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Chitin-Chitosan

Myriad Functionalities in Science and Technology

Edited by Rajendra Sukhadeorao Dongre

CHITIN-CHITOSAN - MYRIAD FUNCTIONALITIES IN SCIENCE AND TECHNOLOGY

Edited by **Rajendra Sukhadeorao Dongre**

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Notice

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Meet the editor

Rajendra S. Dongre, born in Nagpur, India in 1974, completed his MSc degree with Gold Medal from Nagpur University in 1996 and PhD degree in 2010. He was a scientist in CSIR, National Environmental Engineering Research Institute (NEERI), Nagpur, from 2000 to 2003. He became an Assistant Professor in 2003, at Chemistry Department, Nagpur University. His research includes chitin-chitosan biocomposite synthesis and characterization and remediation of toxic-hazardous pollutants like fluoride, nitrate and Pb (II). He has expertise in advance water treatment technique developments besides wastewater analysis. He received the sixth national award, for technology innovation in petrochemical-downstream plastic process industry in polymer science and technology, by petrochemical and fertilizer ministry, Government of India. He was honoured with the fifth national science and technology award, by EET-CRS, India, for his contribution to science. He is an editorial board member of 5 international journals and has published 46 research publications. Four researchers were awarded their PhD degree under his guidance.

Contents

Foreword 1

The modern technical and industrial prosperities besides unprecedented progress in contemporary natural sciences are due to enormous contributions by synthetically developed advanced novel materials. Biopolymers appear to have endurance to our existence, the environment and eventually life; thus scientists explored and utilized inherent inventive matrixes of distinguished natural polymers, viz., agar, algin, carrageenan, glycogen, pectin and chitin for attainment of myriad applications in embryonic science and technology.

Chitin is *N*-acetyl glucosamine polymer functioning as structural unit and provides strengths to most of the invertebrates, e.g., crab, lobster, snail, sea urchins, yeast, bacteria and fungi. In contrast to other polysaccharides, chitin has an average molecular weight of (1 to 2.5) × 106 Da and enriched 7 % of nitrogen aid which makes it an effective ingredient for pharmaceutical, clinical, paper, textile and photography usages. Natively, chitin is insoluble in water and a common organic solvent, while chitosan is soluble in aqueous organic acid, it is seldom applied.

Way back in 1811, a French scientist, Henri Braconnot, isolated chitin from mushrooms. The name "chitin" was coined in the 1830s when it was isolated from pests. Lassaigne in 1831 wrote an article on chitin stating that it was derived from insects and plant sources. In 1843, Lassaigne evaluated the presence of nitrogen in chitin, and Ledderhose in 1878 reported the combination of glucosamine and acetic acid unit frameworks. Later in 1859, Prof C. Rouget discovered deacetylated form of chitin called chitosan (Kite-O-San) as named by Hoppe-Seyler in 1894. In 1930, Rammelberg's study found that hydrolysing chitin in different conditions and depending on degree of deacetylation yield various chitosan confirmers. In 1950, x-ray analysis confirmed chitin's presence as a structural component in fungi. In 1951, Henri Braconnot had published the first book on chitosan; afterwards, the global scientists focussed on chitosan to exploit its vast applications and it resulted in more than 2000 patents till date. From 1920 onwards, researchers and industry derived chitin from natural sources, viz., arthropod/crustacean exoskeleton, insects and fungi.

The book entitled *Chitin-Chitosan - Myriad Functionalities in Science and Technology* has ably identified certain crucial research issues and scientific problems besides compiling some case studies on chitin-chitosan in order to tender viable solutions with ascertain credibility and screening benefits. I hope this book boosts its reader's zest in chitin-chitosan chemistry.

I congratulate the editor for his first international venture and also offer best wish to all the contributing authors for endeavours to make this a fine reference book for chitosan chemistry.

Prof. L. J. Paliwal and Prof. (Mrs.) J. S. Meshram
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Foreword 2

Natural polymers are the energy source for the procurement of assorted products/materials usually utilized in sustainable growth of life sciences. Out of the various alternatives, polysaccharides like cellulose and chitin are the top most abundant biopolymers that have received commercial interests in the past few decades. Large number of academic and industrial researchers are working in new potential applications of these materials due to very specific properties and unique capabilities for various applications.

The present book comprehensively summarizes the most recent technical and research accomplishments in chitosan chemistry covering various areas such as processing, permeation methods, characterizations and physicochemical modifications, etc. The contents and the compiled data highlight the challenging role and contributions of chitin-chitosan in advancement of science and technology. Chitosan-based material characterization is very properly included and discussed in this book. The authors have properly discussed the very specific applications of chitosan in textile industry, agricultural systems, food processing, pharmacological applications, etc.

This book also contributes to critical and recent R&D literature on various areas of chitin-chitosan for the new hybrid composite applications in advanced materials. I am sure this book and authors' own contribution in this area will certainly provide the best compilation of all data of chitosan in one place. I hope that this book edited by Dr. Rajendra S. Dongre will be able to highlight the emerging chitosan technology to better equip us to address the challenges of tomorrow.

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Preface

Chitin is β-(1-4)-N-acetyl-d-glucosamine polysaccharide and is the second most abundant biopolymer after cellulose mainly obtained from crustacean shell being structural components as ordered crystalline microfibril/whisker in the exoskeleton of arthropods and fungi/yeast cell walls. Chitin was first discovered in 1859, while partial N-deacetylated chitin under alkaline/deacetylase enzyme hydrolysis yields chitosan. In industrial and technological viewpoints, naturally resourceful chitin-chitosan is an optional low-cost and renewable biomaterial. Raw chitin-chitosan is semi-crystalline owing to heterogeneous laminar acetyl chains and resembles keratin protein in its biological functions. In the beginning of 19th century, chitin interest was developed due to inherent/exceptional biological properties, viz., solubility, polycationic, biodegradation, biocompatibility, bioadhesivity and immunological, antibacterial and wound-healing features that especially support gene/drug release, cell culture and tissue engineering. Nevertheless, chitosan studies amplified drastically in the past two decades accordingly; several frontiers surmount to open new research fields.

Chitosan is facile for assorted chemical/physical modifications at free reactive groups, without changing bulk properties achieved through various processing like film, membrane, hollow fibre, composite, hybrid, nanofibre, nanoparticle, hydrogel and scaffold. Recent global R&D is focused on novel functional material development from chitosan matrix in order to cater wide utility/demands in many fields including medicine, pharmaceutics, nanotechnology and biotechnology.

Renewable eco-friendly characteristics of chitin-chitosan polysaccharide are the driving force for embryonic novel and myriad applications in S&T. The academia and scientists have faced a great challenge to explore innovative practical functions of chitin-chitosan. Current rapidly lessening resource/supply, consequently augmented awareness for copious optional bioresource, brings chitin/chitosan progressively in the domain of fundamental and applied research. Economy and versatility are key factors that stimulated scientists' curiosity in chitin-chitosan in countless fields, viz., fertilizer to pharmaceutical. Now, chitin is not just seafood processing wastes but a major feedstock vastly exploited by biotechnology to resolve/face many threshold problems and budding challenges besides acquiring better existing products or creating new stuffs owing to interest in chemistry, material science, microbiology, food biopharmaceutical, bioengineering, biochemistry, bioprocessing and environment sector.

This book signifies chitin-chitosan in all the above perspectives and contents that encompass few reports and latest R&D owing to fundamental perceptions gained from foremost global scientists, academia and industry professionals. The book provides assessment of up-to-date potential chitin-chitosan-derived material, which specifically displays comprehensive fundamental techniques and technologies as spotlighted in developing assorted composites im-

parting various biomedical/clinical utilities. Readership enfolds scientists, engineers, postgraduates and academicians working in nanotechnology, biomaterials, biomedicine, therapeutics, tissue engineering and regenerative medicines. The book is structured with prospective innovations in chitin-chitosan matrix perceiving comprehensive technical and scientific efficacy as vital for growth of modern science and technology.

Editor's introductory chapter portrays unequivocal multitask portfolio of chitin-chitosan matrix, all the way from stoichiometric adsorbent: for exchange of solute from bulk to indicator biomarker/biosensor and for detection of biological/physicochemical state, to the end use as tiny quantum dot, a central nanotechnology theme. The explicit functionality of chitin-chitosan framework is depicted thoroughly.

Other chapters cover investigative current and systematic progressive research on the aspects of chitin-chitosan chemistry including definition, history, resource, techniques of synthesis, characterization, extraction, processing and fabrication practices for productive materials own myriad applications, viz., scaffolds for the skin cartilage, bone, liver, nerve, blood vessel, orthopaedic/bones and nanofibre/particle/capsule/film pervaporation membrane, microfluidic device, bioimaging, drug/gene delivery and agricultural, chemical, environment and engineering sectors. Cited work may inspire academia and researchers to implement a few existing and to develop futuristic chitin-chitosan-based techniques and technologies.

In a nut shell, the book has explicated chitin-chitosan advantages over many such polysaccharides and other significant biopolymers/molecules available in almighty nature. I am warmly grateful to all the contributory authors for providing their informative and elucidative chitin-chitosan studies, which may boost budding innovations and future attempts.

Lastly, I owe to *InTechOpen Publisher* staff members for constant endurance and support.

I presume that the book enhances readers' understanding and maintains scientific awareness in chitin-chitosan chemistry owing to unequivocal accumulated research profile with extensive utility all the way from quantum dot to biosensor/biomarker, which may tender noteworthy impression for its myriad functions that ably improve existing techniques, functioning technologies in growth of modern S&T and ultimately our life.

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Last but not the least, I'm deeply grateful to my loving parents, dear wife and daughter *Isha and son Om* for sustained emotional backing during span of the book drafting work. The book is d**edicated to** *Sir Stephen William Hawking*, **ex-**physicist, cosmologist and Director of Research at Centre for Theoretical Cosmology, University of Cambridge.

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Introduction

Chapter 1

Introductory Chapter: Multitask Portfolio of Chitin/Chitosan: Biomatrix to Quantum Dot

Rajendra Sukhadeorao Dongre

Additional information is available at the end of the chapter

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1. Introduction

Rational designing of novel materials has addressed diverse technical needs and industrial problems in addition to their own sizeable share in the prosperity of modern sciences. Biomolecule/polymer yields from natural sources like fungi, bacteria, animals and plants is fascinating due to the innate endurance of the environment and life [1]. Hence, scientists have searched many natural polysaccharides and biopolymers, including agar, algin, carrageenan, glycogen, glycan, pectin and chitin, because of their embryonic/innovative potential to cater for futuristic demands in science and technology (S&T) [1, 2].

In this regard, natural polymers/biopolymers and biomaterials can provide all such aspiring demands for current advances in scientific development. Hence, the scope of this book is targeted precisely, with a concise introductory chapter on the multitask portfolio of chitin/chitosan from biomatrix to quantum dot. The uniqueness of this book is prevalent in all the chapters, which ensure the utmost coherence and relatedness to vital issues allied to the material chemistry of chitin/chitosan, in addition to offering a true analysis of the challenges currently faced by the scientific community in material sciences. The book provides a motivating theoretical and diagnostic R&D framework, and readers will be able to easily understand its contents as well as examine its research claims. In fact, the outcome of this book will hopefully manifest the unequivocal multitask portfolio of chitin/chitosan, ranging from biomatrix to quantum dot.

2. Chitin/chitosan biopolymer: multitask portfolio from biomatrix to quantum dot

Chitin is the second most copious biomolecule after cellulose and is found in arthropod exoskeletons like crustaceans (crabs, lobsters and shrimps), radulae of the phylum Mollusca, cephalopod beaks, lissamphibian scales, tetrapods, fish, as well as in the framework of fungi cell walls [1, 2]. Chitin is like cellulose in structure but differs at the C2 hydroxyl position and substitutes poly-(1 → 4)-β-*N*-acetyl-D-glucosamine links to yield an *N*,*N*′-diacetylchitobiose helix where each sugar unit is mutually inverted with a neighbour via 180° rotation [2]. Such structural features of chitin impart high stability/rigidity due to skeletal interconnected hydrogen bonding. Ubiquitous chitin is accumulated as a structural constituent in organisms and is prevalent in the biosphere and fossilized matter like Pogonophora and insect wings found in Cambrian fossil: amber [3]. The multitask portfolio of chitin ranges from biomatrix/biosensor to quantum dot, all of which possess vital qualities, and include antioxidants, hydrogels, adsorbents, diagnostic testing/therapy, drug delivery, coating/process film, cosmetics, tissue templates, active pharmaceutical ingredients, biomedical scaffolds like wound dressings, contact lenses, microspheres, etc. [4]. Chitin's average molecular weight of 2×10^6 Da with 7% w/w-enriched nitrogen is a vital raw material for the medical, paper/pulp, food, textile, photography and environment industries [1–8]. Chitin has α, β and γ allomorphs self-assembled via legitimated crystallization as microfibrils, as shown in **Figure 1**. Natural chitin is the α form and has antiparallel *N*,*N*′-diacetylchitobiose units [1–3], while the β and γ forms are less vital and seldom observed in nature, e.g., mushrooms.

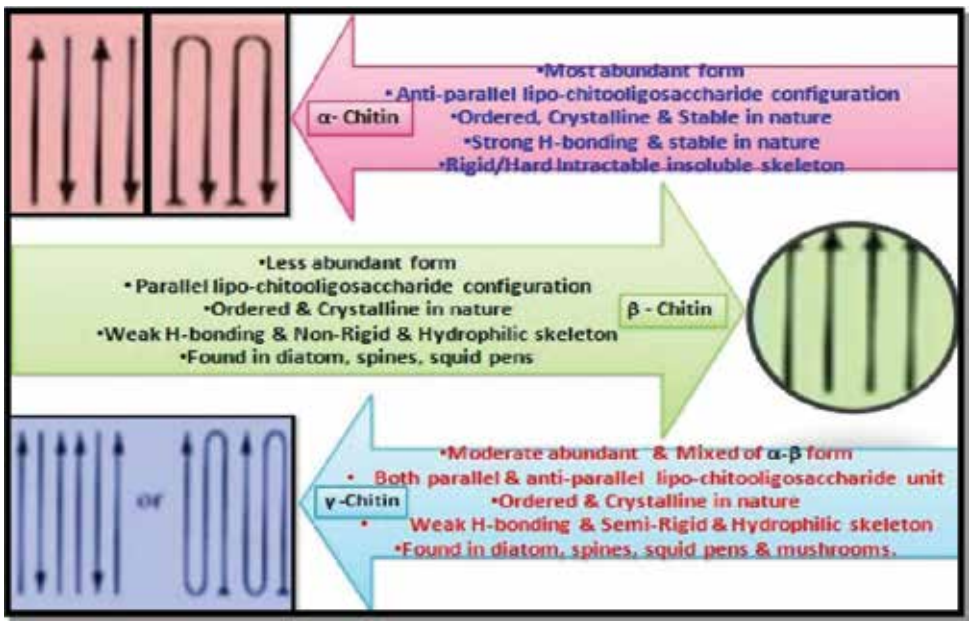

Figure 1. Self-assembled, legitimated, crystallized α, β and γ allomorphs of chitin.

Chitosan is daecetylated chitin, and there numerous papers and patents that signify its utility in biology, genetics, physics, chemistry, polymers, tissue engineering and biomedicine. Assorted physicochemical alterations in flexible chitosan offer unique matrixes like blends, hybrids, films, sheets, dendrites, composites and gels pertaining to superior prospective, unparalleled competency in modern scientific growth [1–8]. Hence, chitin/chitosan biomatrixes have been explored and summarized in unequivocal portfolio applications in S&T.

Raw chitin/chitosan is hydrophobic in nature and has limited solubility in many organic solvents, so possesses a narrow utility. Yet, both chitin and chitosan own special innate features, e.g., they are hydrophobic, polycationic, less immunogenic, highly porous, haemostatic, non-toxic, biodegradable, biocompatible, bioadhesive, antibacterial and antimicrobial [8–10]. Chitosan holds proactive $–NH_2$/–OH groups as free and fragile for assorted chemical modifications, namely, C6 carboxylation, *N*-acylation/alkylation, *N*-quaternization, protonation to NH_3^+, which further improves its pH dependency (acid to alkaline), resultant solubility and utilities [10, 11]. The chitin/chitosan market was augmented to US$2900 million in 2017; furthermore, Global Industry Analysts Inc. expects to boost that figure to US$63 billion by 2024. However, the current global need for chitin is over 6000 tonnes, while all-inclusive production is at 28,000 tonnes [1, 2].

Chitosan has free NH_2/OH proactive groups, which on protonation aids metal chelation at neutral pH, whereas anionic complexation occurs due to coagulation and flocculation of contaminants at pH > pK_a [8, 10]. Such coagulation/flocculation imparts a stoichiometric charge restabilization of particulate suspensions along with patch destabilization and bridging with dissolved solutes, resulting in remediation of hazardous/toxic metal. Nanotechnology has

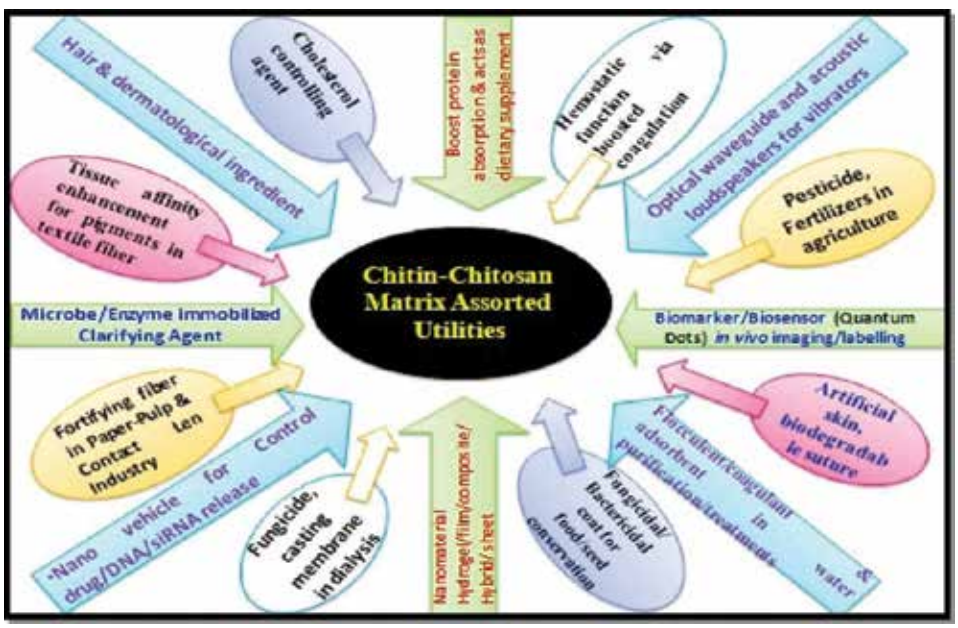

Figure 2. Assorted products and a wide range of uses of chitosan in modern science (incomplete list).

manipulated the chitosan skeleton to yield tubular templates/scaffolds that optimize regenerative relevance, including repair, replacement, maintenance and enhancement of injured cells/organs via tissue engineering [2, 10]. Such a tailored chitosan matrix has high porosity, suitable porosity/collapse aversion and unique structural integrity, which impart a multitasking portfolio ranging from biomarkers/biosensors to quantum dots [10]. Thus, chitosan chemistry offers myriad uses, namely, neo-tissue degradation/creation, cell differentiation, interactive adhesive proliferation and overall migration, as depicted in **Figure 2**.

3. Chemistry of chitin/chitosan

3.1. Biosynthesis of chitin

Several extremely complex biosynthesis steps resulting in a chitinous supramacromolecular skeleton in an arthropod cuticle and fungus cell wall are mentioned below:

- Trehalose/glucose sugar units undergo sequential biotransformation, including phosphorylation, amination and uridine diphosphate (UDP)-*N*-acetylglucosamine substrate formation.
- Enzyme chitin synthase yields a chain as part of protein/carbohydrate cluster counting via intimate topologic packing, which gives a budding chitin coalescence into crystalline fibrils.
- The chitin conformational orientated is continued till long chain polymer translocation across the plasma membrane occurs.
- Lastly, microfibril formations and crystallization are achieved via interchain hydrogen bonding, and an alliance with cuticular protein/carbohydrate yields toughness.

Chitosome enzymes in endoplasmic reticula, Golgi organelles and vesicles enclosed in zymogenic clusters that have cytoplasmic microvesicles at the hyphal tip play a crucial role in predetermined chitin trafficking [11]. Chitosome fused with a plasma membrane activates raw/crude chitosan (CS) units via a proteolytic reaction; further CS insertion engrosses intercession of targeted proteins. Chitosome in epidermal cell-free insects via UDP-*N*-acetyl-D-glucosamine: chitin4-β-*N*-acetylglucosaminyl-transferase; EC 2.4.1.16 *in vivo* yields chitin. In 1962, scientist Candy-Kilby had first proposed metabolic pathway as progressed with glucose and ended with UDP-GlcNAc unit observed in southern armyworm Spodoptera eridania (cell-free extracts), which aids to ascertain total chitin biosynthesis [7, 11, 12].

3.2. Origin and biofunctions

Chitin is a linear homopolysaccharide of *N*-acetylglucosamine, while chitosan is *N*-deacetylated chitin: both offer chemically stable *nitrogen* as a nutrient and an energy source for rumen microbe degradation, in addition to inducing molecular signals responsible for defensive stimulation in plants/animals. Lipo-chitooligosaccharide and chitin induce nitrogen fixation

and regulate gene nodulation/Nod factor because of assorted bacteria via host–guest symbiosis in leguminous plants. Chitin also boosts the anionic exchange capacity of soil, lessens nutrients like nitrate and phosphate leaching, and recovers pesticide delivery and efficacy.

Crustacea possess chitins, proteins and calcium carbonate responsible for rigid exoskeleton creation, keeping the inner soft tissue safe from injury, offering defense against predators, avoiding dryness of delicate tissues and aiding survival. Chitin in protozoa, fungi cell walls, arthropods, nematodes and pathogenic organism skeletons offers defense against the exterior atmosphere [10].

3.3. Processing

Chitin yields via seafood processing from crabs, shrimps, shellfish, krill, clams, oysters and squid contain high protein, nitrogen and calcium carbonate, which are recovered via stepwise processing, as given in the schematic representation of **Figure 3**.

3.4. Formulation/grafting

The degree of deacetylation and drastic acid hydrolysis controls the cleavage of the β-D-glucosamine unit of chitin and yields assorted deacetylated chitosan. Chitosan on skeletal formulation reduces hydrogen bonding and under aqueous conditions forms swelled films in spite of hydrophobic alkyl links, thus dissolving in aqueous mineral acids yields polyelectrolytic matrixes like salts, films, hybrids, chelates/complexes and gels. Chitosan undergoes facile chemical adaptation such as esterification/etherification, hydrogenation, amidation, mono−/di−/tri-*N*-alkylation/acylation and aldimine/ketimine, Schiff-base formation in addition to sodium borohydride reduction to 1,3-melanin/1,6-β-D-glucan keto-amino acid. Certain formulated products of chitosan are immunostimulants that boost immunity in the host [2, 10].

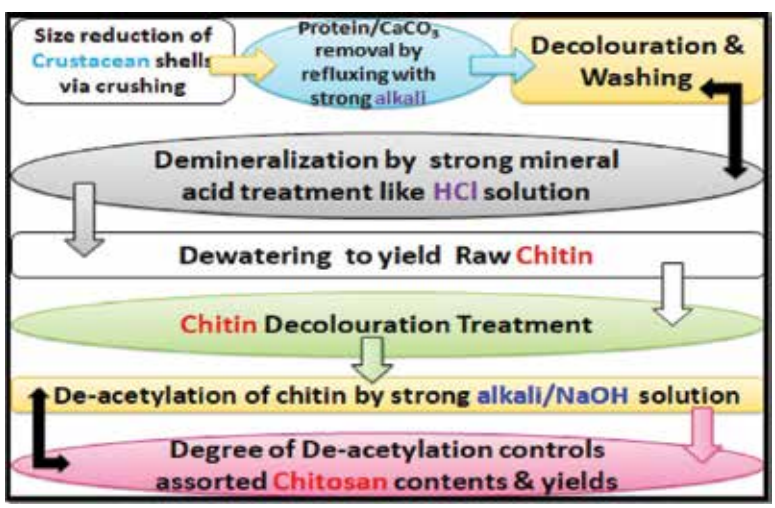

Figure 3. Schematic representation of crustacean processing to obtain chitin/chitosan.

3.5. Solubility

Chitin/chitosan contains a highly hydrophobic anhydroglucoside framework, which also has limited solubility in organic solvents. However, mixtures of organic solvents like hexafluoroisopropanol, hexafluoroacetone, chloroalcohol, 5% LiCl-dimethylacetamide and certain aqueous acetic acids, *N*-methyl morpholine-*N*-oxide and mineral acids were found to enhance the solubility of chitin/chitosan. Raw chitosan's solubility is affected by the degree of deacetylation and it absorbs moisture from the atmosphere. Chitin with 50% deacetylation is hydrophilic but a higher degree of deacetylation (>50%) is hydrophobic and immiscible in ordinary solvents, so it mostly reacts in the solid state [2].

3.6. Chemical and biological properties

Even if the β-(1 → 4)-anhydroglucosidic links of chitin are similar to cellulose, their innate nature is different. Chitin is a white, inelastic, inert, non-toxic, renewable, water-insoluble amino-polysaccharide that binds to the cell wall of phospholipids of Gram +ve bacteria and modifies cell permeability as well as inhibits certain enzymes. The high density (1.35–1.40 g/cm^3), slight basicity (pH >7) and moderate glass transition temperature (203°C) of chitosan aids selective fabrication of permeable membrane matrixes under acidic conditions. Being biocompatible with mammalian/microbial cells, chitosan assists connective gum tissue regeneration as well as accelerates osteoblasts better than other counterparts. Chitin is hemostatic, fungistatic, spermicidal, antimicrobial, antitumoral, anticholesteremic, and a CNS depressant and immunoadjuvant in nature, and is thus facile to binding mammalian/microbial cells [11]. Chitin/chitosan is a highly viscous and polyelectrolytic skeleton only soluble in aqueous solutions of some acids and lipids. Linear polyamine chitosan has proactive amino/hydroxyl functionality and is vulnerable to chemical modification/grafting. Body fluid lysozyme is facile and easily accumulated in chitin. It has myriad therapeutic uses, including fibroplasia-inhibited wound healing/dressing, absorbable stitches, and supports tissue/cell growth and differentiates cells. Chitosan sutures hasten and enhance certain clinical phenomena like wound healing and dressing texture, which are not easily attained in other counterparts. Chitosan scaffolds/templates chelate transition metals and exhibit enzyme immobilizations [2].

3.7. Derivatives

Derivative formation, phase transformation and significant polyfunctional alterations at NH_2, the primary/secondary OH group of chitosan, yields matrixes like composites, blends, gels, films and polyampholytes, as mentioned below [2–10].

3.7.1. N-Phthaloylated chitosan

Chitosan in phthalic anhydride-DMF solution undergoes *N*-phthaloylation, boosts solubility and fastens bulkiness due to its aversion to hydrogen bonding. Water discriminates against functional selective and quantitative *N*-phthaloylation and has a superior reactivity via tritylation/detritylation and the alcoholysis precursor for C6 substitution over *N*,*O*-phthaloylation/*O*-phthaloyl. It offers easy and suitable chemoselective protection for chitosan amino groups in scaffolds used in electrochemical devices.

3.7.2. Sialic acid/chitosan dendron

The water philicity of chitosan is enhanced effectively via gallic acid as a branching part, and triethylene glycol as a spacer arm yields a dendronized form. Residual amines undergo *N*-succinylation and boost the water solubility of resultant dendrites as chitosan is conjugated to preformed dendrons viable for effective drug/gene delivery.

