed, with amperometric analysis by flow injection [202]. The use of chitosan-based polymeric platforms to immobilize enzymes for bio-sensitivity has been further conveyed [203].

4.6. Applications in the Cosmetics Sector

Currently, in the cosmetics industry, the integration of natural substances in both products and formulations is considered, given the increasingly demanding regulations concerning public health and the environment, in addition to consumers' awareness. Among nature-derived microbial growth-inhibiting compounds of aquatic derivation, chitosan has been broadly utilized in cosmetic products [204,205]. In this context, chitosan has been proposed as an antimicrobial polymeric matrix with hydroxyapatite ceramics in a multifunctional sunscreen

713 [206]. The prepared gel exhibits optical absorption of ultraviolet light at 254 nm, and
714 antibacterial activity against *S. aureus*, *Klebsiella pneumoniae*, and *P. aeruginosa*, in addition
715 to a significant effect on the growth of multidrug-resistant bacteria. In another study, Wongkom
716 and Jimtaisong [207] prepared carboxymethyl cellulose-based biocomposites from the peels of
717 *Ananas comosus* and carboxymethyl chitosan cross-linked with ferulic acid, for use as novel
718 matrices for hydrophilic sunscreens, following concerns about the effect of ultraviolet radiation
719 on skin cancers, sunburn and photo-aging. Hydrophilic TiO_2 and phenylbenzimidazole sulfonic
720 acid have been applied as sunblock and protecting agents at a proportion of 2:1 (w/w). The
721 prepared biocomposites have proven their ability as good matrices for anti-UV agents whose
722 concentration can be modified to fix the required protection factor.

723 Chitosan was further operated for the encapsulation and stabilization of bio-functional
724 constituents in beauty and personal care commodities. Indeed, uniform and well-balanced
725 microcapsules involving a fluid aquaphobic nucleus containing linoleic acid, encircled by an
726 envelope of chitosan and lactoionic acid, by the simple association in water, were prepared, and
727 their ability to encapsulate phenyl ethyl resorcinol, a hydrophilic skin brightening or
728 depigmenting agent, was evaluated. The prepared microparticles were revealed as suitable for
729 encapsulating phenylethyl resorcinol as a skin lightener [208]. Otherwise, Libio et al. [209]
730 prepared chitosan films with or without glycerol to determine the composition best suited to
731 physical integrity and skin biocompatibility for makeup removal applications. After evaluation
732 of their physicochemical properties, glycerol-free chitosan films were selected to perform the
733 release experiments using a pigskin model. Although hyaluronic acid interacts with chitosan
734 reducing the moisturizing effect of the film, a noteworthy intensification in the degree of
735 hydration of the skin was distinguished.

736 Recently, some studies on the encapsulation of fragrance compounds in chitosan-based
737 nanoparticles have been established and reported to be capable of avoiding the loss of highly

volatile fragrance ingredients. Xiao et al. [210] developed a tuberose fragrance compound encapsulated in chitosan nanoparticles for cosmetic applications. The microbial growth inhibition activity of nanoparticles against *S. aureus*, *E. coli*, and *Bacillus subtilis* has been explored, in addition to their sustained-release property of nanoparticles, indicating a promising application of these nanoparticles as controlled-release vectors, not only for fragrance but also for antimicrobials [11,211].

5. Conclusions and Perspectives

Chitosan is a naturally occurring, bio-decomposable, bio-safe, biologically compatible hydrophilic polymeric material, derived from chitin, which is among Earth's most abundant and sustainable natural materials. Chitosan and its derivatives propound very interesting innovative matters and platforms with a broad spectrum of encouraging biotechnological applicability and relevance. In recent decades, the marketing of chitosan as a nutritional additive product has enlarged primarily due to its advantageous antioxidative potential, anti-lipidemic action, and ability to enable body mass loss. Overall, such kind of bio-functionalities have prompted the utilization and implementation of chitosan-based materials for the anticipation and handling of long-lasting sicknesses. There are significant obstacles to the commercial exploitation of chitosan, as it is difficult to prepare uniformly reproducible chitosan products in large quantities from a variety of aquatic-based resources all over the world. The adaptation and modification of chitosan additionally complement the global net value and conceivable distinctions in the homogeneousness of the characteristics. Although a range of specimens of chitosan subsidiary polymeric products are being evaluated for uses in pharmacy and medicine-related fields, just a limited number of them, counting carboxymethyl chitosan, trimethyl chitosan, and PEGylated chitosan, have attained a deep-documented and adequately acknowledged implementation profile. Overall, there is still tremendous research work to be

762 performed and completed for the entire exploitation of the interest and convenience of chitosan
763 and its derivatives in pharmacy and medicine-related domains.

Acknowledgments

The funding of this research by the Qingdao University of Science and Technology through a postdoctoral project, Qingdao City through a 2024 postdoctoral funds-3 project grant, and the Science and technology department of Qingdao Municipality (project number: 24-1-4-xxgg-11-nsh) is extremely acknowledged.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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