3.7.3. Thiocarbamoyl chitosan

Methyl/phenylthiocarbamoyl substitution obtained via thiocyanate/thiourea in a eutectic ammonium mixture grafted onto chitosan was found to selectively entrap assorted metal adsorption, e.g., Au^{+3}/Au^{+1}/Au and Pt^{+5}/Pt^{+2} adsorption from contaminated water. This matrix showed augmented adsorption of metal ions onto the monodentate sulfide ligand coordination bond, and/or chelation often showed elevated affinity as per the Pearson principle.

3.7.4. Chitosan hydrogels

Hydrogels are polymeric systems that are found to be puffy in aqueous conditions, but retard water solubility due to cross-linked chains via one or more monomer interlockings. Such hydrophilic gels/hydrogels have polymeric chain networks as colloidal crystals dispersed in water medium. Hydrogels have been substantially noticed in past decades by virtue of their unique features like innate flexibility, normal tissue and bulk water content and outstanding guaranteed applications. Inherent hydrophobic, chitosan-based hydrogels are superior due to their longer durability, high water absorption, elevated gel strength and progressively substituted synthetic hydrogels. Chitosan has a well-defined skeleton ideal for the design of biodegradable and functionalized hydrogels that are stable in variable fluctuating conditions of temperature and pressure. Chitosan-derived hydrogels are more selective, cheap and environmentally friendly because of their high intrinsic sorption affinity and performance, which are elevated by physicochemical formulations intended for focused usage, namely, endotoxin separation of protein, lipid, lipopolysaccharide and chiral drugs. 3D chitosan, laminar, crossed-linked, two-/multicomponent hydrogels are developed in water to fill voids/spaces that vary with density and degree of acetylation of chitosan. Delivery of localized drugs/genes is achieved via slow release hydrogels, which consequently reduce off-targeted side effects of drugs. Chitosan hydrogels are straight grafted via D,L-lactic/glycolic acid treatment, impart huge interfacial water interactions as side chains and are cross-linked/aggregated to yield pH-sensitive sites. Chitosan hydrogels have impending clinical utilities, including wound dressing/healing and as cell and tissue carriers/arrays.

3.7.5. Chitosan microcapsule

The microcapsules are spherical empty particles of varying size from 50 nm to 2 mm. A surface-active chitosan base microcapsule is similar to a quantum dot, which conveys bulk and discrete electronic properties, namely, it holds the electron hole and has tuneable optical activity, long fluorescence and photostability, which are more beneficial than other fluorophores used in the recognition, tagging and imaging in biomedicals [1–5]. The laminar cationic $–NH_2$/–OH linkages of chitosan aid microcapsule creation via anionic knits with quite stable hybrids

[6], e.g., chitosan/sodium alginate and CS/nano-ZnS microcapsules offer efficient bioimaging/labelling as well as controlled drug/gene delivery. Rationally homogenized microcapsules of chitosan can be obtained by coacervation, emulsification, solvent evaporation and gas–liquid, microfluidic and layer-by-layer assembly techniques [7]. Chitosan microcapsules can entrap surfactants like CdS, ZnS cyclodextrin and sodium dodecyl sulfate to yield host–guest interactive external stimuli-sensitive hydrophobic cavities employed for the detection of toxic/hazardous contaminants. Surfactants induce certain skeletal changes that slightly control the shape and size in corresponding monodisperse microcapsules for the detection of pollutants.

Proactive amines in acidic conditions induce protonation and aid efficient $Ca(OH)_2$ coating onto chitosan to achieve pH-trigger microcapsules that impart an enduring antibacterial profile against *Enterococcus faecalis* microbial refractory strains in endodontic treatment and controlled drug delivery. A chitosan microcapsule that recuperates with native $Ca(OH)_2$ is practicable for osteogenesis and is viable for low inflammatory responses such as in bone defect healing as it evades bone resorption.

3.7.6. Biosensor/biomarker

Sensors respond and convert signals into magnetic/electrical fields that are easy to detect by other devices. Advanced biotechnology has prepared many biosensors to coalesce through chitosan to be used for the detection of diverse species, namely, tissues, cells, microorganisms, organelles, enzymes, antibodies, enzymes and nucleic acid [9]. Chitosan biosensors are preferred due to their uniqueness; they are cheap, biocompatible, ecofriendly, flexible, portable, more sensitive, naturally selective and respond more rapidly than other counterparts [2–10]. Certain nanocarbon-doped chitosan amperometric biosensors have huge surface areas, electrical conductivity and easy diffusion and are effective for enzyme immobilization, glucose estimation and the creation of fuel cell bioelectrochemical devices [13, 14].

A biomarker indicator shows the measurement/valediction of biostate, organism survival, pathogenic process and therapeutic prime response, and supervises cancer stages, clinical screening prior to diagnosis, risk assessment and disease detection as well as staging, grading and initial treatment in auxiliary therapy [15, 16]. Gold-coated chitosan/xanthan and graphene nanosphere-derived biomarkers are used for bioimaging, analysis in signal-improved melanoma cancer diagnoses, α-fetoprotein detection and carcinoembryonic antigen discovery, and are more accurate than electrochemical sensors and the ELISA test [17, 18]. Chitosan biomarkers are preferred for their due-advantage services in disease prevention, drug/tissue/cell delivery, dentistry, orthopaedics, ophthalmology, surgery and as an optical-wave guide, as shown in **Figure 4**.

3.7.7. Chitosan-based quantum dot

A quantum dot is 'nanometric/zero-dimensional particle' pertaining to semiconductor, optical-electronic quality, tuned via the size, shape and arrangements of dots/particles of used material. Nanotechnology aids in the encapsulation of quantum dots in the chitosan skeleton, which show remarkable high thermal/mechanical stability, better water solubility exploited for tumor-targeted delivery, anticancer therapy and designed drug release/loading [2–11, 13–17, 19]. Chitosan's amino-functionalized carbon quantum dots (CDs) possessed enviable

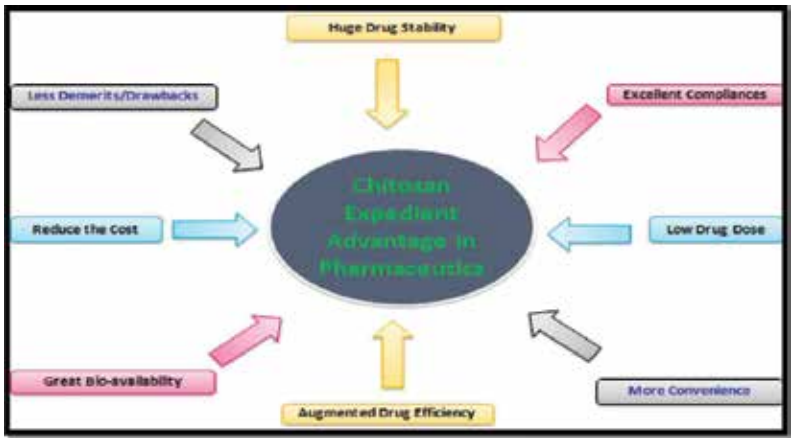

Figure 4. Chitosan expedient advantages in pharmaceutics.

surface proactive amino functionality which can exhibit bright luminescence with high quantum yield of 5% with up-conversion fluorescence effects. Such CDs possess robust UV rays obstacle detection as vital for autonomous navigations in solar devices/cells. Besides, exhibited elevated features than other commercial materials viz; low cytotoxicity, great hydrophilicity, biocompatible and well photo-stable which jointly tenders fluorescent biosensing desired in cancer diagnosis therepy along with cellular imaging and effective drug delivery [2–4]. Nano-ZnO-coated chitosan/glucose-encapsulated carbon quantum dots are designed for layer-by-layer sensitized nanosolar cells. Chitosan-doped cysteine/cadmium/tellurium quantum dots are favoured over counter-immune sensors for their antibacterial profile, electrochemical/luminescent offender DNA biosensing in chronic myelogenous leukemia, antibody immobility and specific protein detection [2–11, 13–17, 19]. Chitosan-formatted quantum dots are quick to generate safe/effectual delivery, silence unwanted gene expression/defects in curing diseases and can deliver genetic plasmids, DNA, si-RNA and oligonucleotides [13–20]. Nanometal-homogenized chitosan quantum dots offer controlled catalytic activity for multiple site selective gaseous adsorption/desorption [20, 21]. Platinum-entrapped polyacrylonitrile/chitosan quantum dots are deposited onto pencil graphite electrodes to be used in synergic water electrolysis and are excellent for facilitated hydrogen evolution reactions and skilful hydrogen production [21]. Chitosan-stabilized hyperbranched ligand quantum dots are multiresponsive drug delivery agents, luminous bioimaging sensors and semiconductors.

3.7.8. Chitosan nanoparticles

Selective and automatic morphological controls for shape/size-distributed chitosan-based nanoparticles are formulated via surface chemistry modifications. Laminar chitosan avoids nanoparticle aggregation and reduces involuntary stress via lyophilize-freezing and spray-drying techniques, but desired/safe characteristics can be formulated by pure ionotropic gelation (onto low-molecular chitosan). Chitosan/gadopentetic acid nanoparticles obtained via gadolinium neutran capture are thermally stable, robust and integrated, and thus helpful for molecular signalling, exogenous gene/drug delivery and intratumoral cancer therapy. Chitosan-doped nanoparticles augment self-branching in the resultant matrix, e.g., *N*,*N*,*N*-trimethylated

chitosan scaffolds impart extracellular competent intracellular drug release and compel better DNA transfer, superior cellular uptake and fine gene silencing [1–8]. The chitosan-blended nanopolyglycolide template has vital utility by entrapping doxorubicin drug, adorning dose-dependent non-viral devices for pulmonary si-RNA delivery, controlling H1299 gene silencing and exhibiting fluorescent protein cell expression [8–11, 13–20]. Nanotechnology aids the fabrication of advanced 2D/3D chitosan nanoscaffolds, namely sponges, foams, gels and fibres/films, for significant and precise encapsulation of nutrient/drug/tissue, which does not affect healthy cells, and is preferred in cancer chemoprevention procedures [1–11, 13–20].

3.7.9. Cosmetic uses

Biopolymer chitosan-derived hydrocolloid systems are solely cationic in its physicochemical character and gets viscous on aqueous acid neutralization, thus can prefer to intervene skin covers and artificial hairs over commercial polyanionic colloids. Chitosan is inherently fungicidal and fungistatic hence, preferred as raw/feedstock for preparation of assorted marketable beauty and cosmetic products [19]. A chitosan/alginate microcapsule has been developed to embody many species like hydrophobics, dyes and harmful UV absorbing agents. Sonat Company, USA, inserted antioxidant, antiallergic and antiinflammatory agents in chitosan to develop novel depilatory compositions to be used in cosmetics for curling hair and for skin and oral care. Biomaterial human hair composed of α-keratin owing few disulfide/$^{-}$S–S^{-} anionic linkages, and chitosan being polycationic thus utilize in synthetically developed formulations viz; elastic foams/emulsions employed in in many shampoo/conditioning products for synchronize boosting, soften/smoothen and strengthen of hairs. Chitosan-formulated hydrogels are employed in shampoos, rinses, styling lotions, hairsprays/colorants and permanent wave agents in hair-care products. Diacid anhydride-treated chitosan imparts a cationic charge and its high molecular weight stops skin infiltration, therefore it can compete with hyaluronic acid in skin-care products/cosmetics such as moisturizers, pastes, mouthwashes, chewing gums, packs, lotions, foundations, eye shadows, lipsticks, cleansing/bathing agents and nail enamels/lacquers. Chitosan mask silicon oxide salts are supplemented in toothpaste as a powder binder to uphold its granular shape. Chitin-based dental fillers are developed to stop candida/thican sticking to teeth and for cleaning false teeth [2, 10].

3.7.10. Chitosan in science and technology

The annual synthetic plastic consumption rate is 300 million tonnes; moreover, artificial polymers are merely 3% recyclable and 97% of plastic waste accumulates in the seas and oceans or in landfills, which harms our planet. Nevertheless, natural polysaccharide chitosan-based bioplastics are more biocompatible and biodegradable, and have equal utility with synthetic plastics. Encouraged by chitin, Boston researchers at the Wyss Institute for Biologically Inspired Engineering, USA, developed a silk protein and chitin-based, biodegradable, cheap, versatile, reinforced and tough plastic alternative called 'Shrilk' [10]. It is claimed that Shrilk would replace plastic in all consumer products, including suture wounds and scaffolds in tissue/cell revival. Shrilk composite consists of laminar plywood-like layers altered via

mechanical and chemical interactions of chitosan/fibroin laminates. Shrilk is composed of fibroin protein derived from silk and chitin, usually extracted from discarded shrimp shells. Shrilk has strength and toughness similar to aluminium alloy, but it is only half the weight and can easily stretch from elastic to rigid complex shapes/sizes such as tubes, sheets, films, rubbish bags, packaging, nappies, etc. Shrilk is cheap, environmentally friendly, remarkably hard, biocompatible and bears high loads, thus it is employed in suture wounds in hernia repair and is a versatile template for tissue regeneration. Shrilk offers potential environmental solutions and is emerging as a stepping-stone toward noteworthy therapeutic advancements like Food and Drug Administration-approved implantable medical devices, bone-tissue gallows, laminar silk fibroin, biocomposts/fertilizers to release N/P nutrients, surgical closure scaffolds and wound healing. Nanotechnology has altered chitosan's characteristics, including biological, physico/electrochemical cellular response and molecular motions, which aid in yielding biodegradable, biocompatible, non-toxic, antimicrobial and immunogenic matrixes for sustainable intracellular drug/protein delivery, cell array and the uptake of hydrophilic agents across epithelial layers [1–10].

4. Limitation and remedy

Apart from its vast uses, chitosan has a few disadvantages, e.g., a weak base (pK_a 6.2), little affinity for acids, low mechanical strength and it is immiscible in aqueous and many organic solvents [11]. Moreover, chitosan-based materials exhibit major puffiness in water and cause unusually fast drug delivery; hence, its parent skeleton seeks assorted physicochemical alterations. Some limitations of raw chitosan can be conquered via proactive amine/hydroxyl formulations to achieve the desired applicability.

5. Futuristic applications of the chitin/chitosan matrix

Nanotechnology has helped to design rational chitin/chitosan biocomposites with myriad applications in modern S&T [2, 14, 21]. Chitosan-integrated membranes in contrast to traditional membranes assist in the retention and remediation of toxic/hazardous contaminants [2]. Also, chitosan-based material imparts unique features, namely, progressive working efficiency, great adsorption profile for desalination and water/wastewater treatment processes, along with compliant large-scale utility for point-of-use devices [13–21]. Chitosan products like nanofilms/sheets, hydrogels, microcapsules, proliferated high-resolution devices, templates, scaffolds and quantum dots are derived via tailoring structural and chemical functionality with a view to having an unequivocal multitasking portfolio in modern scientific achievements [19], in addition to tackling the global challenges in the mitigation of environmental pollution [14, 18]. Chitosan-based products boost many water purification/desalination systems, show no interfacial limitations and also improve work efficiency in the field of S&T, as depicted in **Table 1**.

Utility	Nature of work	Features
Energy	Sunlight conversion (e.g., Dye sensitized solar cell (DSSCs), and power conversion efficiency (PCE))	Proficient light harvesting, especially in biomaterials/ biocomposites; fast charge separation; more current density; improved gas permeability; high storage density; rapid electron/ion transport; less resistance
Life sciences	Engineered/designed biomaterials, films, sheets, hydrogels, composites, etc.	Biocompatible; biodegradable; supports cell adhesion; controls dimension (shape/size and porosity); good mechanical/thermal stability
Chemical sciences	Preconcentration devices, bioreactors, emulsion/oil-H_2O separation, gas adsorption	More permeable; homogeneous flow via designed porosity; controllable dimensions and surface properties; monolithic column
S&T	Shrilk: biodegradable plastic	Entirely degradable bioplastic derived from shrimp and silk protein as a substitute for synthetic plastic, implantable medical devices
	HemCon® PRO Chitosan Technology: biomaterial product	Material produced and branded by Tricol Biomedical HemCon® having exceptional haemostatic, antibacterial features by virtue of strong polycationic charge onto chitosan-altered matrix and harvested in pristine waters of the North Atlantic. Haemostaticity imparts fast adherence/sealing to injured tissues/cells and promotes clotting. Verifies controlled bleeding in anticoagulated patients, arterial wounds with better efficiency than minerals, cellulose.

Table 1. Chitosan-based product applications in the field of S&T [1–11, 13–21].

6. Summary

Chitosan biopolymer is preferred for pharmacological and industrial purposes due to its innate features, namely, high mucoadhesion, biocompatibility, biodegradability, cheapness, non-toxicity and environmentally benign matrix. Advanced science has accomplished chitosan modality to offer assorted formulations like nanovehicles for cell/gene/DNA/RNA release, quantum dot use for never-ending scientific utility, namely, disease detection/ diagnosis, engendering new therapeutic techniques and tissue engineering for both life and Mother Nature.

The scientific facts, findings and fundamental aspects of chitosan chemistry imparts vital commercial applications which fascinated basic and applied research, resulted numerous papers, books and patents in chitin/chitosan sciences every year and this chapter/book is one of such endeavor. Thus, key challenges are highlighted as being compiled with current informative data to understand and create the enormous interest in the chemistry and science of chitosan. Chitin, like many polysaccharides, does not display requisite characteristics crucial for desired applications and thus it is mandatory to perform certain skeleton cationic, anionic, amphiphilic and crosslink formulations at free/proactive amino/hydroxyl functionalities as discussed. All such rationally tailored modifications endow the desired applicability to encompass the wide fields of biomedical/clinical research, pharmaceutics, cosmetics, foods, paper/pulp, textiles, agriculture, water treatment and permeation. This book will contribute to the literature on chitin/chitosan principally on the progress

demonstrated with novel scaffolds, templates and matrixes, which have myriad utilities. The book will inspire researchers to carry on discreet efforts so that chitosan can occupy its worthy status within the field of biopolymers.

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Section 2

Fabricated Chitosan Materials

Chapter 2

Carboxymethyl-Chitosan Cross-Linked 3-Aminopropyltriethoxysilane Membrane for Speciation of Toxic Chromium from Water

Naoki Kano

Additional information is available at the end of the chapter

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Abstract

Adsorption of Cr(VI) from aqueous solution onto the nanomaterials prepared by modified chitosan was investigated in a batch system to evaluate the efficiency of biomass as an adsorbent. The crosslinking materials of chitosan & silicon dioxide and carboxymethyl chitosan & silicon dioxide were synthesized, respectively, as new adsorbent materials for the removal of Cr(VI) from aqueous solutions. The adsorption potential of Cr(VI) by the nanomaterials for desalination was investigated by varying experimental conditions such as pH, contact time and the dosage of the nanomaterials. Adsorption isotherms of Cr(VI) onto the membrane were studied with varying initial concentrations under optimum experiment conditions. The surface property of the membrane was characterized by SEM (scanning electron microscope) and Fourier transform infrared spectrometer (FT-IR). The concentrations of Cr(VI) in solution are determined by ICP-AES (inductively coupled plasma atomic emission spectrometry). The membrane of carboxymethyl chitosan & silicon dioxide exhibited higher adsorption capacity than the membrane of chitosan & silicon dioxide for Cr(VI). The adsorption sites and specific surface area may be increased by changing from chitosan to carboxymethyl chitosan. The maximum adsorption capacity was estimated as 80.7 $mg \cdot g^{-1}$ for Cr(VI) under the optimum conditions.

Keywords: nanomaterials, carboxymethyl chitosan, silicon dioxide, adsorption isotherms, kinetic model

1. Introduction

With the rapid growth of mankind, society, science and technology, the environmental disorder with a big pollution problem has become one of the most important issues in the past half

century [1]. One of the intractable environmental problems is water pollution by heavy metals [2], and has become a challenge for life on earth because of the anthropogenic activities. Heavy metals in environmental water have been a major preoccupation of their toxicity towards aquatic life, human beings and the environment [3].

Due to serious hazardous effects of heavy metal ions on human health and toxicity in the environment [4], it is important to develop a simple and highly effective removal method as well as sensitive analytical method for environmental pollutants to improve the quality of environment and human life.

The environmental conservation is of increasing social and economic importance. Various treatment technologies such as ion exchange, precipitation, ultrafiltration, reverse osmosis and electro dialysis have been used for the removal of heavy metal ions from aqueous solution [5]. However, these processes have some disadvantages, such as high consumption of reagent and energy, low selectivity, high operational cost.

Many works for the removal of heavy metals by adsorption has been reported [6, 7]. Particularly, the development of high efficiency and low cost adsorbents has been aroused general interest in recent years. Biological materials as adsorbent for water purification have become a hot research topic [8, 9]. Biological adsorbent has the advantages of recyclable, low cost, easy operation and little possibility of secondary pollution [10, 11].

Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements [12]. Heavy metals are not biodegradable and tend to accumulate in living organisms, causing various diseases and disorders [13]. They enter the body system through food, air, and water and bioaccumulate over a period of time. Although adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and is even increasing in some areas [14], such as metal plating facilities, mining operations, and tanneries. Among these heavy metals, Cr is one of the top priority list of toxic pollutants defined by the U.S. Environmental Protection Agency.

Cr mainly consists of two stable oxidation states such as trivalent state Cr(III) and hexavalent state Cr(VI) in natural aqueous environment [15]. Cr(VI) is more toxic, carcinogenic and mutagenic. The typical mobile forms of Cr(VI) in natural environment are CrO_4^{2-}, $HCrO_4^-$; and the relative distribution of each species depends on the solution pH, on the concentration of Cr(VI) and redox potential [16]. Cr(III) tends to form $[Cr(H_2O)_6]^{3+}$, $Cr(H_2O)_5(OH)^{2+}$, $Cr(H_2O)_4(OH)_2^+$, or Cr(III) organic complexes. The use of Cr and its compounds in several industrial processes (automobile manufacturing, production of steel and alloys, mining of chrome ore, plating, and electroplating, etc.) leads to contamination of natural waters mainly due to improper disposal methods [17]. They can be taken up by plants and easily be leached out into the deeper soil layers, leading to ground and surface water pollution. It is well known that Cr(III) is essential materials for living organisms, whereas Cr(VI) is the most toxic form. Cr(VI) can diffuse as CrO_4^{2-} or $HCrO_4^-$ through cell membranes [18] leading to carcinogenic, mutagenic, liver damage, pulmonary congestion, and causes skin irritation resulting in ulcer formation to living organisms [19–22].

From the above-mentioned, Cr(VI) must be substantially removed from the waste water before being discharged into the aquatic system. Therefore the separation and reduction of Cr in waste water is very important for environmental protection and human health.

Different technologies for the removal of heavy metal ions are available such as chemical precipitation, coagulation, ion exchange, membrane technologies, and adsorption. Adsorption has been proved as one of the most efficient methods for the removal of heavy metals from aqueous media [23]. The major advantages of biosorption are its high effectiveness, easy operation, no two pollution, and the use of inexpensive biomaterials.

Chitosan has proven to be very efficient biosorbent for the removal of several toxic metals such as mercury (Hg), uranium (U), molybdenum (Mo), vanadium (V) and platinum (Pt) [24–26]. Chitosan, which full chemical name is known as (1,4)-2-amino-2-deoxy-β-D-glucose, can be environmentally friendly adsorbent due to the low price and no second pollution. Chitosan is produced by the alkaline deacetylation of chitin, and the preparation process of chitosan is shown in **Figure 1**. Chitosan is the most abundant biopolymer in nature originated from cellulose that can be obtained from the shells of seafood such as prawns, crabs, and lobsters [27]. The biopolymer is characterized by its high content of nitrogen, and is existed in the form of amine groups, free amino groups and hydroxyl groups, which are responsible for metal ion binding through chelation mechanisms [28].

However, chitosan had some defects such as notable swelling in aqueous media and nonporous structure resulting in a very low surface area [29]. Therefore, many types of chemical modification can be undertaken to produce some chitosan derivatives for improving the removal efficiency of heavy metal [30]. For example, silicon dioxide can be one of the materials for offsetting the defects of chitosan because it has many characteristics such as rigid structure, porosity and high surface area.

Silica gels are low-density solids, consisting of silicon oxide. The study of silica gels has attained considerable attention due to open mesoporic structure, high surface area, large pore volume and good performance as effective adsorbents [31]. Silicon dioxide is a synthetic amorphous polymer with silanol groups on the surface allowing metal adsorption [32, 33]. In case of silicon dioxide, the modified silicon dioxide through the graft between silanol groups and ligands has been developed [34–36]. At present, an interest has grown in the field of organic and inorganic hybrid materials. The silica gels doped with some organic or inorganic material possess a number of novel properties [37].

Due to above-mentioned reason, novel adsorption materials were designed to combine the beneficial properties of silica gel and chitosan. The membrane of cross-linked chitosan with

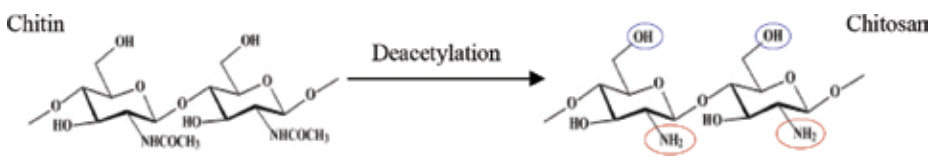

Figure 1. The preparation process of chitosan using chitin.

silicon dioxide was synthesized in this work to enhance the adsorption potential of heavy metal ions. Furthermore, carboxymethyl chitosan has been prepared by using chloroacetic acid (and chitosan) under alkaline conditions to improve the removal efficiency. Using the carboxymethyl chitosan, the membrane of carboxymethyl chitosan & silicon dioxide was also synthesized, and was employed to remove heavy metal ions from aqueous solution. In present study, the adsorption capacity of the membrane was investigated for the removal of toxic chromium ions from aqueous solution under varying experimental conditions.

Moreover, the surface morphology of the cross-linked membrane was determined to characterize these nanomaterials for desalination, and regeneration experiments were also conducted using the membrane.

2. Experimental sections

2.1. Materials, reagent and apparatus

3-Aminopropyltriethoxysilane was purchased from Nacalal Tesque., Inc. (Tokyo, Japan), and chitosan was from Tokyo Chemical Industry Co. (Tokyo, Japan). Cr(VI) standard solutions were prepared by diluting a standard solution (1.005 $mg \cdot dm^{-3}$ $K_2Cr_2O_7$ solution) purchased from Kanto Chemical Co., Inc. All other chemical reagents were also bought from Kanto Chemical Co., Inc. All reagents used were of analytical grade, and water (>18.2 MΩ in electrical resistance) which was treated by an ultrapure water system (Advantec aquarius: RFU 424TA, Advantec Toyo, Japan), was employed throughout the work.

The pH of Cr(VI) aqueous solution were measured by the pH meter (HORIBA UJXT 06 T8, Japan). The surface property of the membrane of carboxymethyl chitosan & silicon dioxide was characterized by SEM (JEOL, JSM-5800, Japan) and Fourier transform infrared spectroscopy in pressed KBr pellets (FTIR-4200, Jasco, Japan). The concentrations of Cr(VI) in solution were determined by ICP-AES (inductively coupled plasma atomic emission spectrometry).

2.2. Prepared the membrane of chitosan & silicon dioxide

The solution of chitosan (3%, w/v) was prepared by dissolving 3 g of chitosan in 100 ml of 0.2 $mol \cdot dm^{-3}$ acetic acid solution. Silica sols (which was prepared by dissolving 2 ml of 3-aminopropyltriethoxysilane in 100 ml ethanol) was added into the solution of chitosan (3%, w/v) at 25°C, and was stirred for 24 h. The membrane of cross-linked chitosan with silicon dioxide was dried at 25°C.

2.3. Prepared the membrane of carboxymethyl chitosan & silicon dioxide

Under alkaline conditions, chitosan can react with chloroacetic acid to obtain the carboxymethyl chitosan. Chitosan (5 g) was accurately weighed into a round-bottomed flask containing 75 ml isopropanol and 25 ml ultrapure water, and then 6.75 g of sodium hydroxide was added for alkalization. The mixed solution was stirred in a water bath at 50°C for 2 h, and was cooled to

room temperature after continued stirring for 4 h. In addition, chloroacetic acid solution was prepared by dissolving 6 g of chloroacetic acid in 25 ml isopropanol solution, and slowly dropped into the round-bottomed flask under stirring for 4 h. The solution was adjusted to neutral using hydrochloric acid, and washed three times with 70% isopropanol, and then filtered. After washing completely with 90% isopropanol again, the solution was filtered. Then, carboxymethyl chitosan was dried at 50°C and used for preparation of membrane.

The reaction process of membrane synthesized from carboxymethyl chitosan & silicon dioxide is shown in **Figure 2**. The solution of carboxymethyl chitosan (3%, w/v) was prepared by dissolving 3 g of carboxymethyl chitosan in 100 ml ultrapure water. Silica sols (prepared by dissolving 5 ml of 3-Aminopropyltriethoxysilane in 100 ml ethanol) was added into the solution of carboxymethyl chitosan (3%, w/v) at 25°C, and the solution was stirred for 24 h. The membrane of carboxymethyl chitosan & silicon dioxide was dried at 25°C.

2.4. Adsorption experiment of Cr(VI) using the membrane

The adsorption capacities of Cr(VI) from aqueous solution using the membrane were investigated by a batch method. The membrane was thoroughly mixed with 50 ml of containing known concentrations of Cr(VI) in a 200 ml conical flask. According to the above-mentioned procedure, Cr(VI) were adsorbed at different pH values (1–7), contact time (20–120 min) and sorbent dosage (0.05–0.3 g dm^{-3}). The pH of each solution was adjusted by using 0.1 mol dm^{-3} NaOH and 0.1 mol·dm^{-3} HCl. Adsorption isotherms of Cr(VI) onto the membrane of chitosan & silicon dioxide were measured at varying initial Cr(VI) concentrations (10–50 ppm) under optimized conditions.

The adsorption capacity of adsorbents for heavy metal ion was calculated using the mass balance equation:

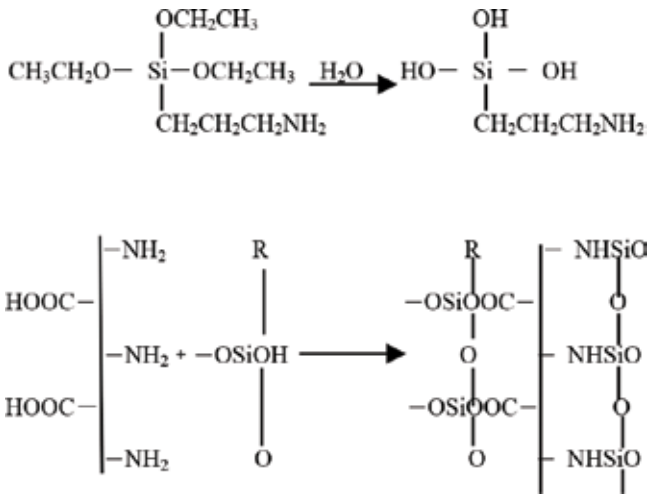

Figure 2. The reaction principle of the carboxymethyl chitosan crosslinked with 3-aminopropyltriethoxysilane.

$$q_{\mathrm{e}} = \frac{(Ci - Ce)}{m} \cdot V \tag{1}$$

where q_e is the adsorption capacity ($mg{\cdot}g^{-1}$) of heavy metal ion by the adsorbents at equilibrium, C_i and C_e are the concentrations of heavy metal ion at initial and equilibrium in a batch system respectively ($mg{\cdot}dm^{-3}$), V (dm^{-3}) is the volume of the heavy metal solution, and m (g) is the mass of the adsorbents.

2.5. Langmuir and Freundlich isotherm models

Langmuir and Freundlich isotherms were modeled in order to evaluate the performance of adsorbents in adsorption processes by the relationship between the metal uptake (q_e) and the concentration of heavy metal ion (C_e) at equilibrium.

The Langmuir isotherm equation is defined as follows:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{\max}} + \frac{1}{K_L q_{\max}} \tag{2}$$

where C_{e} is the concentration of heavy metal ion at equilibrium ($mg{\cdot}dm^{-3}$), q_e and q_{max} are the amount of adsorption of heavy metal ion at equilibrium ($mg{\cdot}g^{-1}$) and the maximum adsorption capacity by the adsorbents ($mg{\cdot}g^{-1}$) respectively, K_L ($dm^{-3}{\cdot}mg^{-1}$) is the adsorption constant of Langmuir isotherm.

The linearized Freundlich isotherm equation is defined as follows:

$$\log_{10} q_e = \log_{10} K_F + (1/n)\ \log_{10} C_e \tag{3}$$

In this equation, K_F is the adsorption capacity [$(mg{\cdot}g^{-1}){\cdot}(dm^{-3}{\cdot}mg^{-1})^{1/n}$], $1/n$ is the adsorption intensity. The values of $1/n$ and K_F were determined on the basis of the plots of q_e versus C_e in log scale.

2.6. Kinetic models

Kinetic models have been proposed to determine the rate of adsorption of the adsorbent. In addition, the process of kinetic study is very important for understanding the reaction process and the rate of adsorption reactions.

The pseudo first-order model is given by the following equation:

$$\ln\left(q_e - q_t\right) = \ln\left(q_e\right) - k_1 t \tag{4}$$

where q_e and q_t are the adsorption capacity of heavy metal ion using the adsorbents at equilibrium and time t, respectively ($mg{\cdot}g^{-1}$), and k_1 is the rate constant of the pseudo-first-order adsorption (h^{-1}).

The pseudo-second order rate equation is expressed as follows:

$$\frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{t}{q_e} \tag{5}$$

where k ($g \cdot mg^{-1} \cdot h^{-1}$) is the rate constant of the second-order model, and q_e and q_t are the adsorption capacities of heavy metal ion using the adsorbents at equilibrium and time t, respectively ($mg \cdot g^{-1}$).

3. Results and discussion

3.1. Characteristics of the cross-linked membrane

The SEM pictures of the membrane of cross-linked chitosan with silicon dioxide are shown in **Figure 3**. It can be observed that the nanomaterial exists in the form of particles. The theory of chitosan and silica network where chitosan moieties were combined through silica groups via both ionic and covalent bonds was proposed [38]. The adsorbents synthesized in this work also may contain free amino groups that are responsible for metal ion binding through chelation mechanisms.

The surface property of the membrane of carboxymethyl chitosan & silicon dioxide was also investigated by SEM, and SEM images are shown in **Figure 4**. The surface morphology of the membrane showed the form of grain coalescence, which may be due to the crosslinking among adjacent carboxymethyl chitosan groups. Moreover, there was the porous structure in the surface of the membrane. It indicates that silicon dioxide was incorporated into the carboxymethyl chitosan definitely, and thereby the porous structure increased. Carboxymethyl has a high chelating ability for metal ions to form stable metal chelates. The lone pair electrons on the nitrogen atom can also constitute coordination bonds with the metal ions to form the

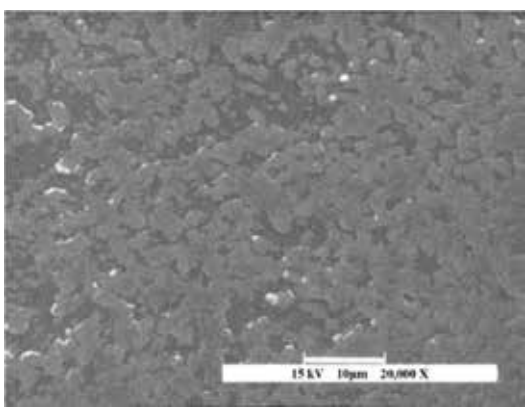

Figure 3. SEM pictures of the membrane of chitosan and silicon dioxide.

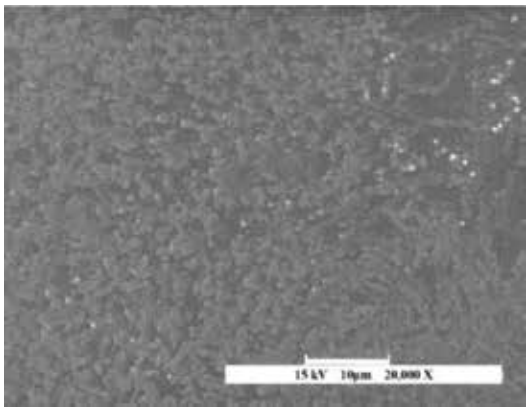

Figure 4. SEM pictures of the membrane of carboxymethyl chitosan and silicon dioxide.

complex precipitation. The molecule also may contain free amino groups and hydroxyl groups, which can remove the heavy metal ions by chelation mechanisms.

The FT-IR spectroscopy is an important technique of characterization used to explain the changes in chemical structures (i.e., the functional group on the surface of the samples).

FTIR spectra of the membrane of carboxymethyl chitosan & silicon dioxide are presented in **Figure 5**. The strong broad band at the wave number region of 3300–3500 cm^{-1} is the characteristic of $–NH_2$ stretching vibration, and the band at 3400 cm^{-1} are related to symmetrical valent vibration of free NH_2 and –OH groups. The –CH stretching vibration in –CH and $–CH_2$ were observed at 2916 and 1376 cm^{-1}. The $–NH_2$ bending vibration was observed at 1652 cm^{-1} shifted to lower frequencies (The lower frequencies observed in the membrane may be explained by the presence of primary amine salt $-NH_3^+$ [39]). A strong C=O stretching band at 1655 cm^{-1} may be related to the carboxymethyl group. Others bands at 1090 cm^{-1} are

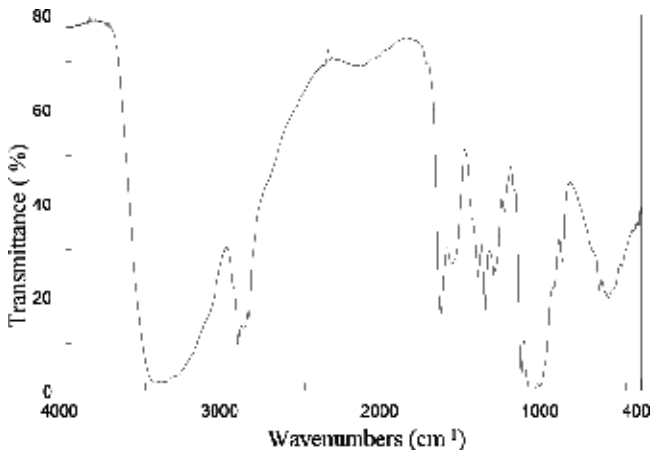

Figure 5. FTIR spectra of membrane of carboxymethyl chitosan & silicon dioxide.

related to Si-O-Si valent vibrations. The results of FTIR analysis show that the membrane of carboxymethyl chitosan & silicon dioxide were prepared successfully in this study.

3.2. Effect of parameters on adsorption

3.2.1. Effect of pH

For obtaining the optimum conditions regarding the adsorption of Cr(VI) onto the membrane, the effects of pH on the removal of Cr(VI) were investigated under the following condition: initial concentration of Cr(VI) for 50 $mg \cdot dm^{-3}$, the contact time of 100 or 120 min, and the dosage of the adsorbent for 0.2 $g \cdot dm^{-3}$.

The effect of pH on the removal of Cr(VI) using these membranes are shown in **Figures 6** and **7** (**Figure 6**: chitosan & silicon dioxide, **Figure 7**: carboxymethyl chitosan & silicon dioxide).

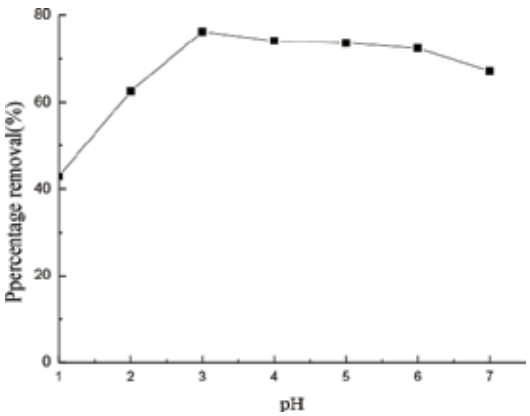

Figure 6. Effect of pH on the removal of Cr(VI) using the membrane of chitosan & silicon dioxide.

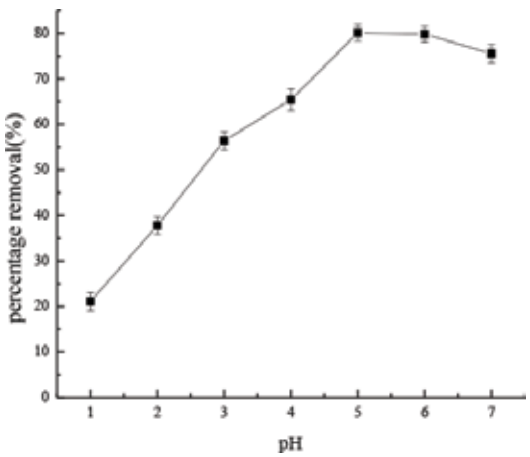

Figure 7. Effect of pH on the removal of Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide.

In case of cross-linking membrane of chitosan & silicon dioxide, the removal of Cr(VI) more than 76% was observed at pH 3 (**Figure 6**). It is well known that pH influences significantly in the adsorption processes by affecting both the protonation of the surface groups and the degree of the ionization of the adsorbates [40]. The surface of the adsorbent will be positively charged at lower pH, and it will not favor the adsorption of positively charged ions. Then it will favor the adsorption of Cr(VI) in the anionic form as $HCrO_4^-$ [41]. As shown in **Figure 8** taken from Irgolic et al. [42], the dominant form of Cr(VI) exists as hydrogen chromate anions ($HCrO_4^-$) between pH 2 and 6. With the increase of pH, the dominant species will change from $HCrO_4^-$ to other form CrO_4^{2-} [43].

Then, pH 3 was selected as the optimal pH in case of the membrane of chitosan & silicon dioxide for further work. It is well known that pH influences significantly the adsorption processes by affecting both the protonation of the surface groups and the chemical form of Cr (VI). Cr(VI) exist in variety of form with different pH, Cr(VI) exist in the form of H_2CrO_4 at pH 1 [44], and different forms such as $Cr_2O_7^-$, $HCrO_4^-$, $Cr_3O_{10}^{2-}$, $Cr_4O_{13}^{2-}$, while $HCrO_4^-$ predominates at the pH range from 2.0 to 6.0. Furthermore, this form shifts to CrO_4^{2-} and $Cr_2O_7^{2-}$ when pH increases [45, 46]. The process of shifts is given Eqs. (6)–(8):

$$H_2CrO_4 \leftrightarrow H^+ + HCrO_4^- \tag{6}$$

$$HCrO_4^{2-} \leftrightarrow H^+ + CrO_4^{2-} \tag{7}$$

$$2HCrO_4^- \leftrightarrow Cr_2O_7^{2-} + H_2O \tag{8}$$

It is found that the adsorption capacity was relatively low at pH 1. It may attributable to the strong competition between H_2CrO_4 and protons for adsorption sites. In case of carboxymethyl chitosan & silicon dioxide, the adsorption efficiency of Cr(VI) increased with the increase of pH, and reached maximum at pH 5 (80%). It is considered that the ($-NH_2$) in the adsorbent may be protonated to form ($-NH_3^+$) at pH 2–6. The surface of the membrane become positively-charged due to strong protonation at these pH range, which leads to a

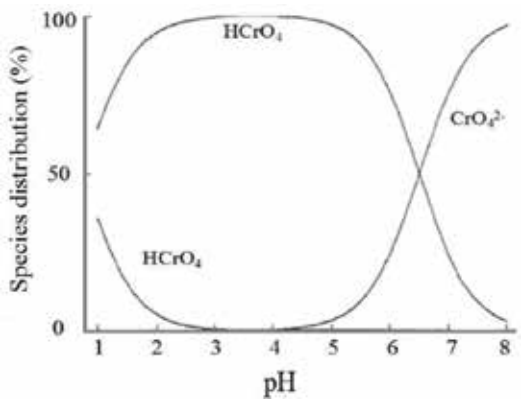

Figure 8. Species distribution curves of Cr(VI) in environmental water.

stronger attraction between the positively-charged surface and the negatively-charged $Cr_2O_7^{2-}$ and $HCrO_4^-$. Then protonation will enhance the Cr(VI) adsorption at pH 5–6. However, at higher pH, Cr may precipitate from the solution as its hydroxides. Hence, pH 5 was considered as optimum pH for further work.

3.2.2. Effect of contact time

Adsorption experiments were performed in order to determine the optimum contact time at optimal pH under the condition of the concentration of Cr(VI) for 50 mg·dm^{-3}, and the dosage of the adsorbent for 0.2 g·dm^{-3}.

The effect of contact time on the removal of Cr(VI) using the membrane of chitosan & silicon dioxide in **Figure 9**, the adsorption capacity of the membrane for Cr(VI) reached adsorption equilibrium at 80 min, and after that there are a slight decrease due to the swelling properties of the membrane absorbent. Therefore, the optimized contact time was selected for 80 min.

The effect of contact time on the removal of Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide is shown in **Figure 10**. It can be observed that the adsorption capacity of Cr(VI) increases with increasing time within 60 min. The removal rate for Cr(VI) reached approximately 80% at 60 min, and after that there is no appreciable increase Then, 60 min was selected as the optimized contact time.

3.2.3. Effect of the membrane dosage

In order to estimate the optimal dosage of the membrane, the adsorption experiments were carried out with the range of 0.05–0.3 g·dm^{-3} for the adsorbent under the optimum conditions of pH, contact time, and the concentration of Cr(VI) for 50 mg·dm^{-3}.

The effect of dosage on the removal of Cr(VI) using the membrane of the membrane of chitosan & silicon dioxide are shown in **Figure 11**. The adsorption capacity of membrane for Cr(VI) reached adsorption equilibrium at 0.2 g dm^{-3}, and the removal rate reached 78.6%.

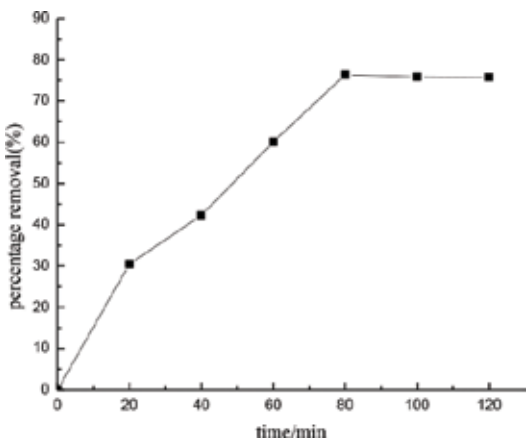

Figure 9. Effect of contact time for Cr(VI) adsorption using the membrane of chitosan & silicon dioxide.

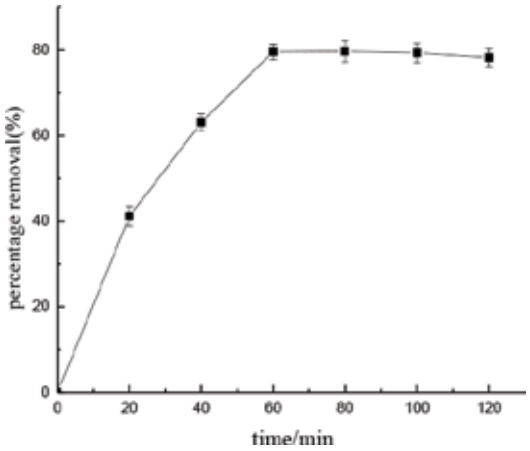

Figure 10. Effect of contact time for Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide.

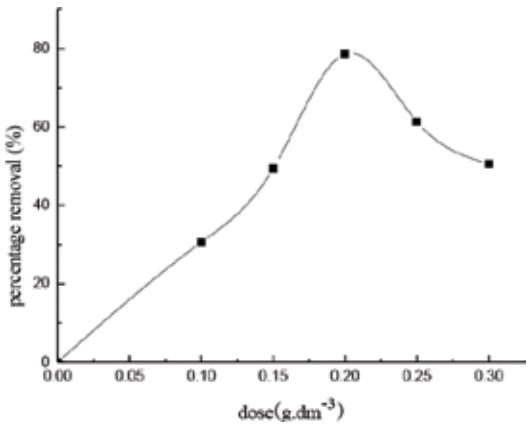

Figure 11. Effect of dose on percent removal of Cr(VI) using the membrane of chitosan & silicon dioxide.

However, remarkable decrease is observed at a dosage more than 0.2 g·dm^{-3}. Thus, 0.2 g dm^{-3} was considered as optimized dose.

The effect of dosage on the removal of Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide is shown in **Figure 12**. The results indicate that the adsorption capacity of the membrane for Cr(VI) reached adsorption equilibrium at the dosage of 0.25 g·dm^{-3}, and that no significant change is observed at a dosage from 0.2 to 0.3 g·dm^{-3}. The removal rate reached about 80% at 0.25 g·dm^{-3}, and 0.25 g·dm^{-3} was selected as the optimal dosage.

3.2.4. Effect of initial concentration

The experiments were performed by varying concentrations from 10 to 50 mg·dm^{-3} under optimized condition of pH, contact time and adsorbent dosage.

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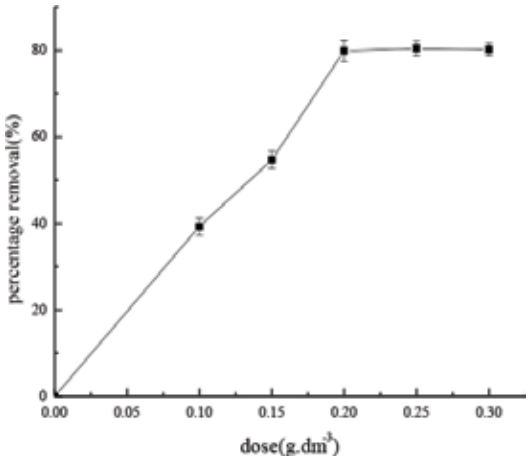

Figure 12. Effect of dosage of adsorbent for Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide.

The effect of initial concentration on the removal of Cr(VI) using the membrane of chitosan & silicon dioxide is shown in **Figure 13**. There was a continuous increase in the uptake of Cr(VI) per gram of adsorbent up to the concentration of 40 mg·dm^{-3}, but the uptake is almost constant at further higher concentrations. The removal rate reached 78.7%. Then, 40 mg·dm^{-3} was considered as optimum initial concentration for Cr(VI).

The effect of initial concentration on the removal of Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide is shown in **Figure 14**. There was a slight increase from 20 to 50 mg·dm^{-3} except at the concentrations of 30 mg·dm^{-3}. The initial concentration was taken as 40 mg·dm^{-3}.

Data from these studies were fitted to the Langmuir and Freundlich isotherm equations.

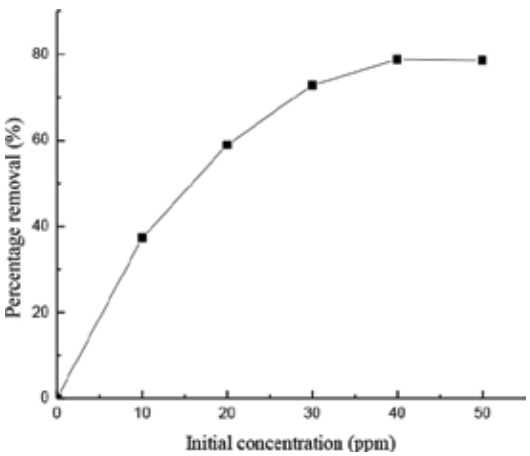

Figure 13. Effect of initial concentration for Cr(VI) adsorption using the membrane of chitosan & silicon dioxide.

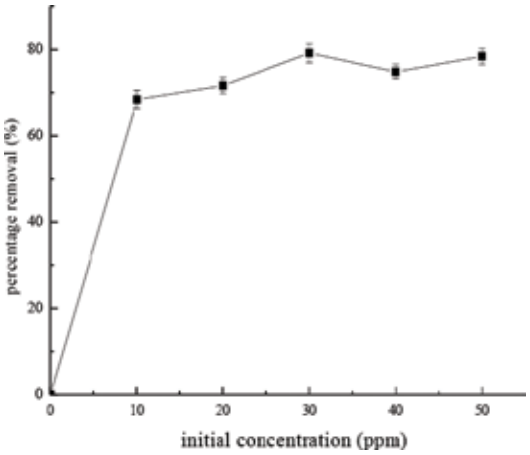

Figure 14. Effect of initial concentration for Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide.

3.3. Adsorption isotherms

Adsorption isotherms are commonly used to reflect the performance of adsorbents in adsorption processes [47]. To understand the adsorption process of Cr(VI) using the membrane, adsorption isotherms of Langmuir and Freundlich were investigated under the optimal conditions.

The adsorption data obtained for Cr(VI) using the membrane of chitosan & silicon dioxide were analyzed by Langmuir (**Figure 15**) and Freundlich equations (**Figure 16**). The correlation coefficient (R^2) of these isotherms for Cr(VI) on the membrane is shown in **Table 1** along with other relevant parameters. From **Table 1**, it is found that R^2 value for Cr(VI) is comparatively large, and favorable adsorption of Cr(VI) on the membrane was presented. Particularly, R^2 values in Langmuir isotherm are larger than that in Freundlich isotherm. The maximum adsorption capacity (q_{max}) calculated from Langmuir model was 21.2 mg·g^{-1}. This result

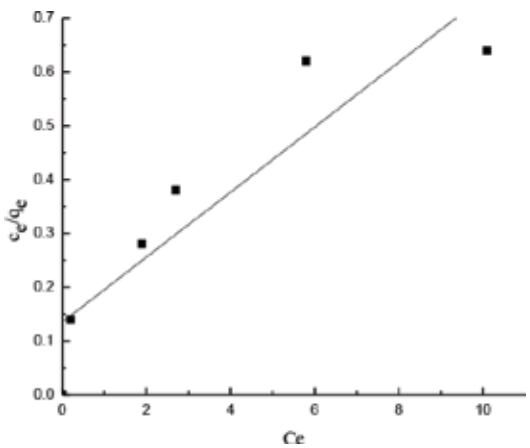

Figure 15. Langmuir isotherm of Cr(VI) adsorption onto the membrane of chitosan & silicon dioxide.

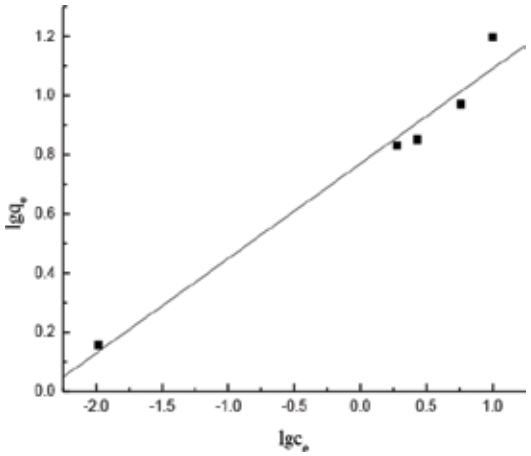

Figure 16. Freundlich isotherm of Cr(VI) adsorption onto the membrane of chitosan & silicon dioxide.

	Langmuir isotherm			Freundlich isotherm		
	q_{max} [mg·g^{-1}]	K_L [dm^{-3}·mg^{-1}]	R^2	K_F [(mg·g^{-1})·(dm^{-3}·mg^{-1})$^{1/n}$]	$1/n$	R^2
Membrane (chitosan & silicon dioxide)	21.2	1.32–01	0.985	3.21	0.78	0.912

Table 1. Coefficient of Langmuir and Freundlich isotherms for Cr(VI) using the membrane of chitosan & silicon dioxide.

suggests that the adsorption of Cr(VI) on the membrane of chitosan & silicon dioxide mainly occurred by monolayer reaction.

The adsorption data obtained for Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide were analyzed by Langmuir (**Figure 17**) and Freundlich equations (**Figure 18**). The correlation coefficient (R^2) of Langmuir and Freundlich isotherms for Cr(VI) using the membrane is shown in **Table 2** along with other relevant parameters.

The maximum adsorption capacity (q_{max}) calculated from Langmuir model was 80.7 mg·g^{-1}. Based on **Table 2**, it is found that R^2 value of Langmuir isotherm is larger than that of Freundlich isotherm. This result suggests that the adsorption of Cr(VI) on the membrane of carboxymethyl chitosan & silicon dioxide mainly occurred by monolayer reaction.

Moreover, the adsorption isotherm of Cr(VI) by the membrane was more suitably described by Langmuir model, indicating that monolayer adsorption of Cr(VI) on the membrane is more dominant.

3.4. Kinetic studies

Kinetic models were tested in this study for the adsorption of Cr(VI) onto the membrane under the optimized experimental conditions. Adsorption time is one of the important factors which

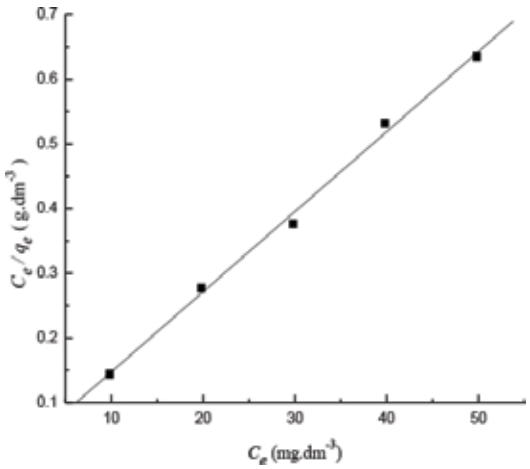

Figure 17. Langmuir isotherm of Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide.

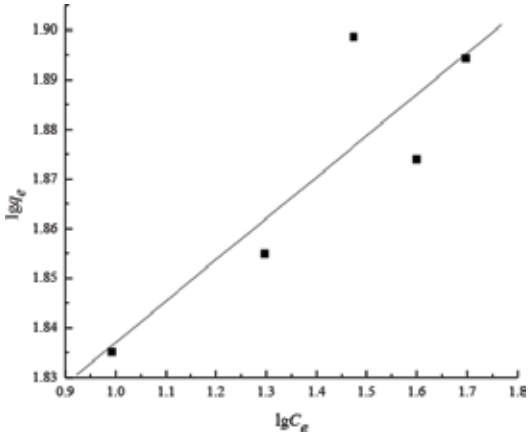

Figure 18. Freundlich isotherm of Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide.

	Langmuir isotherm			**Freundlich isotherm**		
	q_{max} **[mg·g^{-1}]**	K_L **[dm^{-3}·mg^{-1}]**	R^2	K_F **[(mg·g^{-1})·(dm^{-3}·mg^{-1})$^{1/n}$]**	*1/n*	R^2
Membrane (carboxymethyl chitosan & silicon dioxide)	80.7	0.531	0.998	56.7	0.0834	0.867

Table 2. Coefficient of Langmuir and Freundlich isotherms for Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide.

help us to predict kinetics as well as the mechanism of the uptake of heavy metals on material surface [47].

The adsorption data obtained for Cr(VI) using the membrane of chitosan & silicon dioxide were analyzed by kinetic studies are shown in **Figure 19**. Based on the data in **Figure 18**, the pseudo second-order kinetic coefficients for Cr(VI) by the membrane are estimated (**Table 3**). The rate constant of second-order equation (k) diffusion are 1.17×10^{-2} g·mol^{-1}·h^{-1} for Cr(VI). The correlation coefficients (R^2) were 0.996 for Cr(VI) adsorption on the membrane.

The results for rate constant (k) and the amount of adsorbed Cr(VI) (q_e) are shown in **Table 4** along with the regression coefficients (R^2). From **Table 4**, it is found that R^2 value of the pseudo second-order is larger than that of pseudo first-order, therefore, the pseudo second-order kinetic model provided more comparable.

Then, the pseudo first-order and pseudo second-order kinetic model of Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide are shown in **Figures 20** and **21**. It implies that the adsorption kinetics based on the experimental values is in good agreement with the pseudo second-order kinetic model, and that the rate constant of second-order equation (k) are 3.4×10^{-2} g·mg^{-1}·h^{-1} in this work.

From the kinetic studies, it is found that the pseudo second-order model provided more comparable, the pseudo second-order model implies that the adsorption process for Cr(VI)

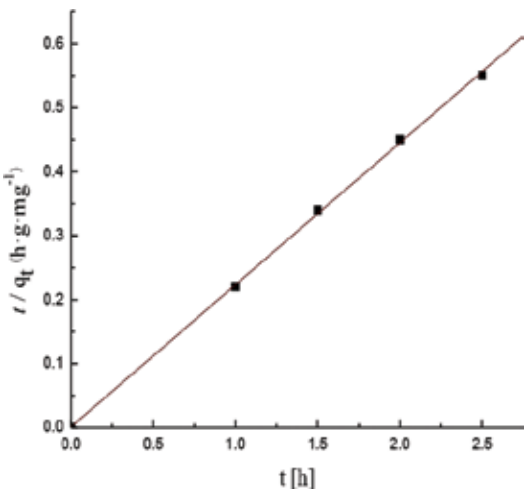

Figure 19. The pseudo second-order kinetic model on the membrane of chitosan & silicon dioxide.

	q_e (mg·g^{-1})	K_2 (g·mol^{-1}·h^{-1})	R^2
Cr(VI)	0.106	1.17×10^{-3}	0.996

Table 3. The pseudo second-order kinetic coefficient for Cr(VI) using the membrane of chitosan & silicon dioxide.

	Pseudo-first-order			Pseudo-second-order		
	q_e (mg·g^{-1})	K_1 (h^{-1})	R^2	q_e (mg·g^{-1})	K_2	R^2
Cr(VI)	79.7	8.91	0.924	94.4	3.42×10^{-2}	0.990

Table 4. The kinetic coefficient for Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide.

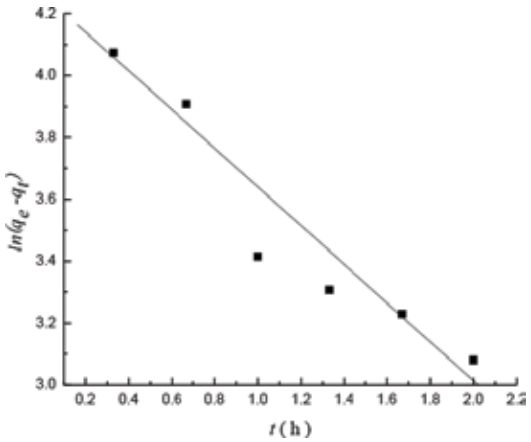

Figure 20. The pseudo first-order kinetic model of Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide.

was mainly chemical, and that the adsorption process involves the valency forces through sharing electrons between the metal ions and adsorbent.

It is obvious that the adsorption capacity of Cr(VI) by the membrane of carboxymethyl chitosan & silicon dioxide is higher than that by the membrane of chitosan & silicon dioxide from the comparison of each maximum adsorption capacity.

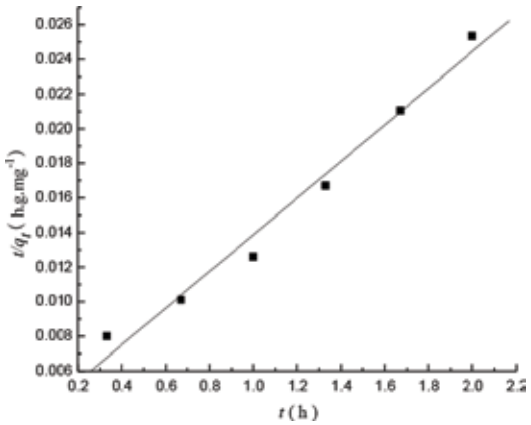

Figure 21. The pseudo second-order kinetic model of Cr(VI) adsorption using the membrane of carboxymethyl chitosan & silicon dioxide.

Adsorbent	Adsorption capacity ($mg \cdot g^{-1}$)	References
Cross-linked chitosan bentonite composite	89.1	[48]
Chitosan/polyvinyl alcohol/containing cerium(III)	52.9	[49]
STAC-modified rectorite	21.0	[50]
Ethylenediamine-modified cross-linked magnetic chitosan	51.8	[44]
Clarified sludge	26.3	[51]
A novel modified graphene oxide/chitosan	86.2	[52]
Chitosan-g-poly/silica gel nanocomposite	55.7	[53]
Membrane of chitosan & silicon dioxide	21.2	This study
Membrane of carboxymethyl chitosan & silicon dioxide	80.7	This study

Table 5. Comparison of adsorption capacity for Cr(VI) by different adsorbents.

The comparison of maximum adsorption capacity of Cr(VI) by these membranes in present study with that of another adsorbents in literatures are presented in **Table 5**. As seen in **Table 5**, the adsorption capacity of the membrane for Cr(VI) in this work is on a level with that of another adsorbents in previous works.

3.5. Effect of competitive ions on the adsorption of Cr(VI)

Competitive experiment for Cr(VI) was performed at optimized pH (pH 5), contact time (60 min) and sorbent dosage (0.25 $g \cdot dm^{-3}$) under the presence of competitive ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}) at different concentrations 0, 10, 20, 30 and 40 ppm (**Figure 22**), and the presence of common ions (Cl^-, NO_3^- and SO_4^{2-}) at different concentrations 0, 10, 20 and 40 ppm (**Figure 23**). From **Figures 22** and **23**, it is suggested that adsorption capacity of Cr(VI) by the

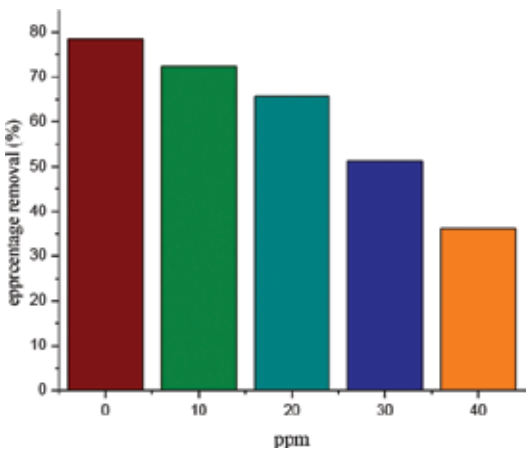

Figure 22. Effect of competitive ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}) on the adsorption of Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide.

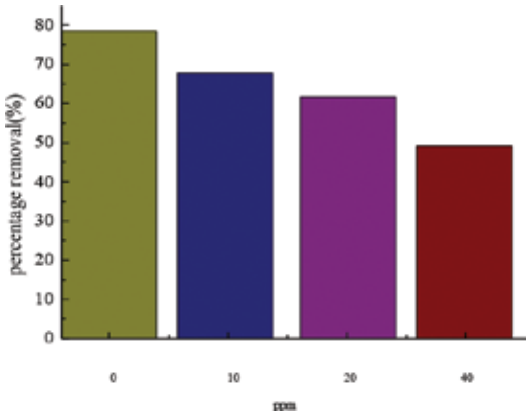

Figure 23. Effect of competitive ions (cl^-, NO_3^- and SO_4^{2-}) on the adsorption of Cr(VI) using the membrane of carboxymethyl chitosan & silicon dioxide.

membrane decreases as the concentration of competitive ion increases. However, it can be effective adsorbent for Cr(VI) even under the presence of low concentration competition ions.

3.6. Adsorption mechanism

Novel adsorbent for Cr(VI) was synthesized by chitosan & silicon dioxide. Adsorption mechanism chromium onto the membrane is shown in **Figure 24**.

The membrane has carboxymethyl, free amino group and hydroxyl groups on its surface as the adsorption site. It can remove Cr(VI) by forming stable metal chelates, and the porous structure of the membrane enhance the adsorption capacity of Cr(VI). Silanol groups (Si-OH) on the silica surface cross-linked with amino groups and carboxyl groups on the carboxymethyl chitosan were reacted to prepare the membrane. The main role of silicon dioxide is as follows: (1) the specific surface area of the membrane is increased by the porous structure of silicon dioxide, (2) the hydroxyl groups in silicon dioxide may enhance the adsorption capacity of the membrane for the removal of Cr(VI). Carboxymethyl chitosan as well as chitosan was used to enhance the adsorption capacity in this work, and the adsorption sites and specific surface area will increase by changing from chitosan to carboxymethyl chitosan.

3.7. Regeneration studies

From industrial and technological point of view, it is desirable to recover and reuse the adsorbed material. Then, regeneration experiments were conducted using the membrane of carboxymethyl chitosan & silicon dioxide after adsorption of Cr(VI) at pH 13.5. In each desorption experiment, 75 mg of the spent adsorbent after adsorption was treated with 200 ml of 0.5 $mol \cdot dm^{-3}$ NaOH and 2 $mol \cdot dm^{-3}$ NaCl solution as desorption agent, and then filtered. Cr(VI) content in the filtrate was determined by ICP-AES. Adsorption and desorption studies have been continued during five cycles at room temperature for 4 h as eluent. The

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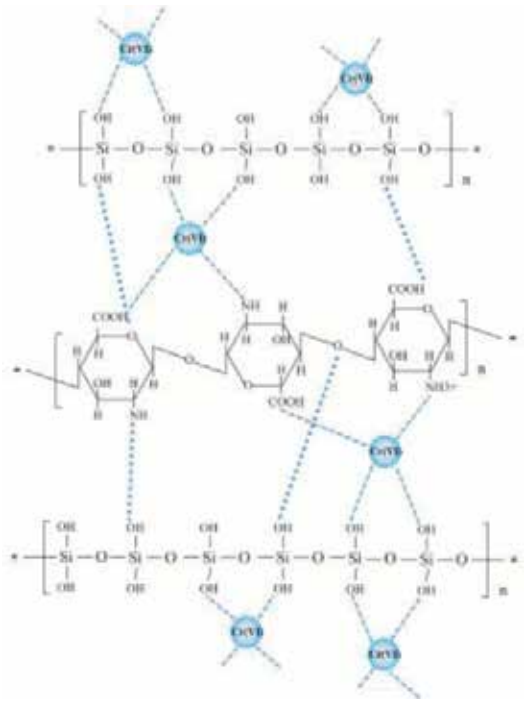

Figure 24. Adsorption mechanism for the removal chromium onto the membrane of carboxymethyl chitosan & silicon dioxide.

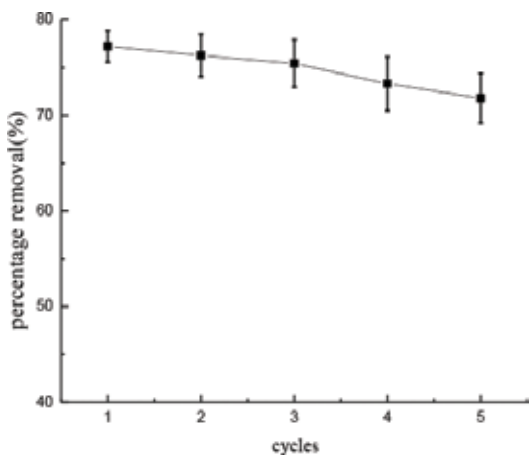

Figure 25. Adsorption capacity after desorption using the membrane of carboxymethyl chitosan & silicon dioxide.

adsorption capacity after desorption using the above leaching agent is shown in **Figure 25**. From this **Figure 25**, it is found that the membrane still present the high adsorption capacity (74.6%) towards Cr(VI) within three cycles.

4. Conclusions

The efficiency of the membrane synthesized as an adsorbent for Cr(VI) was investigated by batch techniques. The following conclusions can be drawn considering the results of this work:

1. The optimal conditions of adsorption Cr(VI) using the membrane of chitosan & silicon dioxide are determined. The optimal pH is pH 3; the optimal contact time is 80 min; the optimal dosage is 2.0 g dm^{-3} and 40 $mg \cdot dm^{-3}$ was considered as optimum initial concentration. The removal of Cr(VI) by the membrane was more than 80% under the optimal experimental conditions (at pH 5, contact time of 60 min, dosage of 0.25 g dm^{-3} and initial Cr(VI) concentration of 40 $mg \cdot dm^{-3}$). The adsorption of Cr(VI) using the membrane conforms to the Langmuir isotherm adsorption equation, and the correlation coefficients was 0.985. The maximum adsorption capacity of Cr(VI) calculated by Langmuir model was 21.2 $mg \cdot g^{-1}$.
2. The adsorption isotherm of Cr(VI) by the membrane of carboxymethyl chitosan & silicon dioxide was also more suitably described by Langmuir model, and the correlation coefficients was 0.998. It suggests that monolayer chemical adsorption of Cr(VI) on the membrane is more dominant. The maximum adsorption capacity was estimated as 80.7 $mg \cdot g^{-1}$ for Cr(VI) under the optimum conditions. The adsorption capacity of the membrane for Cr (VI) in this work is on a level with that of another adsorbents in previous works. The best fit was obtained with a pseudo-second order kinetic model while investigating the adsorption kinetics of Cr(VI) adsorption on the membrane, and the correlation coefficients was 0.990. The rate constant (k) are 3.4×10^{-2} $g \cdot mg^{-1} \cdot h^{-1}$.
3. From regeneration experiments (repetition of adsorption and desorption experiment), it is found that the membrane of chitosan & silicon dioxide still presents the high adsorption capacity (74.6%) towards Cr(VI) within three cycles.

From this work, it was quantitatively found that nanomaterials for desalination synthesized in this study can be an efficient adsorbent for Cr(VI). It is very significant information from the viewpoint of environmental protection, and can be used for treating industrial wastewaters including pollutants.

Acknowledgements

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Chapter 3

Chitosan-Clay Based (CS-NaBNT) Biodegradable Nanocomposite Films for Potential Utility in Food and Environment

Asmae Laaraibi, Fatiha Moughaoui, Fouad Damiri, Amine Ouakit, Imane Charhouf, Souad Hamdouch, Abdelhafid Jaafari, Abdelmjid Abourriche, Noureddine Knouzi, Ahmed Bennamara and Mohammed Berrada

Additional information is available at the end of the chapter

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Abstract

The aim of this work is to design newer material for food packaging applications and to valorize the Moroccan marine wastes using chitosan (CS) prepared from exoskeletons of shrimps. Biodegradable and uniform nanocomposite films developed from sodium bentonite nanoparticles dispersed in chitosan matrix were carefully studied. The montmorillonite is used as nanofiller, and aqueous acetic acid solution is employed as a medium for dissolving and dispersing chitosan and montmorillonite. The existence of dialdehyde chitosan as cross-linking agent was examined. Morphology, thermal behavior, and mechanical properties of the nanocomposite films have been studied using FTIR, TGA, FEGSEM, TEM, XRD, and a tensile test. The XRD results indicate the formation of an intercalated and exfoliated nanostructure at low bentonite content and an intercalated and flocculated nanostructure at high bentonite content. Plastic deformation of the chitosan film is carried out using a thermomechanical treatment in the presence of a solvent and a plasticizer. The nanocomposite films obtained show a good tensile strength due to the reinforcement of chitosan intercalation in the silicate, which is an interesting mechanical property needed for food packaging applications. These nanocomposite films made from naturally occurring materials might play an important role in advanced research in food and environmental science.

Keywords: chitosan, food, environment, montmorillonite, sodium bentonite, films, nanocomposite, biodegradable

1. Introduction

The increase in living standards, changing consumer habits, and industrial development led to a high consumption of biodegradable plastic materials [1]. One of the best options to reduce current packaging waste is the use of biodegradable films that allow the final replacement of plastic packaging bags which are not recycled and are thus a pollution source. The present research is directed toward the development of biodegradable ecofriendly materials with enhanced properties [2]. Chitosan (CS) is a deacetylated derivative of chitin, which is the most abundant polysaccharide in nature after cellulose; it is a natural polysaccharide, biocompatible, and biodegradable in addition to the antibacterial properties that can be useful in many areas as the food packaging industry. Chitosan which consists of a linear (1–4) linked 2-amino-2-deoxy-D glucan as shown in **Figure 1** is a relatively inexpensive material, next to cellulose.

Chitin and chitosan are biopolymers having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions, and applications [3, 4], as biomedicine [5, 6], pharmaceuticals [7–9], metal chelation [10, 11], and food additives [12] and in the fabrication of sensors or biosensors [13].

Chitosan is highly soluble in acid aqueous solution. The positive charge and molecular arrangement confer to chitosan's interesting properties [14]. **Figures 2** and **3** illustrate the protonation of chitosan which leads to soluble material. **Figure 4** shows the simulation of protonated chitosan backbone (positive charges of NH^{3+} onto chitosan polymer) in acid aqueous solution. These positive charges cause a mutual repulsion and thus a swelling behavior and good solubility of chitosan. The protonation constants p*K*a of chitosan decrease slightly, from 6.3 when the molecular weight reduces. The degree of deacetylation effects on p*K*a values. The decrease in degree of deacetylation increases the p*K*a. The degree of deacetylation influenced the balance of hydrophobic interactions and hydrogen bondings on chitosan [15].

In the same way, bentonite clays are also abundant and low-cost natural materials. Sodium bentonite is the name for the ore whose major constituent is the mineral, sodium montmorillonite. Montmorillonite is a three-layer mineral consisting of two tetrahedral layers sandwiched around a central octahedral layer. Bentonite is rich in montmorillonite (usually more

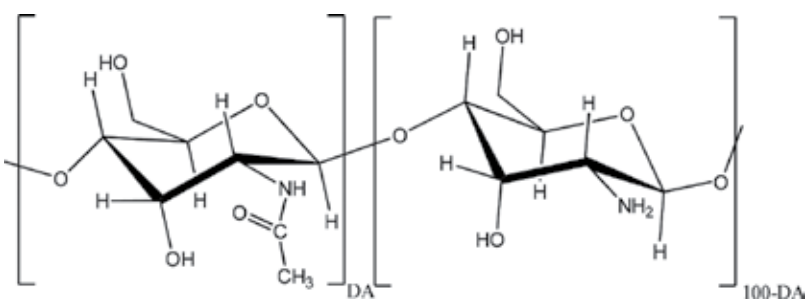

Figure 1. Chemical structure of Chitosan.

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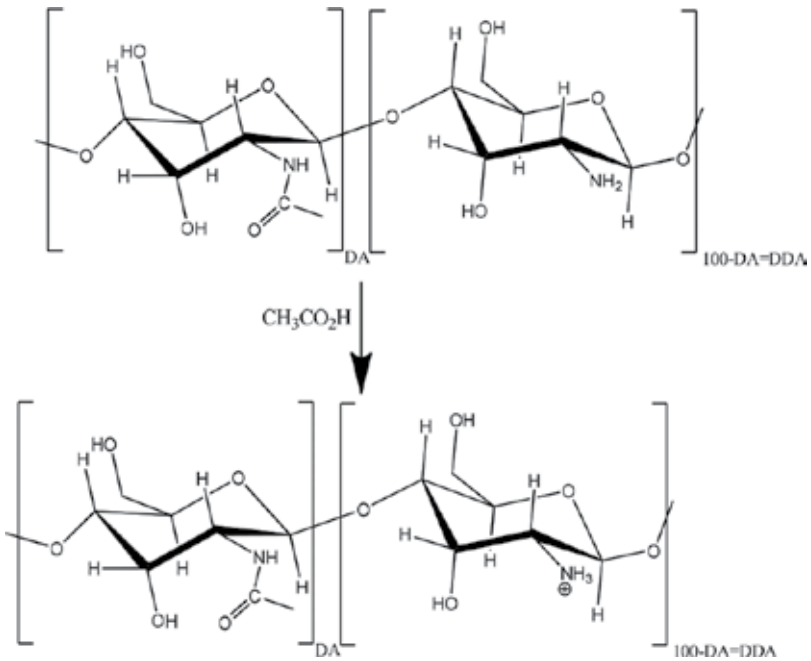

Figure 2. Protonation of chitosan in acetic acid aqueous solution.

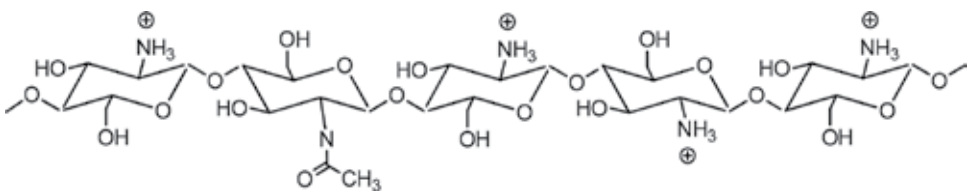

Figure 3. Polymer backbone of protonated chitosan in acid aqueous solution.

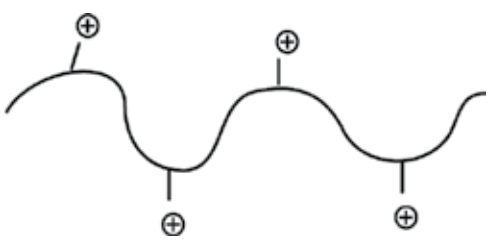

Figure 4. Simulation of protonated chitosan backbone in acid aqueous solution.

than 80%) [16–19]. Bentonite and montmorillonite names are often used interchangeably. However, the terms represent materials with different degrees of purity. Bentonite is the ore that comprises montmorillonite, inessential minerals, and other impurities.

When sodium bentonite comes into contact with water, the atoms and molecules dissolve, and ions with negative charges develop. These negative charges cause a mutual repulsion and thus a swelling within the clay structure. **Figure 5** shows the three-layer structure of a particle of bentonite and its exfoliation in sodium hydroxide aqueous solution.

The cations residing between the layers of sodium bentonite (Na-BNT or simply BNT) are exchangeable with ammonium ions of chitosan. This process converts the hydrophilic surface of the layer into a hydrophobic one, thereby improving the compatibility of nanoclay into polymer matrix.

The three processes that may occur in chitosan polymer and clay mixture, as shown in **Figure 6**, are intercalation, exfoliation, and conventional distribution. Intercalation is a physical process by which a macromolecule like a polymer is inserted in the clay sheets. Such a molecule is flanked by two clay layers and is immobilized and shielded. Exfoliation is a delaminating process wherein the gallery is expanded from its normal size of 1 nm to about 20 nm or higher. Thus there is a clear disruption of the layers which get spatially separated apart bringing about nanoscale dispersion in the polymer matrix. Thus exfoliated clays represent true nanomaterials. Intercalation and exfoliation of the clays can be accomplished using polymer from its solution or a melt [20].

Chitosan/bentonite composites are economically interesting because they are easy to prepare and involve inexpensive chemical reagents. Nanocomposites prepared from chitosan/bentonite shape natural films with a great potential and provide physical protection, they are biocompatible and biologically active toward microbial growth while being nontoxic and biodegradable. These nanobiocomposites obtained by adding nanofillers to biopolymers like chitosan result in very promising materials since they show improved properties with preservation of the material biodegradability without eco-toxicity [21].

Although chitosan/clay nanocomposites are very interesting materials, they were not extensively investigated as potential film packaging for food application. Thus, the aim of this work is to analyze the role of the chitosan/bentonite ratio, the DDA of chitosan, and a plasticizer

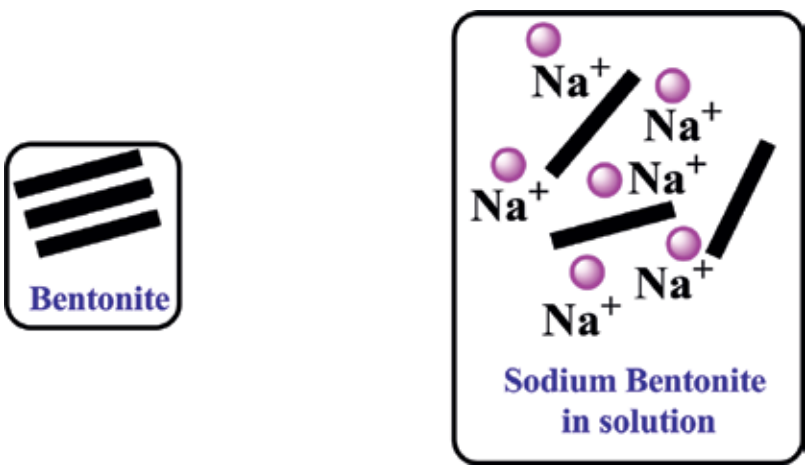

Figure 5. The three sheet structure of a particle of bentonite.

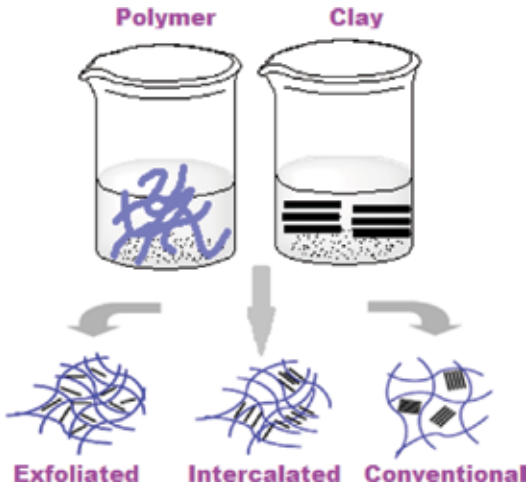

Figure 6. The three processes that may occur in polymer and clay mixture.

specially glycerol in the solution casting process for the achievement of chitosan/clay nanocomposite films. In order to investigate the combined effect of glycerol and unmodified clay on the properties of chitosan-based nanocomposites, films containing different amounts of clay and glycerol were prepared and characterized with particular regard to structural, thermal, and mechanical properties. Finally, new nanocomposite active films were proposed for safe packaging of edibles [22].

2. Material and methods

2.1. Materials

Chitosan (CS) with different degree of deacetylation (DDA) (from 70 to 100%) in powder form was prepared in our laboratory from exoskeletons of shrimp waste and purified [23, 24]. The chitosan chemical structure is schematically shown in **Figure 1**. The degree of deacetylation (DDA %) was determined by conductimetric titration [20].

Analytical grade sodium periodate ACS reagent, 99.8–100% dry basis, and bentonite were purchased from Sigma-Aldrich. Sodium bentonite used in this study was purified according to the method reported by H. Sedighi et al. [25]. The bentonite ore was beneficiated to improve its montmorillonite content by removing the impurities, generally albite, calcite, dolomite, orthoclase cristobalite, and quartz. The crude sample was primarily crushed at the size of 2 mm, and 5 g of bentonite powder was added to 100 mL of hot deionized water and stirred for 2 h. The separation of the impurities is obtained by a sedimentation process of the solution. The settled precipitate is mostly impurities, and the solid collected from the supernatant is generally pure montmorillonite. The slurry and solid phases were separated by filtration, and the remaining solid was dried at 150°C. All the other chemicals used are of analytical grade and used as received.

2.2. Preparation of chitosan film

Chitosan solution was prepared by dissolving 1 g of chitosan powder in 100 ml of aqueous acetic acid solution (1%, v/v), under continuous stirring at room temperature for 2 h followed by vacuum filtering to remove the insoluble residue. This solution was cast into Petri dishes and dried at 50°C for 20 h to evaporate the solvent and form the films. The dried films were soaked with an aqueous solution of 0.05 M NaOH to remove residual acetic acid, followed by rinsing with distilled water to neutralize, and then dried at room temperature.

2.3. Preparation of chitosan/bentonite (CS/Na-BNT) films

Chitosan/Na-BNT (also described as CSBNT) films were prepared using the casting/solvent evaporation technique. Firstly, 1% chitosan solutions were prepared by dissolving 1 g of chitosan powder in 100 ml of aqueous acetic acid solution (1%, v/v), under continuous stirring at room temperature for 2 h followed by vacuum filtering to remove the insoluble residue. Nanocomposite samples were obtained by dispersing selected amounts of bentonite in aqueous solution and stirred at 50°C until swelling was completed. After, the dispersion was slowly added to the CS solution to reach a final clay concentration of 1, 2, 3, and 5 wt% followed by stirring at room temperature for 5 h and then for 30 min at 25°C in ultrasonic bath. The amounts of chitosan, clay, and plasticizer used for each sample are listed in **Table 1**. For example, the composite film CSBNT1% is 1% BNT and 99% CS prepared from 1 g chitosan and 0.0101 g bentonite.

The nanocomposite solutions were then poured into Petri dishes and dried at 50°C for 20 h to evaporate the solvent and form the films. Free chitosan and nanocomposite films plasticized with glycerol were obtained by adding glycerol (30% (wt/wt) on solid CS) to the CS solution while stirring for 20 min at room temperature.

Following the same procedure used for chitosan films, the dried films were soaked with an aqueous solution of 0.05 M NaOH to remove residual acetic acid, followed by rinsing with distilled water to neutralize, and then dried at room temperature.

Chitosan/Na-BNT/cross-linker films were prepared using same method of manufacturing of chitosan/Na-BNT films. The dialdehyde chitosan was used as cross-linker and prepared according to I. Charhouf et al. [26] method and added after dispersing BNT in CS solution.

2.4. Oxidation of chitosan (cross-linker)

2.4.1. Preparation of dialdehyde chitosan

Mix 1 g chitosan ([CS] = 5.34 mM) in suspension with 50 ml HCl (10^{-3} M) (pH ranging from 4 to 5) with magnetic stirring. Mix with 1 ml aqueous solution of sodium periodate 0.534 mM, P0 = 0.1 (P0 = moles of $NaIO_4$ x moles of CS). The reaction was carried out at 4°C in the dark

Sample	Chitosan (g)-wt%	BNT (g)-wt%	Glycerol (g)-wt%
CS	1–100%	—	—
CSBNT1%	1–99%	1%	—
CSBNT2%	1–98%	2%	—
CSBNT3%	1–97%	3%	—
CSBNT5%	1–95%	5%	—
CSG	1–70%	—	30%
CSGBNT1%	1–69%	1%	30%
CSGBNT2%	1–68%	2%	30%
CSGBNT3%	1–67%	3%	30%
CSGBNT5%	1–65%	5%	30%

CS: Chitosan, BNT: bentonite, CSBNT1%: Film chitosan/bentonite 1%, CSBNT2%: Film chitosan/bentonite 2%, CSBNT3%: Film chitosan/bentonite 3%, CSBNT5%: Film chitosan/bentonite 5%, CSG: Film Chitosan/Glycerol, CSGBNT1%: Film Chitosan/Glycerol/bentonite 1%, CSGBNT2%: Film Chitosan/Glycerol/bentonite 2%, CSGBNT3%: Film Chitosan/Glycerol/bentonite 3%, CSGBNT5%: Film Chitosan/Glycerol/bentonite 5%.

Table 1. Amounts (g and wt %) of chitosan (CS), glycerol (G), bentonite (BNT), used for the preparation of chitosan, chitosan/glycerol, chitosan/BNT, and chitosan/glycerol/BNT.

for 30 minutes. After reaction, to eliminate the unreacted periodate, add 1 ml ethylene glycol. The oxidized chitosan was washed by distilled water for 4 h and freeze-dried.

The oxidation of chitosan using NaIO4 was well characterized as reported by I. Charhouf et al. [26]. In this work, we partially oxidized chitosan with a very few amount of sodium periodate. It is clearly seen from **Figure 7** that the oxidation reaction leads to opened structure of chitosan with dialdehyde functions.

2.5. Characterization and measurements

2.5.1. Infrared spectroscopy (FTIR)

Fourier transforms infrared (FTIR) spectra of the chitosan films and the chitosan/clay films were collected using a Tensor 37 FT-IR spectrophotometer (Spectrum 400 Perkin Elmer) operating in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.5.2. Mechanical properties: tensile measurement

Mechanical properties of chitosan/clay nanocomposite were measured with a Universal Testing Machine Ludwig mpK, tensile strength (TS), and percentage elongation at break (EL) of the films at 25°C according to ASTM D882 standard procedures [27]. The films were cut to a dog bone shape with a rectangular midsection (100 mm long x15 mm wide) flaring to 25 mm x

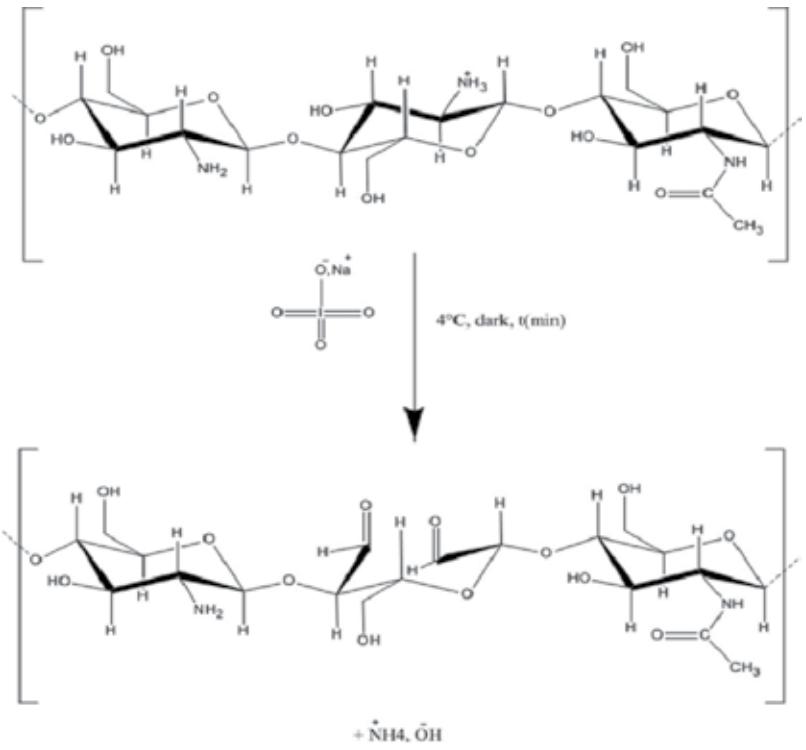

Figure 7. Oxidation reaction of chitosan by sodium periodate.

35 mm sections on each end. The thickness of each sample was measured with micrometer at three different locations and averaged. The films were conditioned at 50% RH for 72 h before each test. A 100 N load cell was used and the extension rate was set at 5 mm/min [28].

The tensile strength (σ) and percentage of elongation at break (E) were calculated using the following equations:

$$\sigma = F_{max}/A. \quad (1)$$

$$E = \Delta l/L_0 \times 100\,\%. \quad (2)$$

where F maxis the maximum load (N), A is the initial cross-sectional area (m^2), Δl is the extension of film strips (m), and L_0 is the initial length (m).

2.5.3. Thermal stability analysis

The thermal properties of nanocomposites and pure chitosan were investigated by thermogravimetric analysis (TGA). Samples were placed in the balance system and heated from 25 to 600°C at a heating rate of 10°C/min under a nitrogen atmosphere. Three replicates were tested for each sample.

2.5.4. XRD analysis

The X-ray diffraction analysis of the obtained films was performed by diffractometer with Cu Kα radiation (λ = 1.5418 A°) at room temperature. XRD scans were performed on sodium bentonite, chitosan films, and chitosan/bentonite films with a 2θ range between 5°-30°, at a scanning rate of 1°/min and scanning step of 0.01°.

2.5.5. Morphological analysis

Conventional high-vacuum scanning electron microscopy (SEM) images were also taken to visualize the structure of chitosan and oxidized chitosan. Chitosan and oxidized chitosan were freeze and dried for 24 h and were sprayed on silicon wafer substrate, then sputter-coated with gold (Agar Manual Sputter Coater; Marivac Inc., Montreal, QC, Canada), and imaged using a Quanta 200 FEG Environmental Scanning Electron Microscope (FEI Inc., Hillsboro, OR). Observations were performed at 20 kV using the high-vacuum mode.

The microstructural characterization of nanocomposites was carried out on the following samples: CSBNT3%, chitosan/bentonite 3%; CSBNT5%, chitosan/bentonite 5%; CSBNT10%, chitosan/bentonite 10%; CSBNT20%, chitosan/bentonite 20%; and CSG, chitosan/glycerol. Samples in powder form were coated in epoxy and ultramicrotomed with a diamond knife at -100°C. The recovered thin sections were observed at transmission electron microscope (TEM) (JEOL 2011) operating at 200 kV and the remaining blocks at field emission gun scanning electron microscope (FEGSEM) (Hitachi S 4700) at 1 kV after a slight platinum metal deposition.

3. Results and discussion

3.1. Preparation of bionanocomposite films

The intercalation of the cationic biopolymer chitosan into layered silicate clay (bentonite) through a cation exchange process results in nanocomposites with interesting structural and functional properties. Chitosan/Na-BNT films were prepared using the same method of manufacturing of chitosan films. However, Na-BNT was exfoliated in sodium hydroxide aqueous solution, purified, and washed prior to be added to chitosan solution. Organic matter is present in bentonite as intrinsic impurities composed predominantly of humid substances. Since competitive reactions can take place between the organic matter present in the bentonite and the chitosan, the extent of intercalation and polymer/clay interactions can be affected. Purification capable of removing of organic matter from bentonite before intercalation is fundamental.

The plasticization action of water molecules on hydrocolloid-based films has been widely reported in the literature [29, 31, 32]. In addition to water, the most commonly used plasticizer was glycerol (G), thus nearly systematically incorporated in most of biopolymer films [30]. Glycerol is indeed a highly hygroscopic molecule generally added to film-forming solutions to prevent film brittleness [31, 32]. The interest in use of the glycerol is that it acts as plasticizer and reduces the intermolecular forces by increasing the mobility of the biopolymer chains. The

glycerol reduces the extent interactions between Na-BNT stacks making it possible to achieve a better dispersion of nano-sized filler and can modify the ability of water to swell BNT in the aqueous solution, due to the ability to reduce the surface energy of aqueous solution.

In this study as shown in **Figure 8**, chitosan (CS)/(BNT) nanobiocomposite and chitosan (CS)/(BNT)/cross-linker films were prepared by the intercalation of chitosan in bentonite to form miscible, biodegradable nanocomposite material used as packaging films for food preservation.

Periodate oxidation of chitosan have been relatively little explored, with only a few studies on the periodate oxidation reaction and products formed. Recently, Charhouf et al. [33] studied the periodate oxidation and the physical characterization of oxidized chitosan more in detail. The periodate oxidation of chitosan obviously leads to changes in the chemical structure. The cleavage of C2–C3 in chitosan (CS) units leads to the formation of a dialdehyde. Reaction of cross-linking chitosan and dialdehyde chitosan takes place when dialdehyde group reacts with amine moiety of unmodified chitosan as shown in **Figure 9** giving a cross-linked material.

The nanocomposite films prepared by casting technique using two inexpensive resources available and biocompatible (chitosan and Na-bentonite) were obtained as shown in **Figure 10**.

The presence of a group like hydroxyl, on the surface of chitosan, facilitates encapsulation of essential oils (EOs) or bioactive compound. The nanoemulsions were used to stabilize the EOs in the chitosan matrix, without altering its film-forming properties [34]. We investigated different emulsion formulations to encapsulate essential oils and to study their effects on the in vitro antimicrobial activity against various microorganisms. **Figure 11** shows images of antimicrobial films casted from solutions containing modified chitosan (2% w/w), dyes, and essential oils (0.05% w/w).

Rosemary essential oil, with its warm and penetrating aroma, is one of the most stimulating oils used in aromatherapy. Rosemary was one of the earliest plants to be used in medicine, as well as for cooking. It has a very strong antiseptic action so it is terrific to use in aromatherapy recipes for cleaning. Incorporation of essential oils (EOs) in chitosan films was studied in order to prepare antimicrobial barriers to be applied to food surfaces. Essential oils

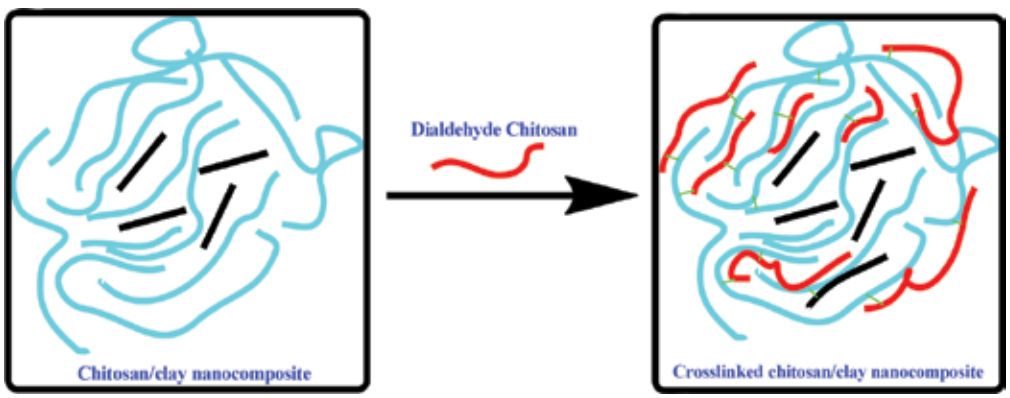

Figure 8. The sheets structure of exfoliated bentonite and dispersed in crosslinked chitosan matrix.

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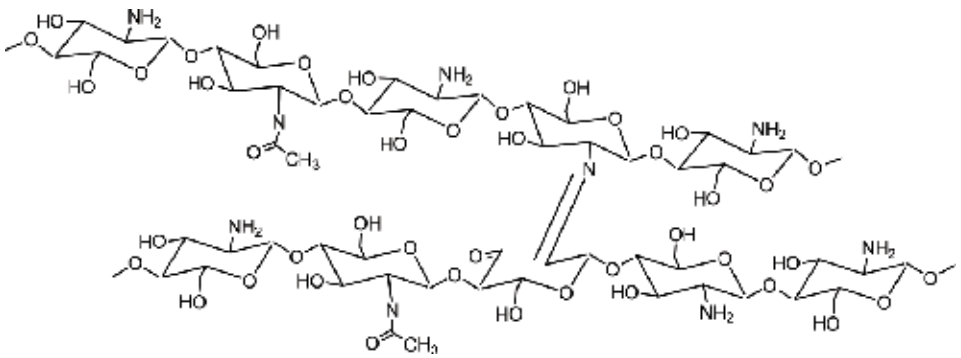

Figure 9. Crosslinking reaction between chitosan and dialdehyde chitosan.

were directly incorporated in the chitosan nanocomposite matrix. The EOs were selected by their ability to develop antimicrobial synergies against *Listeria* bacteria (monocytogenes or innocua) with chitosan, which is characterized by intrinsic antimicrobial properties.

3.2. Infrared spectroscopy (FTIR)

FTIR spectra of chitosan (CS), bentonite (BNT), and chitosan/bentonite nanocomposite (CSBNT) films are displayed in **Figure 12**. The spectrum of chitosan shows a broad peak at 3475.80 cm^{-1} corresponding to amine N–H symmetrical vibration and H bonded O–H group; the peaks at 2924.44 cm^{-1} were assigned to the symmetric and asymmetric $–CH_2$ vibrations of carbohydrate ring. The absorption peak observed at 1618.79 cm^{-1} was assigned to (C = O in amide group, amide I vibration), 1545 cm^{-1} was attributed to ($–NH_2$ bending of amide II), and

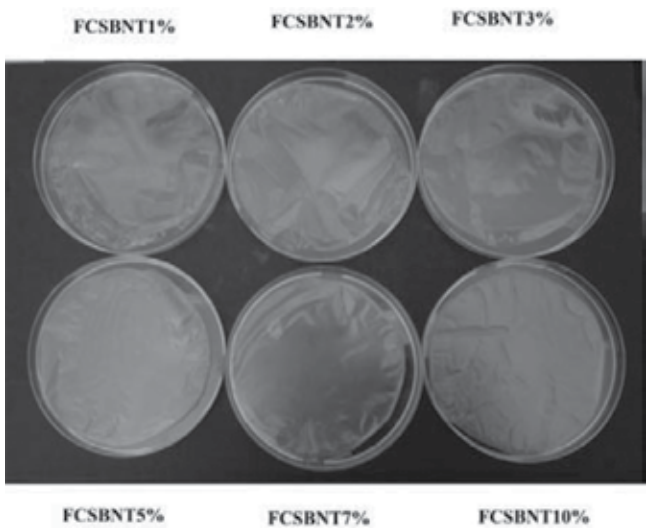

Figure 10. Images of chitosan/bentonite nanocomposite films casted from solutions containing chitosan (2% w/w) and bentonite at various amount of pure clay.

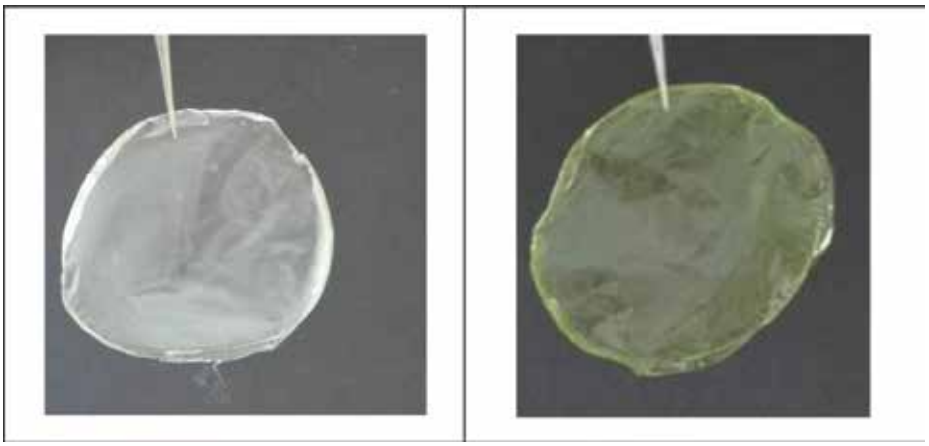

Figure 11. Images of antimicrobial films casted from solutions containing modified chitosan (2% w/w) and essential oils (0.05% w/w) added as pure rosemary essential oil.

1390 cm^{-1} was given to (N–H stretching or C–N bond stretching vibrations, amide III vibration). The peak at 1116.93 cm^{-1} corresponds to the symmetric stretching of C–O–C groups. The absorption peaks in the range 900–1200 cm^{-1} are due to the antisymmetric C–O stretching of saccharide structure of chitosan.

As can be seen in **Figure 12**, the FTIR spectrum of BNT shows a peak at 1010 cm^{-1} that belongs to Si-O-Si linkage. In addition, the characteristic absorption peaks are found at around 3670 cm^{-1} (stretching vibration of Al-OH and OH), at 3465 cm^{-1} (stretching vibration of O-H and H-O-H groups), at 1638 cm^{-1} (H-O-H bending vibration), at 933 cm^{-1} (Al-Al-OH bending frequency), and at 509 cm^{-1} (bending vibration of Si-O).

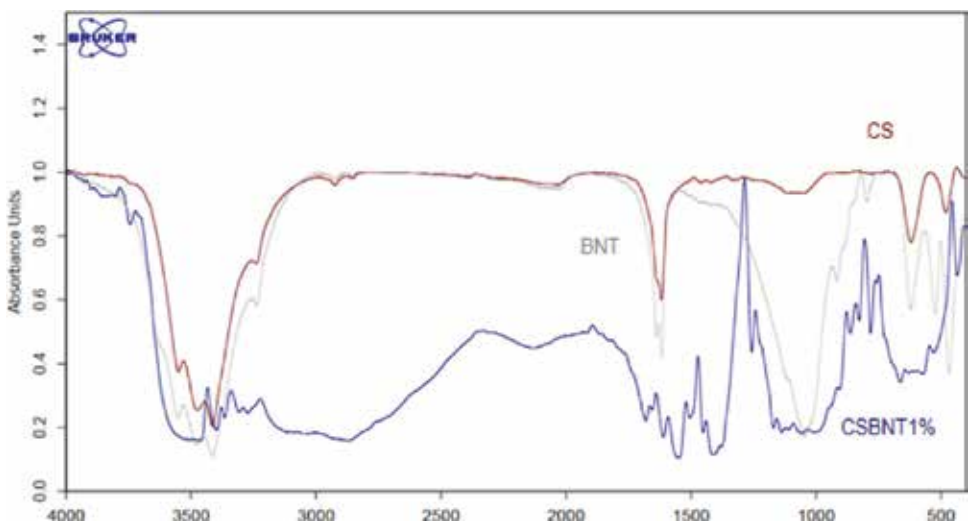

Figure 12. FTIR spectrum of: Chitosan film (FCSBNT0%), Chitosan/BNT films respectively (FCSBNT3%) and (FCSBNT5%).

The FTIR was also used to study the polymer/clay interaction, since a shift in the NH_3^+ vibration may be expected when – NH_3^+ groups interact electrostatically with the negatively charged sites of the clay [35]. Nevertheless, this shift is higher for CSBNT nanocomposite film with the lowest amounts of CS, while the chitosan/clay films with the highest amounts of biopolymer show a frequency value that trends to that observed in the films of pure chitosan (CS). This fact may be related to the $–NH_3^+$ groups that do not interact electrostatically with the clay substrate. The spectrum of CBNT nanocomposite film (**Figure 12**) shows a characteristic band at 3462.78 cm^{-1} attributed to hydrogen bonding formation due to the functional groups of CS (O-H and N-H groups) and BN (O-H groups) [36, 37]. The intensity of the NH_3^+ band also increases for higher amounts of intercalated chitosan. The secondary amide band at 1645 cm^{-1} of chitosan is overlapped with the HOH bending vibration band at 1628 cm^{-1} of the water molecules associated to the chitosan/clay films, which are present as in the starting clay, as expected for a biopolymer with high water retention capability [38, 39].

3.3. Tensile measurements of chitosan (CS), chitosan/bentonite (CSBNT), chitosan/glycerol (CSG), and chitosan/glycerol/bentonite (CSGBNT) films

The stress-strain curves of the tested specimens are being presented in **Figure 13**, while the average values along with the standard deviation of Young's modulus, tensile strength and elongation at break of the films on the stress-strain behavior of the chitosan and chitosan/glycerol films, respectively [40], are shown in **Figure 14**. The higher strength obtained in the case of the CS films can be attributed to more efficient stress transfer between the adjacent chains due to the strong electrostatic interactions between the NH_2 and NH_3^+ groups. The CSG specimen presents almost double strength (at yield and break) and elongation at break. Due to the lower acidity of the diluted films, a weaker hydrogen bond network was established between the amino groups and the glycerol chains. On the other hand, the extensive deformation strengthening in undiluted systems (CSG) suggests the creation of a long-range order and the formation of hydrogen bonding after the addition of glycerol.

The effect of BNT addition on the tensile response of the chitosan and chitosan/glycerol films is being depicted. The stress–strain curves of BNT composite films prepared from the 1 w/v% chitosan solution is being presented. The addition of BNT results in a pronounced enhancement of the stiffness and a dramatic decrease in the elongation at break of all clay-added systems. Further addition of BNT leads in intercalated structures which limited the polymer-clay interactions and thus their reinforcing ability.

The results on mechanical properties showed the increase in the tensile strength (TS) and elastic modulus (EM) of such nanocomposite films can be attributed to the high rigidity and aspect ratio of the nanoclay as well as the high affinity between the chitosan and the bentonite. On the other hand, the CS/BNT nanocomposites have shown significant decrease in elongation at break (EB). This reduction can be attributed to the restricted mobility of macromolecular chains.

In **Figure 14**, effect of BNT addition is being illustrated for the diluted systems (CS nanocomposites). The ductile response of the CS films is maintained after the addition of BNT with strength and a relative lower decrease in the elongation at break. The systems with 3 wt% BNT presented the lowest enhancement in all mechanical properties.

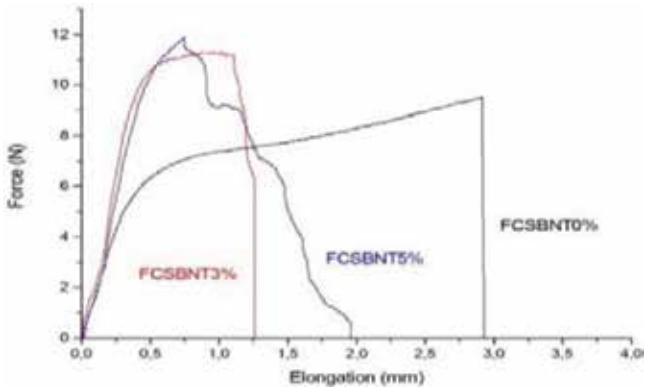

Figure 13. Stress-strain curves of chitosan film (FCSBNT0%), chitosan/BNT films (FCSBNT3%), (FCSBNT5%), respectively.

Figures 13 and **14** present the combined effect of glycerol and BNT on the tensile response of the CS-based nanocomposites. The first observation is that the addition of BNT results in a direct reduction of the strength of the chitosan/glycerol. A completely different stress–strain behavior is being obtained after the addition of BNT in diluted chitosan/glycerol systems (**Figure 14**). The CS/glycerol-based nanocomposites behave like hyperelastic materials rather than like ductile polymers. It is assumed that more water and glycerol are distributed in the chitosan network, inducing a very obvious plasticization effect. The extent of hydrated chitosan crystals was confirmed from the intensities of the XRD patterns. It is very interesting to note that although the mechanical properties of the unreinforced chitosan are comparable before and after the application of the reflux processing, reflux resulted in a fourfold increase of the stiffness and strength of the nanocomposite films.

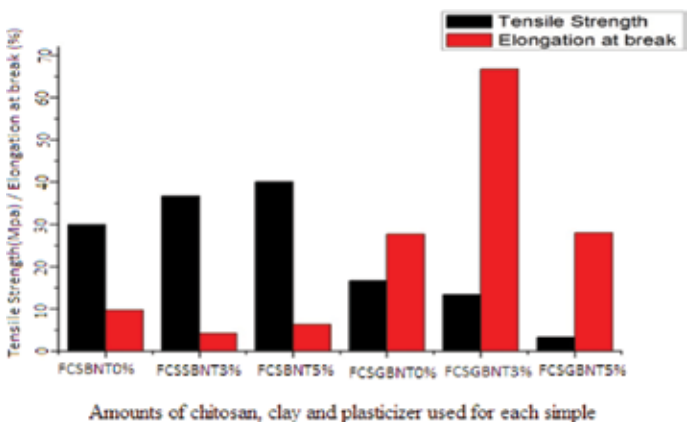

Figure 14. Mechanical properties of chitosan/BNT particles films. Chitosan film (FCSBNT0%), chitosan/BNT films (FCSBNT3%), (FCSBNT5%), respectively, and chitosan/glycerol film (FCSGBNT0%), chitosan/glycerol/BNT films (FCSGBNT3%), (FCSGBNT5%), respectively.

3.4. Thermal stability analysis

The thermal stability of the chitosan (CS) and its nanocomposites has been investigated by TGA under nitrogen (**Figure 15**). There are two steps of degradation. The first range (50–200°C) is associated with the loss of water, whereas the second range at 270°C corresponds to the deacetylation and degradation of chitosan, and the third step, in the temperature range 450–550°C, can be associated with the oxidative degradation of the carbonaceous residue formed during the second step.

The nano-dispersed clay in the chitosan matrix exhibits a significant delay in weight loss. The nanocomposite forms char with a multilayered carbonaceous-silicate structure, which may keep its multilayered structure in the polymer matrix. This high-performance carbonaceous-silicate char builds up on the surface during burning, thus insulating the underlying material and slowing the escape of the volatile products generated during decomposition. The decomposition temperature CS/BNT nanocomposites show higher thermal stability than those of the pure CS. The thermal stability of the nanocomposites increases systematically with increasing clay.

For nanocomposites containing glycerol, a further degradation step at T≈250°C is observed, related to the loss of unbound glycerol, as indicated in **Figure 16**. Furthermore, it can also be observed that the presence of glycerol plasticizer increases of about 20°C the degradation temperature for the third step, irrespective of the presence or not of the clay.

3.5. XRD analysis

The XRD patterns of chitosan and chitosan-based nanocomposite films in the range of 5–30° are shown in (**Figure 17**). The basal plane of BNT shows a reflection peak at about 2θ = 8.8°. After incorporating BNT within CS, with CS/BNT, the basal plane of BNT at 2θ = 8.8° disappears, substituted by a new weakened broad peak at around 2θ = 12.8°–13.0° (CSBNT3%, CSBNT5%). It is suggested that the BNT form intercalated and flocculated structures.

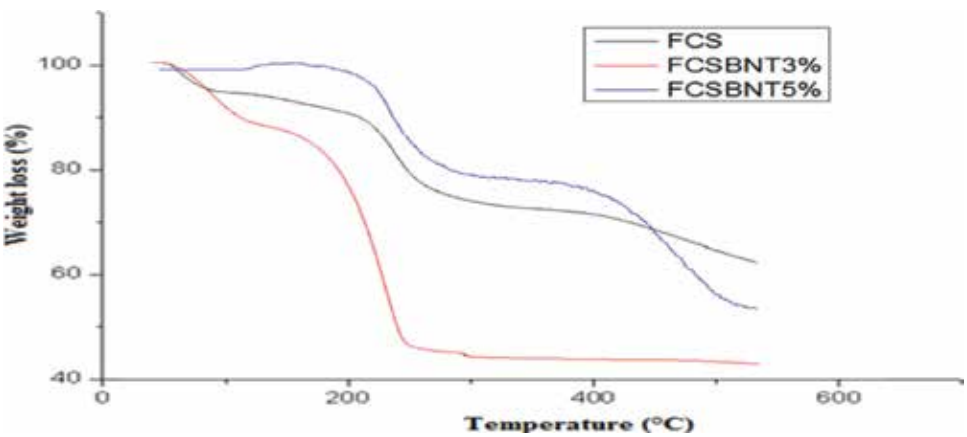

Figure 15. Thermal properties of: Chitosan (FCSBNT0%) and Chitosan/Bentonite films (FCSBNT3%), (FCSBNT5%).

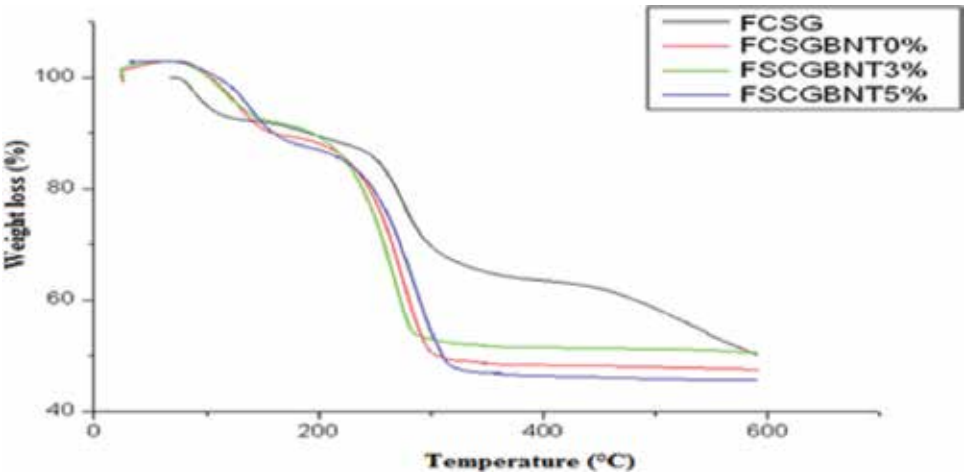

Figure 16. Thermal properties of: Chitosan/Glycerol (FCSG), Chitosan/Glycerol (FCSGBNT0%) and Chitosan/Glycerol/ Bentonite (FCSGBNT3%) and (FCSGBNT5%) films.

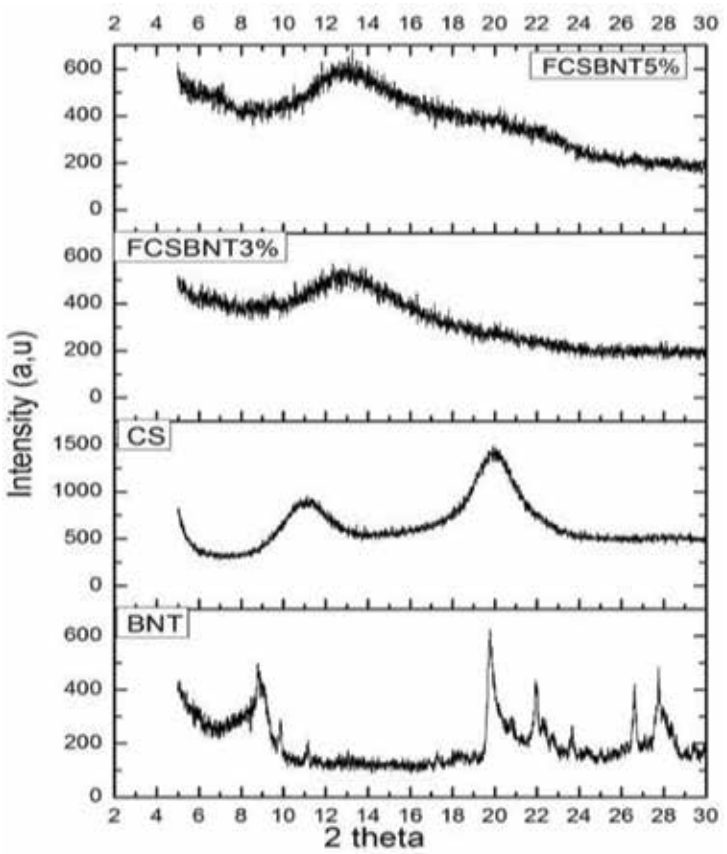

Figure 17. XRD patterns of: Chitosan (CS), Bentonite (BNT) and Chitosan/Bentonite films (FCSBNT3%) and (FCSBNT5%).

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On the base of XRD patterns, it is suggested that the BNT forms intercalated and exfoliated structures at higher CS content (CSBNT5%), while decreasing the CS content (CSBNT3%), clay layers (BNT) form intercalated and flocculated structures. According to [23], the formation of flocculated structure in CS/clay nanocomposites can be due to the hydroxylated edge-edge interactions of the clay layers. Since one chitosan unit possesses one amino and two hydroxyl functional groups, these groups can form hydrogen bonds with the clay hydroxyl edge groups. This strong interaction is believed to be the main driving force for the assembly of BNT in the CS matrix to form flocculated structures.

The XRD patterns of chitosan/glycerol films obtained from 30 w/v% solutions are shown in **Figure 18**. The addition of glycerol results in a pronounced peak at 12.5°. Because of the hydrophilic and polycationic nature of chitosan in acidic media, this biopolymer has good miscibility which is attributed to the interaction of glycerol molecules with chitosan

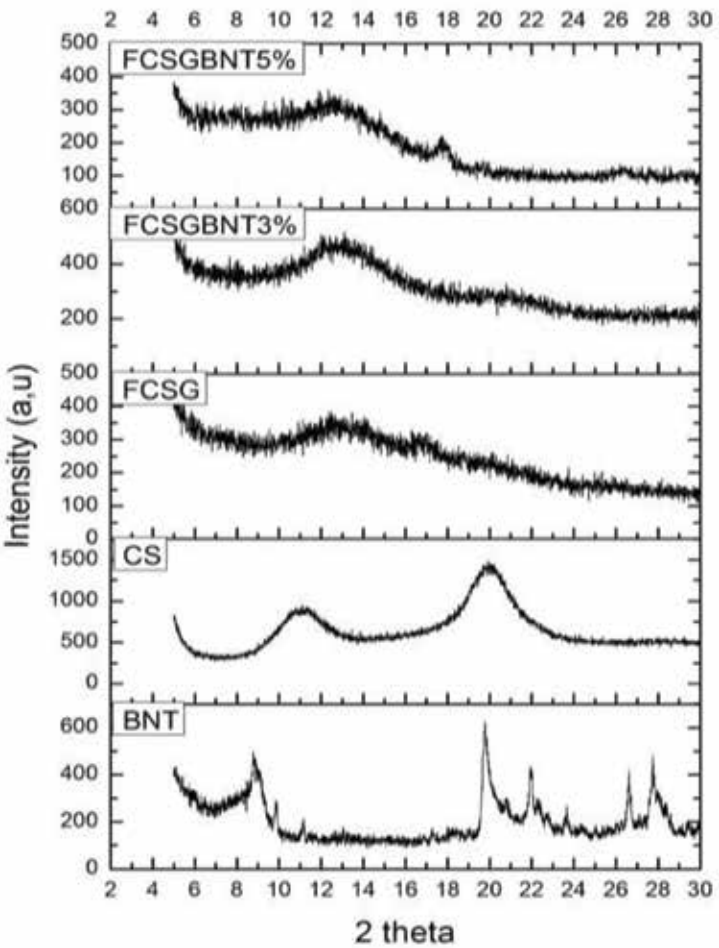

Figure 18. XRD patterns of: Chitosan (CS), Bentonite (BNT), Chitosan/Glycerol (FCSG) and Chitosan/Glycerol/Bentonite films (FCSGBNT3%) and (FCSGBNT5%).

macromolecules. Glycerol favors the chains mobility and thus the chitosan crystallization process in the early stage of the post-processing aging the effect of glycerol addition. The XRD patterns of chitosan/glycerol/BNT films obtained from chitosan solution are shown in **Figure 18**. The combined addition of glycerol and clays resulted in great enhancement of the chitosan crystallinity of the nanocomposite films prepared with 1 w/v% chitosan solution. This indicates that the presence of clay facilitates the distribution of glycerol within the chitosan matrix and the interaction of glycerol molecules with chitosan macromolecules. The combined addition of glycerol and clay had an opposite effect in films obtained from low content chitosan solution leading to decrease of the XRD peaks intensities. In addition a new peak at 18.2° appeared in XRD patterns of all obtained films. This diffraction peak is characteristic for chitosan films prepared using acetic acid solution as solvent.

The addition of glycerol favors the opening of the clay galleries resulting in intercalated nanocomposites in comparison to samples without glycerol.

Chitosan/Na-BNT nanocomposites exhibit an intercalated or intercalated/orientated structure of clays. In particular, the X-ray diffraction results show that in film without glycerol, the BNT stacks lay with their platelet surface parallel to the casting surface. The presence of glycerol, on the other hand, enhances the chitosan intercalation in the silicate galleries and hinders the flocculation process, leaving the BNT stacks randomly orientated in the space.

3.6. SEM and MET images of chitosan, oxidized chitosan, and bionanocomposite materials

The SEM images of chitosan and oxidized chitosan at high vacuum and at different magnifications are shown in **Figure 19**, showing that there is no change of elongated and fibrous network of chitosan, but on the surface of oxidized chitosan, we can see a slight degradation of some leaves.

3.6.1. FEGSEM analysis

We present in this study the microstructural characterization results obtained on the chitosan/Na-BNT nanocomposites too. The dispersion and the exfoliation of the clay were observed at field emission gun scanning electron microscope (FEGSEM) and transmission electron microscopy (TEM).

The results of the observation of the microtome block of chitosan/bentonite (CSBNT3%) and (CSBNT5%) samples are presented in **Figure 20**.

At low magnification, the CSBNT 3% powders were observed, and clusters were found grouped into in the epoxy more matte and dark appearance (**Figure 20a**). Decohesion between the epoxy and the sample powders is visible in greater or lesser proportion, probably due to preparation and cutting. At higher magnification, small clay particles of a few hundred nanometers are observed, which are relatively well dispersed in the polysaccharide (**Figure 20b**). However, a large clay aggregate of about 10 microns was also observed (**Figure 20c** and **d**).

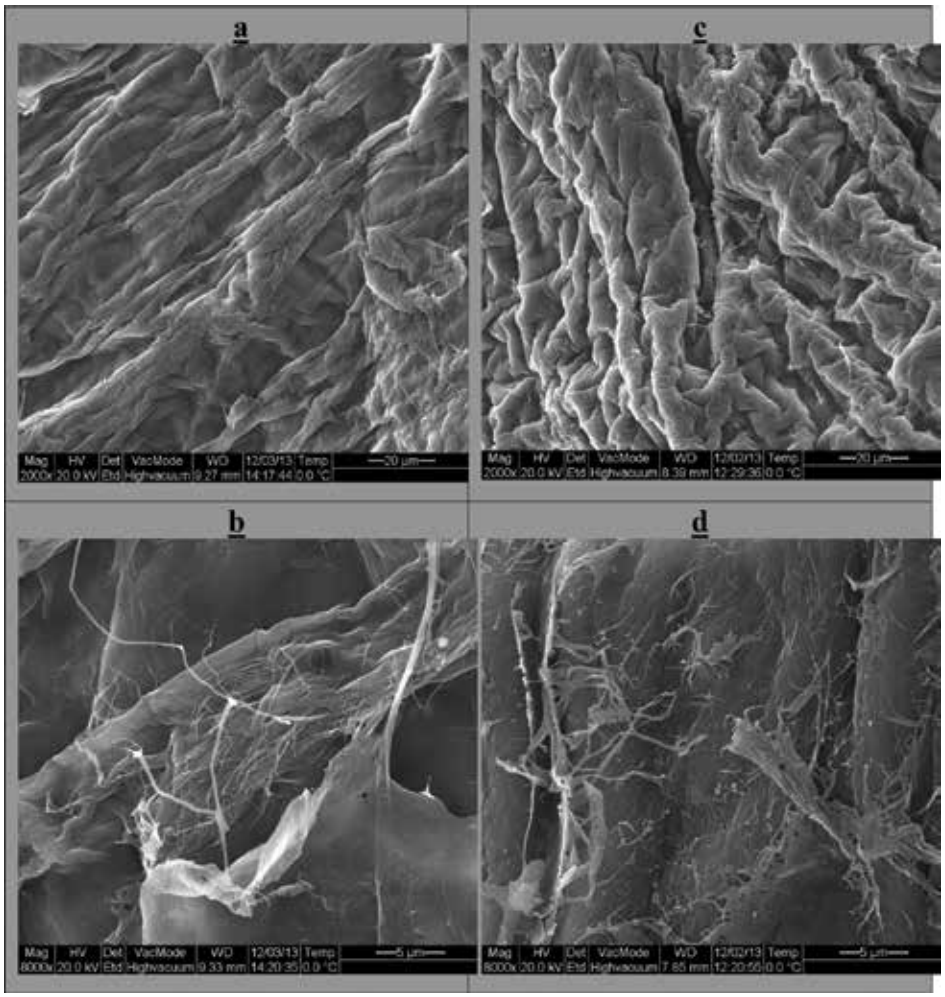

Figure 19. SEM images of (a & b) chitosan and (c & d) oxidized chitosan at different magnifications.

At higher magnification, small clay particles similar in size to those observed in the CSBNT 3% sample are regularly observed with chitosan/BNT 5% as shown in **Figure 21**.

3.6.2. TEM analysis

Transmission electron microscopy (TEM) images indicated that the silicate layers were dispersed in the chitosan matrix. The results of the TEM observation of the chitosan/BNT 3% sample show that small particles of clay from less than 100 nanometers to a few hundred nanometers are observed at low magnification which is consistent with the FEGSEM observations. Larger aggregates of clay are also observed but more rarely.

Depending on the level of the clay particles, the leaflets are sometimes well aligned (**Figure 22**) and sometimes of more unstructured appearance (**Figure 23**). This unstructured aspect of the

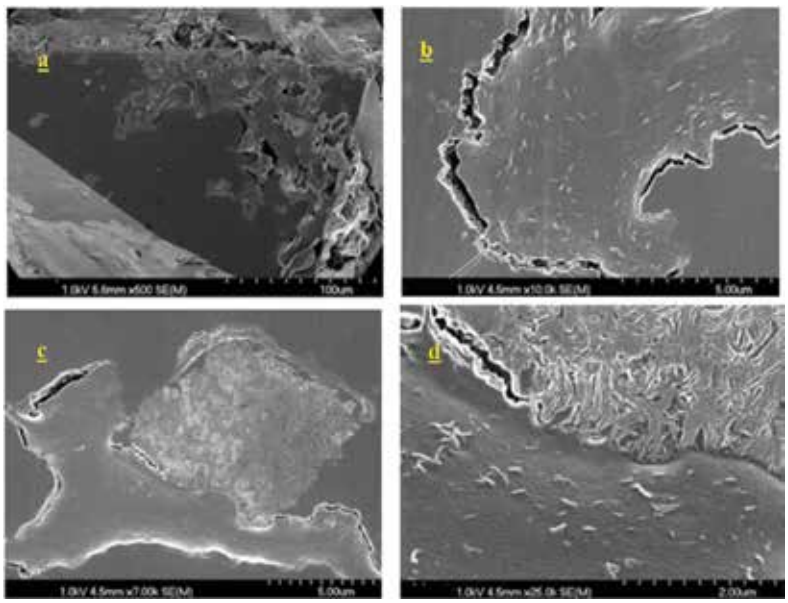

Figure 20. Images of FEGSEM of chitosan/BNT 3%.

clay sheets may be a sign of a more advanced level of intercalation. There are also some isolated single or double leaflets around other clay particles (**Figure 23**), which is a clear sign of exfoliation.

As expected, the concentration of clay in the polysaccharide affects the dispersion of the clay. The higher the concentration, the poorer the dispersion is obtained which is the effect of the greater aggregation of the clay. A good but not always uniform dispersion is observed in chitosan/BNT 3% and 5%. Similarly, the concentration of clay also appears to affect the level

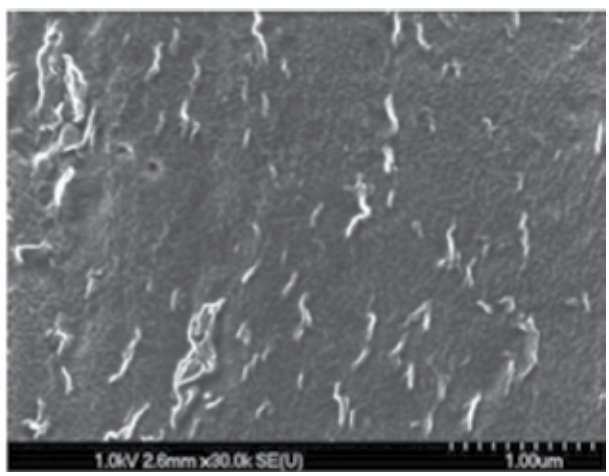

Figure 21. Image of FEGSEM of chitosan/BNT 5%.

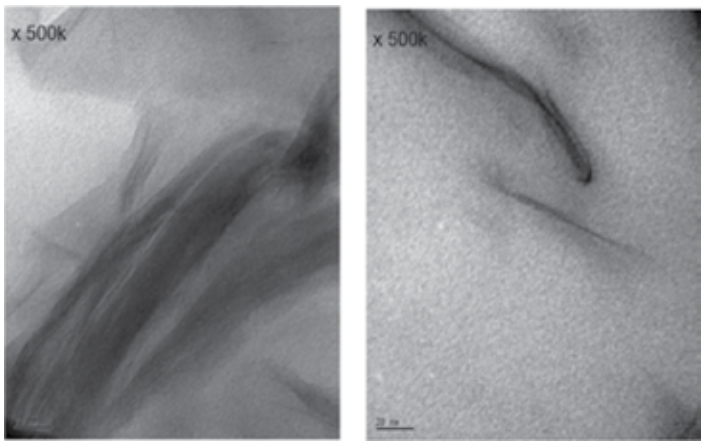

Figure 22. Images TEM of chitosan/BNT 3% (x 500K).

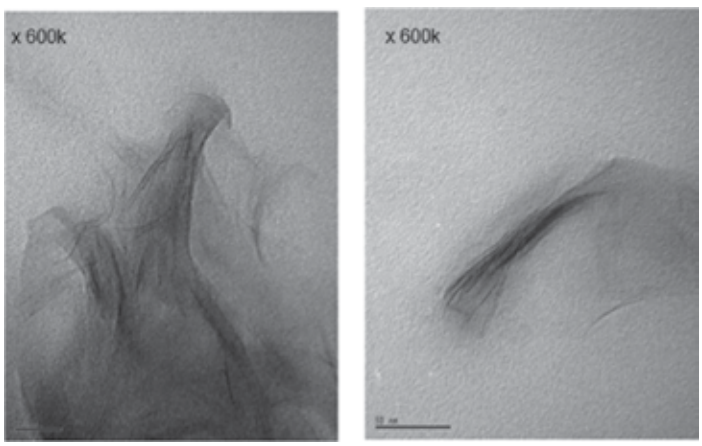

Figure 23. Images TEM of chitosan/BNT 3% (x 600K).

of intercalation/exfoliation. Based on MET observations, signs of exfoliation (the presence of isolated single or double clay leaflets and more unstructured appearance of the leaflets in the particles) are visible in samples of lower clay concentration less than 10%.

4. Conclusion

Natural biopolymer-based biodegradable packaging materials are a new generation of polymers emerging on the packaging market, and driven by the perception that biodegradable plastics are "environmentally friendly," their use is predicted to increase chitosan, a natural material that has interesting antimicrobial and film-forming activities. Its application in films can contribute to food preservation and shelf-life extension. In this study, various films were successfully prepared

by the solution casting technique and characterized with particular regard to structural, thermal, and mechanical properties. Films of chitosan/bentonite, chitosan/glycerol/bentonite, and chitosan/glycerol/bentonite/essential oil nanocomposites were prepared with purified bentonite (BNT), and according to the process used, they might be less expensive than other packaging materials.

Exfoliated chitosan/clay nanocomposites of varying clay contents have been successfully prepared with or without the presence of glycerol (plasticizer) and oxidized chitosan (crosslinker). This approach represents a new route to prepare high-performance nanocomposite materials. The oxidized chitosan could partially react with the amine groups on chitosan; as a result, high mechanical properties can be obtained. The Na-BNT layers are exfoliated by chitosan chains and disorderly dispersed in the chitosan matrix, as confirmed by XRD and TEM characterization. The incorporation of a small amount of clay into the chitosan matrix results in obvious enhancement in the thermal properties of chitosan. The chitosan/clay nanocomposites retain good mechanical properties. Once the clay is exfoliated and efficiently dispersed into the chitosan matrix, the storage modulus and tensile property of the chitosan/clay nanocomposites are significantly improved with respect to that of neat chitosan.

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Chapter 4

A Review of Chitosan-Based Materials for the Removal of Organic Pollution from Water and Bioaugmentation

Carlos Escudero-Oñate and Elena Martínez-Francés

Additional information is available at the end of the chapter

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Abstract

Chitin is a natural polymer extracted mostly from shrimp or crab shells and is the Earth's second most abundant polysaccharide. After a simple deacetylation procedure, chitin is converted into chitosan that consists in a polysaccharide structure of deacetylated-β-glucosamine. Chitosan has been largely employed in wastewater treatment the removal of colloids through coagulation-flocculation processes. Different chitosan based materials have been produced and tested in the removal of inorganic pollutants such as toxic metals and metalloids, nutrients, dyes, micropollutants and hydrocarbons. Sorbents such as magnetic-activated carbon chitosan have been successfully tested in the removal of antibiotics (ciprofloxacin, erythromycin and amoxicillin) from water. Raw chitosan and ZnO nanoparticles entrapped in chitosan have demonstrated an excellent potential for the removal of the insecticide permethrin from aqueous effluents. Chitin and chitosan in flake and powder form have also demonstrated a promising effectiveness in the removal of oil spilled in seawater. Superhydrophobic and superoleophilic sponges modified by thioles have been also prepared from chitosan and used for the removal of oil spills. Chitosan hydrogels have been tested as well as entrapment matrices for the immobilization of hydrocarbon-degrading biomass for oil spills. Strains such as *R. corynebacteriorides* (QBTo), *Bacillus subtilis* LAMI008 and *B. pumilus* have been successfully immobilized and employed in hydrocarbon degradation processes. In this book chapter, the use of chitosan and chitosan-based materials in the removal of organic pollutants from water is reviewed.

Keywords: chitin, chitosan, adsorption, organic pollutants, sorbents, oil spill pollution, bioaugmentation, water treatment

1. Introduction

Chitin is a natural polysaccharide and the second most abundant biopolymer on Earth after cellulose [1]. This biopolymer consists of units of β-(1–4)-N-acetyl-D-glucosamine and is the main component of the exoskeleton of arthropods and crustaceans but also can be found in relevant amount in the cell walls of fungi. Chitin is a structural biopolymer whose role is analogous to that of collagen in the higher animals and cellulose in terrestrial plants [2]. In a similar way, plants produce cellulose in their cell walls, and insects and crustaceans synthetize chitin and accumulate it in their shells. Chitin may be regarded as cellulose with hydroxyl at position C2 replaced by an acetamide group (-$CONH_2$). This similarity partly explains some analogies occurring in chitin and cellulose, such as low solubility and low chemical reactivity [3]. Chitin is found in three polymeric forms, α-, β- and γ-chitin, usually found in shrimp and crab shells, squid pen and stomach cuticles of cephalopod, respectively. From the three aforementioned forms of the biopolymer, α-chitin is the most abundant and stable form. α-, β- and γ-chitin correspond to antiparallel, parallel and alternated arrangements of polymer chains, respectively. A hydrogen bond between the acetamide group on the C2 carbons and the secondary alcoholic hydroxyl groups on the C3 carbon is linked through a water molecule with the primary alcoholic hydroxyl groups on a C6 carbon. As a result of this configuration, chitin possesses a strong crystalline structure, which explains the high chemical and solvent stability of the biopolymer. Due to its crystalline structure, chitin exhibits remarkable differences from cellulose in the solubility and reactivity despite of the relatively similar chemical structure [3].

The production of chitin uses basic raw materials of the cuticles of various crustaceans, principally crabs and shrimps. In regular fishery wastes, the biopolymer chitin is associated with proteins, minerals, lipids and pigments [4]. All these substances are considered impurities, and they all have to be quantitatively removed to achieve the required purity of the chitin. The chitin is normally extracted from the carapaces from crustaceans treating the crushed material with acid to achieve complete dissolution of the calcium carbonate structure. After this process, the material is submitted to an alkaline extraction to achieve the solubilization of the proteins. In a later purification step, the material obtained from the deproteinization process follows a decolorization step to remove residues of pigments to yield an almost colorless product [5]. Partial deacetylation of chitin leads to the formation of the polymer chitosan, consisting of units poly(D-glucosamine). A scheme of the production of chitosan from waste crustacean shells following chemical and biological approaches is presented in **Figure 1**.

When the degree of deacetylation of chitin reaches about 50%, the material obtained starts becoming soluble in aqueous acidic media and is called chitosan [5]. The degree of deacetylation is indicative of the amount of amino groups (-NH_2) along the chitosan chain and refers to the degree of removal of acetyl groups (-$COCH_3$) from the amido moieties. The degree of deacetylation and the degree of polymerization (DP), which in turn decides molecular weight of polymer, are two important parameters dictating the use of chitosans for various applications [1]. A scheme of the deacetylation reaction that converts raw chitin into chitosan is presented in **Figure 2**.

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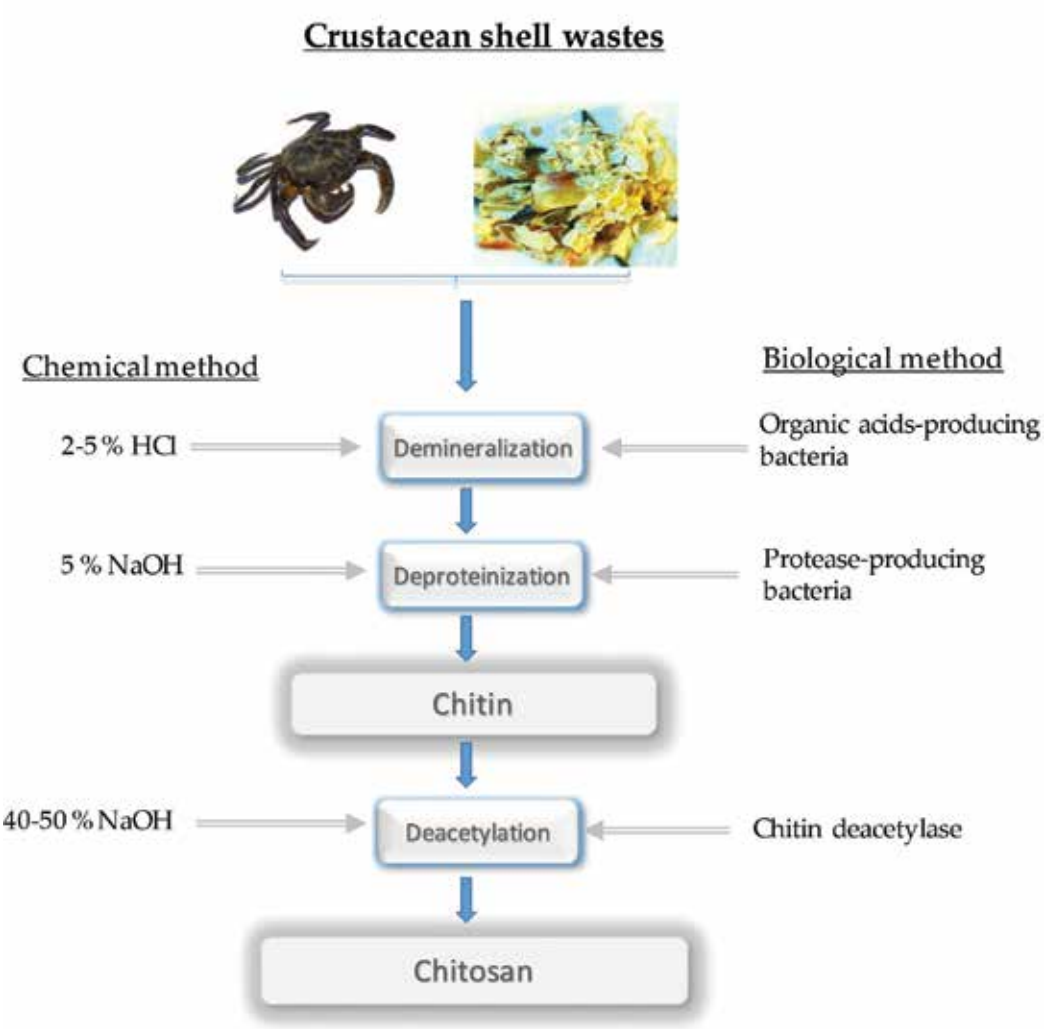

Figure 1. Production of chitin and chitosan using chemical and biological methods (adapted from Jo and co-authors [6]).

Chitosan is insoluble in water, alkali and organic solvents but soluble in most solutions of organic acids when the pH of the solution is lower than 6. Acetic and formic are two of the most widely used acids employed to solubilize chitosan. Some dilute inorganic acids such as nitric acid (HNO_3), hydrochloric acid (HCl), perchloric acid ($HClO_4$) and phosphoric acid (H_3PO_4) can also be used to prepare chitosan solutions but only after prolonged stirring and warming [7]. At low pH chitosan remains as a polycationic species, due to protonation of the amino group according to **Figure 3** [8].

Chitosan exhibits a unique set of properties that makes this polymer a great candidate for the development of water treatment processes. Among them, the most relevant are its high biodegradability, low toxicity, low price and natural availability. The weaknesses exhibited by

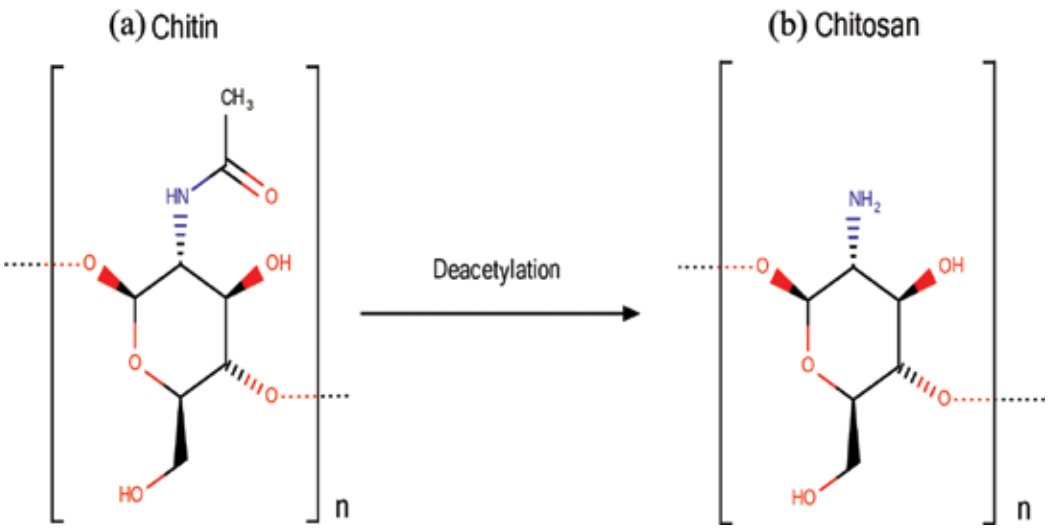

Figure 2. Chemical structure of (a) chitin and (b) chitosan.

this biopolymer however derive from its low acid stability, poor mechanical properties, low thermal stability, resistance to mass transfer, low porosity and surface areas [9, 10]. In order to overcome the drawbacks exhibited by chitosan, a large amount of effort has been devoted to the development of physicochemical modification methods to include different types of functionalization in the polymer. Chemical modifications such as oligomerization, alkylation, acylation, quaternization, hydroxyalkylation, carboxyalkylation, thiolation, sulfation, phosphorylation, enzymatic modifications and graft copolymerization have been carried out, allowing obtaining modified properties for specific end used applications in a large variety of fields [1]. Following physical and chemical methods, researchers have managed to produce a variety of forms of chitosan such as gel beads, membranes, film, fibers, porous frameworks, powders, sponges, hollow fibres and nanoparticles [11–20]. Some chemical modifications have been found capable of enhancing its flexibility and chemical stability and lower its susceptibility to acidic media. Among the most widely employed modifications that contribute modulation of

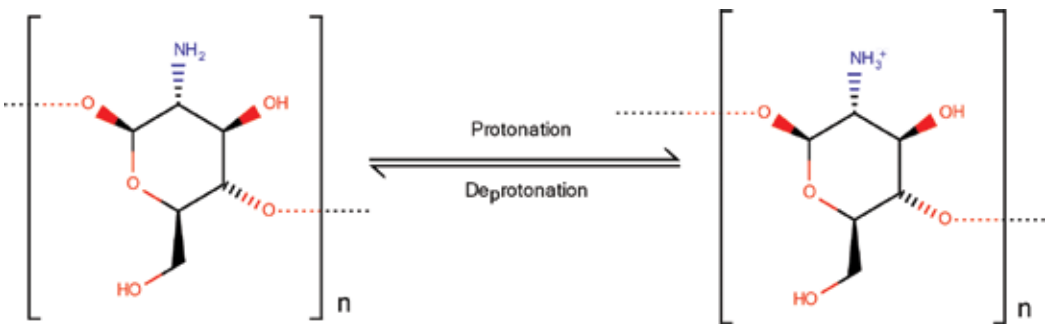

Figure 3. Protonation and deprotonation equilibrium of chitosan.

the functional properties of chitosan while improving the mechanical strength, cross-linking might be found. Several reagents such as glyoxal, glutaraldehyde and epichlorohydrin have been employed to reinforce the structure of chitosan by cross-linking [19, 21–23].

2. Chitosan-based systems for the removal of organic pollutants

Chitosan has revealed a large potential in the detoxification of polluted effluents. This biopolymer on itself and chitosan-based materials have not only shown a high capacity to remove a variety of toxic metals such as Cu(II) [24–27], Pb(II) [28, 29], Cr(VI) [30, 31], As(V) [32], Mo(VI) [33] and Hg(II) [34] but also have demonstrated a large potential to remove other concerning inorganic species from water such as nutrients (NO_3^-, NO_2^- and PO_4^{-3}) [35, 36]. In addition to the abatement of inorganic pollution from water, this biopolymer has been also explored as sorbent against organic pollution. In the next sections, we review the use of raw chitosan and chitosan-based materials in the removal of micropollutants and in the abatement of hydrocarbon pollution associated to oil spills.

2.1. Chitosan-based materials for the removal of organic pollutants

In recent years, chitosan-based composites using metals [37], metal oxides [38] and bimetals [39] have been receiving a large attention as alternative sorbents in water treatment processes. These kinds of materials have been chosen primarily due to their high adsorption capability [40]. Arayne and co-workers in 2011 studied the potential of raw chitosan beads and chitosan beads modified with ZnO nanoparticles (Cs/ZnO NPs) to remove permethrin, an insecticide largely employed in agriculture [41]. Through this study, the authors demonstrated that chitosan beads have an excellent adsorption performance and that the removal efficiency from a 0.1 ppm solution of permethrin increased from 49% when using chitosan beads to 99% when using Cs/ZnO NPs. Despite chitosan demonstrated a high capacity on itself to remove permethrin, including Cs/ZnO NPs in the matrix of the biopolymer, provided a large enhancement that leads to the almost total removal of this pollutant [42]. Saifuddin and co-authors, also in 2011, reported the use of a composite system based on cross-linked chitosan-silver nanoparticles (Cs-Ag NPs) to remove the pesticide atrazine (a very persistant herbicide of the triazine class) [43]. In their study, the kinetics of removal of the pesticide by the developed sorbent was evaluated. The authors reported an equilibrium time at about 65 min for 1, 5, 10, 20 and 25 ppm of atrazine. During their experiments, the authors detected a remarkable increase in the reduction of the pesticide content in water when increasing the sorbent dose. At the dosage of 2.0 g/L of Cs-AgNPs composite, 98% of the initial concentration of atrazine (from 1 ppm solution) was removed [43].

Danalıoğlu and co-authors, in a recent study, developed and tested a novel adsorbent based on a composite magnetic-activated carbon/chitosan system (MACC) for the removal of three widely employed antibiotics: ciprofloxacin, erythromycin and amoxicillin [44]. In their study, MACC nanocomposite demonstrated a good adsorption performance towards the targeted antibiotics [44]. Herein, the initial concentrations of antibiotics tested were 15, 60 and 60 ppm for ciprofloxacin, erythromycin and amoxicillin, respectively. To the initial antibiotic solution,

1 mg of MACC adsorbent was added. As a result, adsorption took place rapidly during the first 30 min, and then the adsorption rate slowed down to reach the equilibrium at about 120 min. The authors performed a set of equilibrium experiments and, by means of the Langmuir isotherm model, managed to calculate the maximum sorption capacity of the material for the different antibiotics (90.01 mg/g for ciprofloxacin, 178.57 mg/g for erythromycin and 526.31 mg/g for amoxicillin [44]). Danalıoğlu et al. also compared the adsorption capacity of MACC for ciprofloxacin to that reported for magnetic alginate-Fe_3O_4 hydrogel fiber and graphene oxide/calcium alginate [44]. Magnetic alginate-Fe_3O_4 hydrogel presented an adsorption capacity for ciprofloxacin ranging from 153 to 555 μg/g [45], and graphene oxide/calcium alginate varied from 18.45 to 39.06 mg/g [46].

In addition to the studies described above, other researchers have reported the removal of phenol and o-chlorophenol using chitosan beads modified with sodium alginate and calcium chloride [47]. These authors reported that such a modification improved the stability of the obtained material as well as the sorption capacity of the beads. The maximum sorption capacity for phenol reported by the authors in this study was 108.69 mg/g, while for o-chlorophenol was 97.08 mg/g. Lie and colleagues investigated the use of raw chitosan, chitosan chemically modified with salicylaldehyde (CS-SA), β-cyclodextrin (CS-CD) and a cross-linked β-cyclodextrin polymer (EPI-CD) in the removal of phenol, p-nitrophenol and p-chlorophenol from aqueous solution [48]. In their study, it was observed that the adsorption capacity of unmodified chitosan for phenol was remarkably lower than that observed for the modified biopolymer. While the chitosan modified by CS-CA was able to achieve a capacity of 8.50, 20.49 and 44.92 mg/g for phenol, p-chlorophenol and p-nitrophenol, respectively, the raw, unmodified chitosan was only able to barely remove about 2 mg/g of the substances. On the other hand, the sorption capacity of chitosan chemically modified by CS-CD was 34.93, 179.73 and 20.562 mg/g for phenol, p-chlorophenol and p-nitrophenol. The last modification by EPI-CD led to the sorption capacities of 131.50 mg/g (phenol), 74.25 mg/g (p-chlorophenol) and 41.11 mg/g (p-nitrophenol) [49].

The removal of phthalate esters (PAEs) [50] by molybdate-impregnated chitosan beads (MICB) in an aqueous solution has also been reported by Chen and co-workers in 2007. The experiments performed by the authors indicated that all PAEs studied were adsorbed by MICB; however, diheptyl phthalate (DHpP) was most efficiently removed, achieving capacity values of 3.01 mg/g and a removal value of 92.5% [51].

2.2. Chitosan-based materials for the removal of oil pollution from water

Among the different types of organic pollution affecting water bodies, a specially concerning type nowadays is the oil pollution. While catastrophic spills such as the Exxon Valdez oil spill in the coast of Alaska (1989) or the BP Deepwater Horizon oil spill in the Gulf of Mexico (2010) caused a very important harm and gathered a large amount of public attention, most of the oil spills are less extraordinary [52]. It is estimated that about 9 million barrels of oil are released globally into the oceans every year. Of this amount, however, more than half come from natural seepage from the ocean floor, and human consumption activities represent the second largest source of oil released into the oceans (about 35%) [52]. Eco-friendly and sustainable approaches to remove oil pollution from water are therefore required to avoid the environmental threat and hazards associated to it.

Different kinds of adsorbents have been explored for the removal of oil droplets from oil-in-water emulsions. For instance, activated carbon, biopolymers, organoclays, sawdust, vermiculite, walnut shell and resins have been tested for this purpose [53–58]. Biopolymer-supported materials have demonstrated being an efficient adsorbent for the removal of several contaminants from aquatic ecosystems. However, some of them have exhibited limitations when facing the scenario of oil removal. Chitosan has demonstrated being one of the most efficient biopolymers for the removal of oil droplets from water. The polymer, in addition to its oil sorption capacity, exhibits a unique structure that is very prone to chemical functionalization, allowing a large versatility in the production of novel sorptive materials with oil-enhanced selectivity and capacity. In addition to this, chitosan has a good biodegradability, biocompatibility, eco-friendliness and low cost [59, 60].

Barros and co-workers [61] investigated the adsorption capacity of chitin flakes, chitin and chitosan powder, chitosan flakes and chitosan solution towards crude oil spilled in seawater. In their study, 5 L of seawater were placed in a plastic container, and 7 g of petroleum were added to it. After 30 min, 50 mL of a 0.5% chitosan solution was sprayed over the oil spill. The results showed that, although chitosan flakes had a better adsorption capacity for oil (0.379 ± 0.030 g oil/g adsorbent) compared to the others, the biopolymer sank after adsorbing the oil. Such a characteristic offered a clear hinder in practical applications. On the other hand, chitosan solution, despite presenting lower adsorption capacity (0.013 ± 0.001 g oil/g adsorbent), did not present the low buoyancy drawback [61]. Elanchezhiyan and colleagues [62] investigated the recovery of oil from oil-in-water emulsion using chitosan/magnesium-aluminum layered double hydroxide hybrid composite (CS-LDHCs) obtained by a single co-precipitation method. CS-LDHC adsorbent was dispersed in 20 mL of deionized water solution containing 4% of oil and the effect of contact time in the oil removal by the CS-LDHCs and the layered double hydroxide hybrid (LDH) was investigated [62]. This was done by varying the contact time from 10 to 120 min at room temperature. Both adsorbents, CS-LDHCs and LDH, reached maximum oil removal saturation at 90 min, and, thus, the authors set 90 min as contact time for both adsorbents in further experiments. The researchers reported an oil removal capacity of 78% for CS-LDHCs, while for the LDH was found to be 30%. Since CS-LDHCs showed a much higher oil adsorption capacity, further studies were performed just targeting this material [62]. The effect of pH was also investigated in their study in the range from 3 to 11. This was done because normally the change in pH of oil-in-water emulsions cause emulsion breaking, which means that demulsification takes place [59, 63]. After their pH study, the authors demonstrated that the adsorption of oil was enhanced in acidic medium (pH 3.0) [62].

Elanchezhiyan and co-workers also studied the effect of contact time on the removal of oil from oil-in-water emulsion using zirconium-chitosan composites (Zr-CS-HC) in time-course experiments at room temperature [59]. The study of the effect of sorbent dosage of Zr-CS-HC indicated that a maximum oil removal percentage of 79% was achieved when exposing 400 mg of sorbent to 25 mL of polluted solution [59]. The authors determined that the maximum oil uptake on chitosan was reached after 4 h contact time. In their study, the authors demonstrated that Zr-CS-HC had a higher removal efficiency compared to chitosan and provided an explanation based on the higher number of vacant sites on the surface of Zr-CS-HC [59].

Grem and co-authors reported that chitosan microspheres produced by ionic gelation of chitosan with sodium tripolyphosphate (STP) were able to separate 90% of the oil from produced water containing 200 ppm oil suspension using packed columns [64].

In another very recent study, Doshi and colleagues [65] studied amphiphilic sodium salt of oleoyl carboxymethyl chitosan (NaO-CMCS) for the removal of oil from a simulated oil spill. Marine diesel was chosen as oil phase for the emulsion studies [65], and in their study, both deionized water and seawater were used to simulate oil-in-water (o/w) emulsions (1:1 v/v). The o/w creamy emulsions were prepared with different dosages of NaO-CMCS (0.5–5 g/L), and a calcium chloride dihydrate solution (0.1%) was also added to that mixture. From the results obtained, the authors concluded that about 75–85 and 19–49% of oil was recovered from the emulsified oil using deionized water and seawater, respectively [65]. The recovery of oil from the polluted aqueous phase was 76% in the case of deionized water containing a 0.5 g/L concentration of NaO-CMCS and 20% from seawater with a 2 g/L dose of NaO-CMCS. It was therefore concluded that this chitosan derivative was an effective material for the removal of oil from spills from polluted seawater [65].

Ummadisingu and co-workers [66] prepared chitosan from seafood industry waste and tested the purified material for the removal of oil from aqueous solutions. The authors explored the effect of contact time, pH, initial concentration and mass of adsorbent. They reported that the sorption equilibrium of oil on chitosan was reached after only 6 min of contact time, and the maximum capacity of chitosan to adsorb oil from oil–water solution was found to be 17.96 g/g of adsorbent [66].

Su and colleagues [67] have recently reported the preparation of a superhydrophobic and superoleophilic chitosan sponge using a freeze-drying method with the assistance of a cross-linking process employing tripolyphosphate/citral followed by octadecanethiol modification. In their paper, the authors describe a procedure that allowed getting a three-dimensional porous structure with large pore volume and good compressive properties. The obtained sponge was able to effectively absorb oil, reaching an absorptive capacity up to 60 times its own weight. The material was able to selectively absorb the emulsified oils in water and achieve continuous oil–water separation. The authors reported an oil–water separation efficiency up to 99% and claimed that the sponge still maintained a highly absorptive capacity after being reused for many cycles while having a good biodegradability.

Bibi and co-workers [68] investigated the adsorption capacity of carbon nanotubes (CNTs) mixed with chitosan (Cs)/poly(vinyl alcohol) (PVA) and cross-linked with silane. In their experimental setup, naphthalene was selected as polycyclic aromatic hydrocarbon (PAH) model, and its removal was studied with two membranes, CM10 and CW with and without CNTs, respectively [68]. A standard solution of 3 mg/L of naphthalene was prepared, and 30 mg of membranes were placed in 40 ml naphthalene-containing solution. The removal percentage of naphthalene with the CW membrane during the first 5 min reported by the authors was 10% and after 150 min 93%. For the CM10 membrane, more than 50% of naphthalene was removed during the first 5 min and 97% after 150 min. These results showed that CM10 membrane had good sorption capacity for naphthalene and that the sorption process took place fast [68]. The correlation between CNT content of the membrane and the removed amount of the naphthalene was 0.16 mg of naphthalene/1 mg CNTs. The authors reported that CW

membrane had equilibrium swelling of 217 g/g and CM10 of 162 g/g and concluded that the best membrane was CM10 due to its excellent sorption capacity and fast removal kinetics [68].

2.3. Chitosan-based materials containing immobilized bacteria for the removal of oil pollution

Among the different clean-up actions and materials employed as a first response against oil spills, the use of booms, skimmers, absorbent materials, controlled burning and vacuum and centrifuges might be found. These techniques cannot however achieve a complete clean-up of the polluted area, and their implementation should be done short after the oil spill occurs [69]. In the last years, the use of bioremediation-based techniques has largely attracted the attention of researchers and industrial stakeholders. The use of microorganisms for these decontamination purposes is considered as an effective and environmentally friendly treatment for, i.e., shorelines contaminated as a result of marine oil spills. Most of the compounds present in crude oil and refined products are prone to biodegradation and therefore might be removed from the environment through consumption by microbes [69]. There are mostly two complementary approaches: bioaugmentation and biostimulation. While the first approach involves addition of oil-degrading bacteria to the polluted system, the second approach intends to support the growth of the indigenous hydrocarbon degraders present in the system by the addition of nutrients and/or other growth-limiting substances [69, 70]. A scheme of a biodegradation is presented in **Figure 4**. The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. Such a process is normally initiated through an intracellular oxidative attack and the activation of the organic molecule through incorporation of oxygen in a reaction catalyzed by oxygenases and peroxidases [71]. A complete oxidation of the target hydrocarbon would lead to the production of CO_2 and water. The generation of different series of structures corresponding to different transformation products should however not be disregarded when this kind of bioremediation techniques is explored.

In order to improve the performance of the degradation of oil-related pollutants, some researchers have proposed strategies that involve the use of biomass in immobilized systems. One of the preferred entrapment systems for these purposes has been chitosan. In addition to the natural trend of chitosan to absorb oil, chitosan hydrogels have excellent water permeability and mass transfer properties (allowing the required access of the biomass to the nutrients they require). In addition to the aforementioned benefits, chitosan contributes to providing shelter to the valuable biomass while helping preserving the integrity of the culture. The use of microorganisms entrapped in chitosan gel matrices is therefore expected to have a very positive impact in both bioaugmentation- and biostimulation-based decontamination processes.

Dellagnezze and co-workers studied a bacterial consortium composed of four metagenomic clones and *Bacillus subtilis* strain CBMAI 707 (all derived from petroleum reservoirs) entrapped in chitosan beads towards hydrocarbon degradation capacity [72]. Experiments were carried out in mesocosm scale (3000 L) with seawater artificially polluted with crude oil. The compounds present in the oil that were the target of the biodegradation studies were benzo (a) pyrene ($C_{20}H_{12}$), benzo (a) anthracene ($C_{18}H_{12}$) and benzo (K) fluoranthene ($C_{20}H_{12}$) [73–75]. The degradation of hydrocarbons was evaluated in two different treatments: bioaugmentation and control. The authors performed time-course experiments, following the system at days 0, 5, 10, 20 and 30. The researchers demonstrated that degradation ratios increased abruptly from

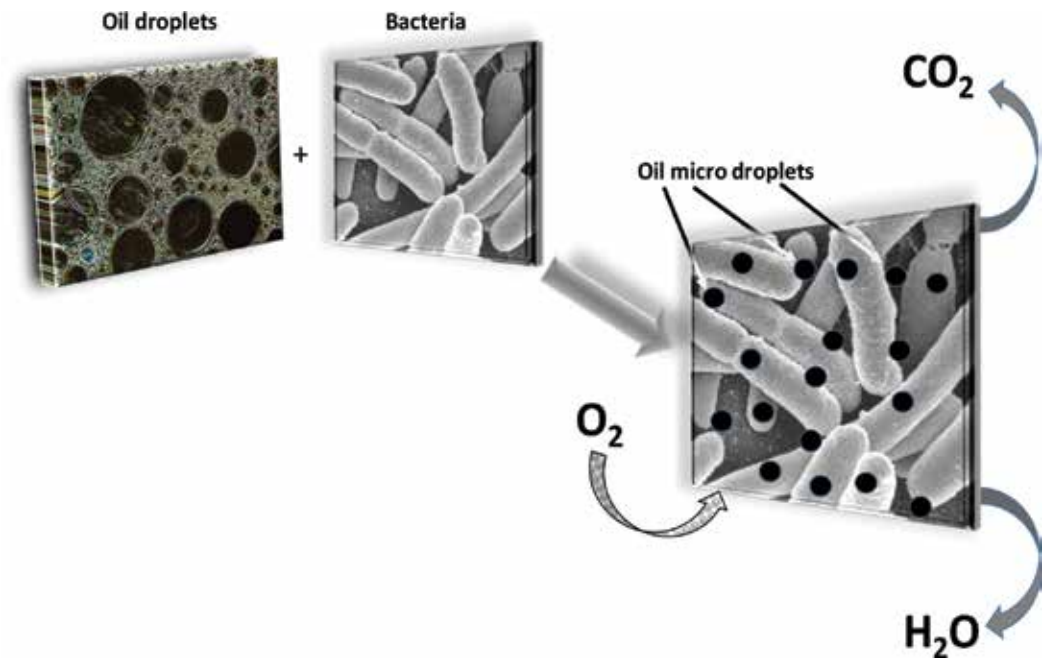

Figure 4. Oil biodegradation scheme under aerobic conditions.

the 5th to the 10th day and then just slightly increased until the end of the experiment. The system remained closed during the first 5 days to allow the acclimation of the bacteria to both treatments, and during this period, the authors did not observe significant biodegradation rates [72]. More than 90% of hydrocarbons' degradation was produced by the 10th day in both treatments. Similar to the 10th day, in day 20 most of the hydrocarbons were totally degraded. After 30 days from the beginning of the assays, the degradation percentages in the bioaugmentation treatment were higher than those observed in the control treatment. For instance, 35% of benzo (k) fluoranthene was degraded in the control treatment, 70% was degraded in the bioaugmentation treatment, and benzo (a) pyrene was almost completely degraded (99%). Likewise, benzo (a) anthracene showed higher degradation percentage, reaching 85% in the bioaugmented series compared to a 68% observed in the control [72].

Sar and Rosenberg [76] studied the biodegradation of n-hexadecane and its biosurfactant recovery. For this purpose, spores of *Bacillus subtilis* LAMI008 were entrapped in 3 mm chitosan beads and cross-linked with 0.3% glutaraldehyde [76]. The authors performed biodegradation assays in 50 mL of mineral medium containing 1% n-hexane (v/v) supplemented with 1% glucose (w/v) and inoculated with 10% of spore suspension (v/v) or with 10% of spore-entrapped chitosan beads (w/v). Both cultures were adjusted to 10^7 CFU/mL [76]. The biodegradation of n-hexadecane by *Bacillus subtilis* LAMI008 entrapped in chitosan beads was compared with that by free cells under similar conditions, and almost 100% of 1% n-hexadecane was degraded within 48 h in both assays.

In another study Gentili and co-authors [77] examined the potential of chitosan flakes as carrier material for the immobilization of *R. corynebacteriorides* (QBTo), a hydrocarbon-degrading

bacterial strain. The authors performed biodegradation tests in a crude oil-polluted seawater microcosms [77]. For this purpose, three different microcosm situations were tested. In the microcosms inoculated with QBTo immobilized onto chitosan, 60% of hydrocarbons in the hexane extracts were removed compared with the control microcosms sample and the seawater microcosms. In the control microcosms, QBTo could not produce a significant reduction in the hydrocarbon concentrations [77]. In the seawater microcosms, QBTo was inoculated without a carrier, and a decrease of 30% of hexane extract was obtained. The degradation of hydrocarbons in the microcosms with the strain QBTo immobilized onto chitosan was higher than that obtained in the microcosms without the carrier. The explanation to this improvement is due to the enhancement of the strain survival, since the carrier material and the biofilm structure that the cells developed on it exert a protective effect [77].

Costa and co-authors [78] investigated the potential of the bacterial strain *B. pumilus* entrapped in chitosan in the degradation of hexadecane. The biodegradation assays were performed with free-living and immobilized bacterial cells with 1% hexadecane (v/v). In addition to this, 5 mL of an adjusted culture with 10^9 CFU/mL were used in free-living cell assays [78]. For the immobilized assays with bacterial cells, 5 g of chitosan beads containing the selected strain were used, and the biodegradation was studied at 0, 48, 96 and 144 h. The biodegradation results indicated that after 48 h, the free-living cells removed 81.83% of the hydrocarbon, while the culture of immobilized cell was able to remove only 38.12%. The authors provided an explanation to this based on the fact that in the assays performed with free-living cells, there was approximately 10^4 times more biomass than in the experiments performed with immobilized cells. The authors pointed out that during the immobilization process there was an important loss of cells. Cell counting performed by the authors during the biodegradation experiments showed that the immobilized biomass grew progressively and removed 84.53% of hexadecane after 96 h. These results, obtained in a larger time span, where similar to the 86.28% removed in assays performed with non-immobilized, free-living cells. Moreover, the authors found that the biomass concentration inside the beads was similar to that observed in the free-living cultures at 144 h and also removed hexadecane efficiently [78].

3. Conclusions

Chitosan offers a large potential in the development of sorbent materials for abatement of organic pollution from water. The biopolymer can be used in many different applications on its raw form or included in the preparation of a large variety of materials such as hydrogel beads, nanoparticles, films, membranes and meshes. Among the different scenarios of severe organic pollution, the pollution caused by hydrocarbon spills into water bodies deserves a special mention. Chitosan and materials based on this polymer have demonstrated an enormous potential to efficiently remove hydrocarbons from contaminated water. In addition to the aforementioned, the potential offered by chitosan to develop biological remediation systems deserves a special mention. The excellent biocompatibility of this polymer makes possible development of oil remediation biosystems based on bacteria entrapped into chitosan.

Conflict of interest

The authors certify that they have no conflict of interest.

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Chapter 5

Chitosan's Wide Profile from Fibre to Fabrics: An Overview

Xue Luo and Li Li

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76196

Abstract

Textile has a high structure capacity, is adaptive to multiple situations and is applied in food, energy, environmental, construction and medical industries. Its stable and flexible characteristics are sure to attract even more attention. Biofunctional textile is one of the most important categories of functional textile, taking up 7% of the total amount, and is expected to be the most promising section of growth. Due to the restrict requirement of fibre production, chitosan is one of the few materials that can be spun into pure fibre. The pure chitosan fibre can be blend with other fibres and produce durable functional fabric suitable for medical as well as daily use. This article also reviewed existed modification on chitosan material prepared for fibre spinning and technology related to chitosan-based textile production and discussed the difficulties and possible solutions in chitosan yarn spinning and possible ways of fabric forming.

Keywords: chitosan, textile, fibres, fabrics, biofunctional materials

1. Introduction

The textile industry accounts for 2% of the world's gross domestic product and is the seventh largest economic sector, according to a recent report by McKinsey & Company [1]. The capabilities and stability of textiles have advanced considerably. Entanglement of fibres in yarns can be designed and fabricated by changing numerous technical factors during production. Furthermore, diverse methods are used for forming fabrics. Knitting and weaving structures differ in elasticity, density and air and liquid permeability. There are numerous methods of enriching textile structures by combining and layering existing structures without adherence between components. Moreover, textiles are considerably more stable than common film

structures because of the elasticity embedded in their structure. Textiles are durable under conditions subjecting them to impact forces and are resistant to abrasion. Therefore, the structure of textiles is favoured for both industrial use and apparel.

Advances in material development offer opportunities for cross discipline studies to further improve the mechanical and functional performance of textiles. Textiles are applied in the food, energy, environmental, construction and medical industries, meaning they are not only a domestic product. Their stable and flexible characteristics have attracted considerable attention. Functional textiles account for 27% of worldwide fabric production, with the functional textile market expected to be worth US$175 billion by 2020.

Biofunctional textiles are among the most valuable categories of functional textiles, accounting for up to 7% of total functional textiles. Developing countries involving growing textile industries, such as China, have lost global textile and apparel market share. The textile market has shifted to more value-added products, namely technical textiles. Biofunctional textiles are categorised according to their usage: in vitro and in vivo use. Biofunctional textiles for in vivo use emphasise biocompatibility and biostability because they come into direct contact with cells and biological fluids. One method of endowing these capabilities in a textile is by coating a textile polymer with functional molecules, which is sometimes enhanced through chemical bonds. However, the coating is thin and vulnerable to abrasion and other physical movements, even when the environment of application is in vivo. Another means of developing biofunctional textiles is by spinning fibres with pure functional materials. Candidate materials for spinning fibre are extremely limited because of both process requirements and mechanical properties of the fibre required for yarn spinning.

The two main methods of forming fibres are melt and wet spinning. Melt spinning is typically applied to thermoplastic polymers that transform to a liquid form under heat and recover to a solid and flexible form after cooling. The mechanical and functional properties of the fibre should not be affected by the high temperatures required in melt spinning.

Such a requirement of melt spinning is hardly achieved by biofunctional fibre materials, such as chitosan and alginate, because they are not thermoplastic and also degrade when heated at elevated temperatures. In such cases, low-temperature wet spinning or gel spinning is used. In wet spinning, the polymer is dissolved in a solvent and then extruded through a spinneret into a nonsolvent in a coagulation bath in which the fibres are precipitated and solidified. The filaments are then washed to remove the remaining solvents and nonsolvents, drawn, dried and lubricated before being wound on a bobbin. Other solvent removal methods exist. Instead of precipitating the fibres in a coagulation bath, dry spinning involves solidifying the fibres by evaporating the solvent in a stream of hot air or hot inert gas. The solvent is recovered and reused in such a method.

The strict requirements of fibre spinning and the fragility of biofunctional materials limit the material choices for pure biofunctional fibres. Typical pure biofunctional fibres are made from corn, bamboo, milk, microorganism products and animal shells. The first four material sources are developed mainly as a substitute for oil-based manmade fibres, whereas the fifth material source has special biofunctions other than biocompatibility and biostability.

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Chitosan and chitin fibres are the products of such technology, which inherent the antibacterial and wound-healing characteristics of chitosan and chitin materials while holding adequate mechanical strength.

2. Chitosan textile production

Traditionally, chitosan is mainly treated on a yarn's or fabric's surface [2–6] and is exposed to harm from abrasion and other movements. Chitosan peals when it wears, and the biofunctions are vulnerable to physical and chemical damage. Producing a pure fibre from chitosan is a reasonable means of improving stability and durability.

Chitosan fibres are derived mainly from wet spinning. Dissolution, deaeration and filtration of chitosan are performed before spinning [7]. The semi-finished fibres are then refined, dried and post-finished. Factors in dissolution, deaeration and drying are vital to control properties of chitosan fibre, including solvent, pH and concentration during dissolution, method and agent of drying, etc. A study [8] shows that methanol drying yielded chitosan fibre has superior mechanical properties to fibres dried using other methods and agent.

The three main methods are available for producing a fabric from fibres. A nonwoven method entails entangling fibres by using water or air jets. This process is similar to wool felting and requires no yarn spinning. The nonwoven method is fast and cheap, and it is suitable for producing cheap, disposable products. Woven and knit fabrics are more durable and are more suitable for daily use because they can be reused hundreds of times, withstanding frequent washing and abrasion [9, 10] (**Figures 1–5**).

Chitosan fibres are weaker than cotton. Typical chitosan fibres have a dry tensile strength of 2.09 cN/dtex, which is close to the minimum dry tensile strength value for cotton fibres (1.9–3.1 cN/dtex). Their wet tensile strength (1.8 cN/dtex) is lower than that of cotton fibres (2.23.1 cN/dtex) (**Table 1**). In a spinning factory, the humidity is usually set to a high level (70–80%), under which conditions chitosan becomes adhesive and weak, causing problems during the spinning process, especially when yarns have a high chitosan fibre ratio.

Chitosan's weakness also affects end-product usage. A weak textile does not withstand tearing, bursting or abrasion. Therefore, the strength and weakness must be balanced by mixing and strengthening the fibre with other materials. The price of chitosan and fibres is expensive at up to $100 per kilogramme, which is comparable to cashmere. The blending of two or more types of fibre solves both the mechanical strength and cost problems.

Blending two or more biofunctional materials or blending biofunctional materials with non-biofunctional materials can be achieved during fibre spinning, yarn spinning or fabric formation. Blending in or before fibre spinning requires property consistency between the two or more biofunctional materials in the spinning process; the ratio of biofunctional materials is settled and less flexible regarding the production process. Blending after yarn production is likely to cause an uneven distribution of the biofunctional materials and influence the performance. A balanced method of forming blended fabric requires the blending of pure fibres during yarn spinning.

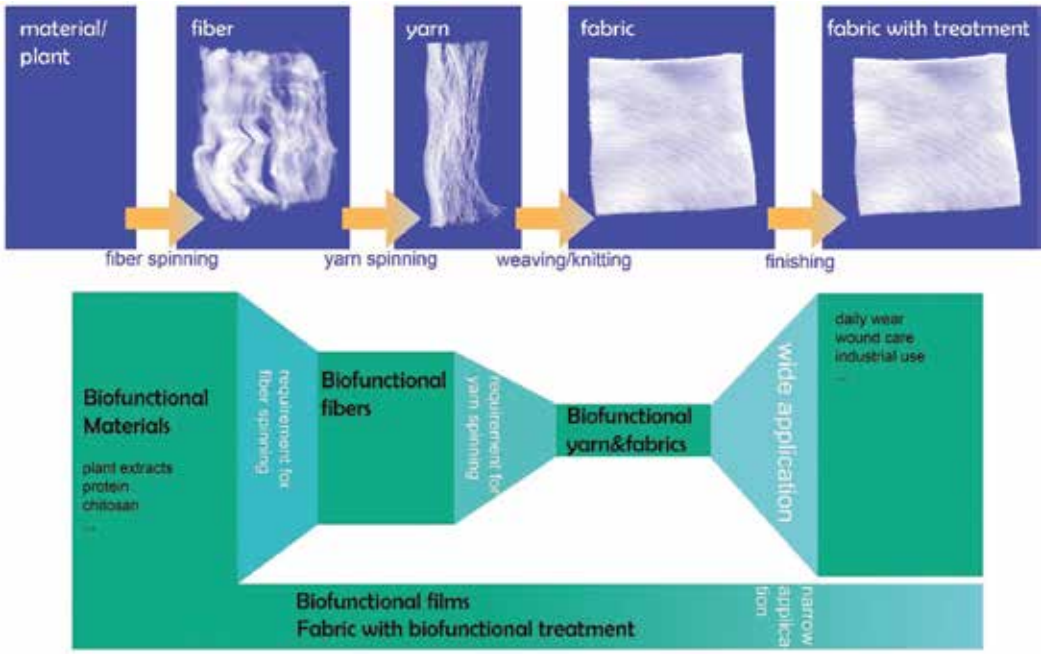

Figure 1. Process of textile production (upper); the strict requirements of textile production heavily restrict the applicable material list (chitosan is an example). The rich structural capability and stability of biofunctional textiles render them versatile.

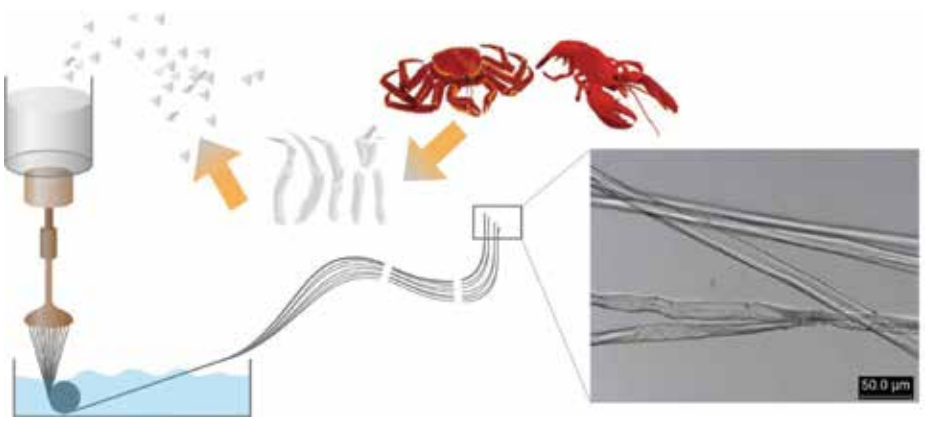

Figure 2. Spinning process of chitosan fibre (left) and chitosan fibre under microscope (right).

Blending biofunctional fibres during yarn spinning may cause problems. Biofunctional fibres normally have weaker mechanical properties than normal fibres, but they may have special properties. Chitosan fibres easily adhere to rubber rollers, which not only causes difficulty in spinning yarns with a high chitosan content but also causes material loss, raising production costs. Therefore, the mechanical properties and biofunction of yarns must be carefully balanced to ensure sufficient strength and comfort handle and maintain stable biofunctions while controlling the production difficulty and costs.

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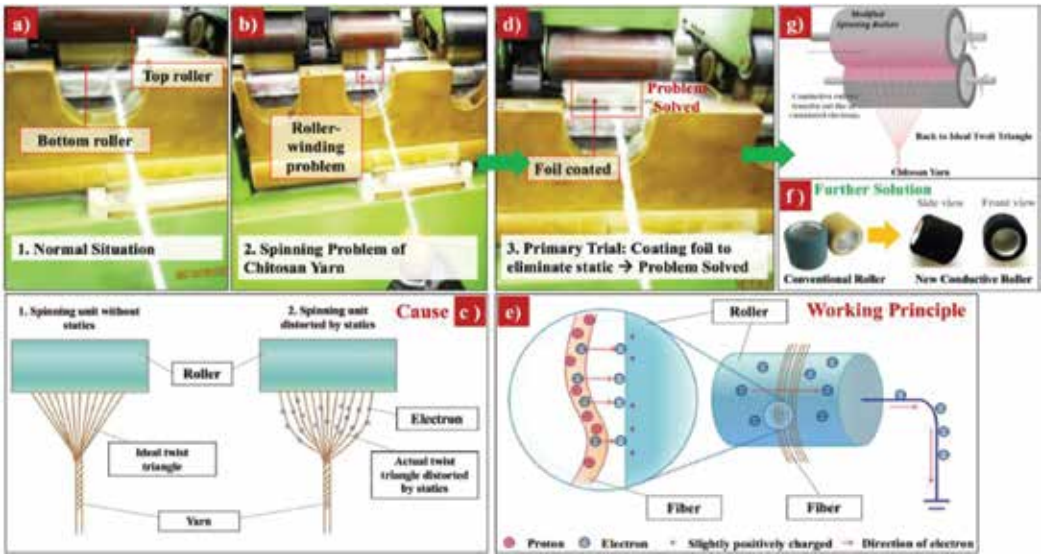

Figure 3. (a) Normal situation of spinning a twist triangle; (b) roller-winding problem faced during the spinning of chitosan fibres; (c) analysing the cause of the problem: adhesion caused by static electricity is easily generated by abrasion (due to the extremely low electronic work function of chitosan fibres); (d) primary trial to solve the problem by using a layer of foil to remove (transfer) accumulated electrons; (e) working principle of the primary trial; (f) further trial: modified conductive roller to remove the static electricity; and (g) a twist triangle turns back to an ideal situation when the modified rollers are applied.

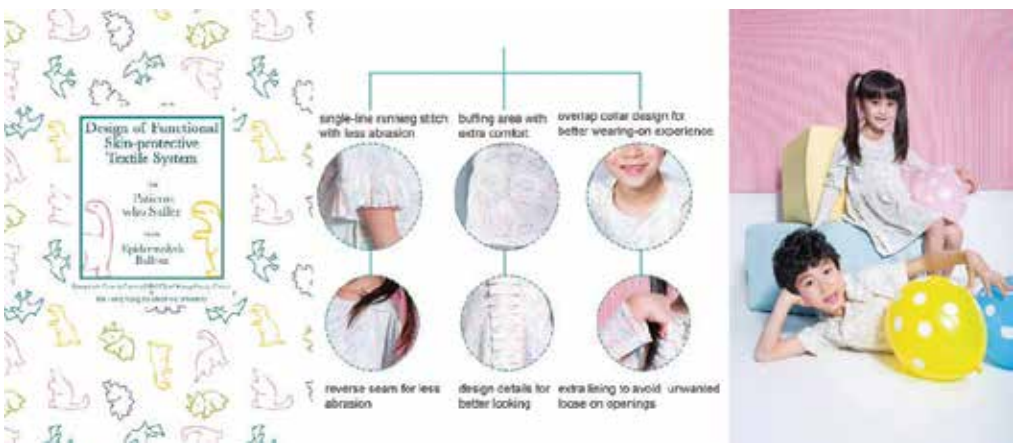

Figure 4. Example of product design targets epidermolysis bullosa patients.

Studies [11–13] have shown that factors associated with the blending process, such as blending time (fibre or sliver blending) and spinning factors, may influence the distribution of yarn fibres. Regarding the ratios of biofunctional fibres, variables must be examined not only for academic purposes but also for industrial and domestic application. Efficient and precise testing methods must be developed to facilitate the regulation of biofunctional textile production, testing and usage.

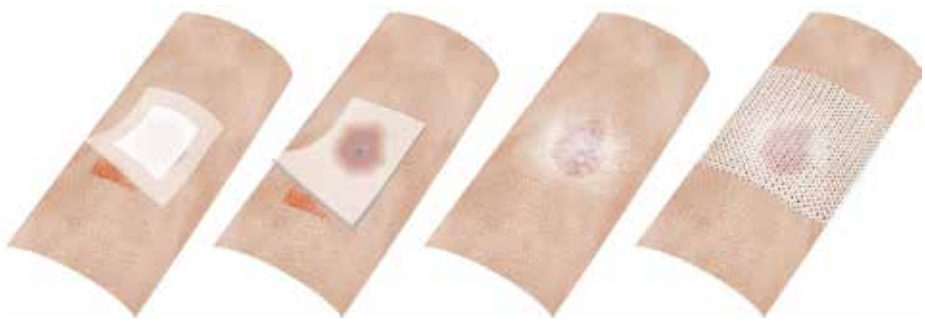

Figure 5. Four common biofunctional materials related to wound care: Band-Aid, gel dressing (artificial skin), powder or cream and textile.

Parameters	Values	Cotton*
Dry tensile strength (cN/dtex)	2.09	1.9–3.1
Dry tensile strength ratio (%)	15.1	7–10
Wet tensile strength (cN/dtex)	1.80	2.2–3.1

*Source from www.intechopen.com

Table 1. Parameters of chitosan fibre.

Besides blending after fibre spinning, blending before fibre spinning and chitosan material modification are other common ways for researchers to reinforce the weak fibres especially in wet and acidic environment. Blending chitosan with other polymers can strengthen the fibre but compatibility needs to be improved, and thus reactive compatibilisers such as epoxy-functionalised LDPE has been introduced to increase the strength of the blend [14].

Biofunctions of chitosan/chitosan-treated fabric can be improved by adding functional groups on chitosan material or adding other ingredients. Some approaches make reaction faster by improving chitosan water solubility by acylation, alkylation, pegylation, hydroxyalkylation, carboxyalkylation and depolymerisation, but the durability is sure to be shortened. Some others improve cationic properties by deacetylation, quaternisation and addition of cationic moieties. Concerning non-toxic requirement of chitosan textile for medical use, some safe additives include neem [15].

Cross-linked chitosan fibre shows equal [16] or better [17] antibacterial effect and possibly has better mechanical property due to the reduction of crystallinity [18]. Stronger antibacterial function is found in N,N,N-trimethyl chitosan fibres [19] and succinic anhydride-modified fibre [20]. N-carboxyethyl chitosan fibres [21], quaternisation-functionalised chitosan fibre [22] and succinic anhydride-modified fibre [20] have better liquid absorption capacity than normal chitosan fibre, but tensile strength and elongation at break are lower. There are few studies that clearly report improvement in both mechanical and biofunction properties.

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3. Application of chitosan textile

Chitosan textiles can be used for medical care to provide safe and continuous protection for patients and caregivers. For patients with skin trauma, particularly those who are bedbound and at risk of developing bedsores, or those with low immunity, chitosan textiles can be used as materials for daily dressing and bedding or as fundamental materials for long-term wound care [23]; this is because such textiles control bacterial growth and accelerate wound healing. Moreover, chitosan textiles can avoid or reduce the side effects of long-term antibiotic or silver fungicide use. The antibacterial function can be controlled by the blending ratio of the chitosan fibres to other fibres to meet different needs.

Chitosan textiles are also required in space and military activities. The extreme environment in space renders the balance of microorganisms even more vital. Excessive or insufficient microorganisms can pose a risk. An excessively high number of microorganisms pose an infection risk. Furthermore, the growth and mutation of bacteria in space are uncontrollable, which may pose a threat to humans and other on-board animals. An excessively low microbial population may cause immune disorders or impaired immune function. Using chitosan textiles in ship interiors and clothing can continuously maintain the number of microorganisms, which is beneficial to the health of shipborne personnel.

In addition to special uses, chitosan textiles can be used for ordinary clothing, especially underwear. The market demand for antibacterial underwear is considerably high. In relatively hostile market environments, functional underwear can be marketed as having advantageous properties over products of competing enterprises, in order to prevent the prices from being trapped at low levels. In contrast to chitosan antibacterial agents coated on the surface of normal fabrics, blended chitosan-knitted fabrics do not lose their biofunctions after friction or washing. The antibacterial performance of the fabrics is still stable after being washed hundreds of times. Therefore, such fabrics are particularly suitable for end products such as underwear, which must be washed frequently. The safety of chitosan-knitted fabrics is also superior to that of traditional silver-based antibacterial fabrics, because they can come into direct contact with the skin.

Chitosan also have antifungal effects [24–29], which render chitosan textile particularly useful for bedding, home decor and personal hygiene products. However, chitosan fibres are not bright or white, which limits the consumer acceptance of chitosan textiles.

4. Conclusion

Further development of relevant technologies will give chitosan textiles a more crucial role in functional textiles in the future. Producing daily wear from a blended fabric with a low proportion of chitosan will ensure that the price is acceptable to consumers. However, chitosan fabrics with special properties generally require a high proportion of chitosan fibres. This requires breakthroughs in spinning technologies, because chitosan fibres are sticky and easily generate

static electricity. The upper and lower limits of the functions of chitosan fabrics deserve attention. Because of the high price of chitosan fibres, increasing their proportion should engender greater functional improvement.

Tuning the biological function of chitosan and increasing the ratio of chitosan in fabrics both require a systematic study of blending technologies. Even in the traditional spinning process, numerous parameters may affect the distribution, feather, yarn thickness and compactness of the final fibre. For chitosan yarns, these parameters may affect wet gas absorption, comfort, biological function and the mechanical properties of yarns and fabrics. Results from studies on the textile industry could be used to strengthen the function of chitosan textiles. Meanwhile, factors influencing the structure and effects of chitosan textiles can be eliminated in yarn spinning and during knitting, weaving or felting processes of nonwoven fabric to improve the effects of the final product.

Relevant test methods and standards for chitosan textiles must be further standardised. Chitosan fibre-blended textiles are different from textiles treated with traditional antibiotics or silver fungicides. In contrast to treated fabrics, bactericidal components are insoluble in chitosan fibre-blended textiles. Furthermore, the antibacterial properties of chitosan fibres may not be detected by certain test methods such as those that entail testing the zone of inhibition.

Meanwhile, the method proposed in most studies for testing the antibacterial effects of chitosan in a liquid form after it has been dissolved in an acetic acid solution is not suitable for chitosan textiles, because this situation would not occur in practical use. After existing test methods and determination methods are compared, a comprehensive test system should be developed for chitosan textiles. Furthermore, the antibacterial principle of chitosan fibres may be different from the antiseptic principle of the acetic acid solution for chitosan raw materials. Therefore, studying their differences and similarities is necessary. Moreover, the production of chitosan raw materials causes pollution problems that must be solved. Chitosan textiles have been continuously refined. It is expected that many new findings and technologies will be discovered and developed in this cross domain.

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Chapter 6

Blended Composites of Chitosan: Adsorption Profile for Mitigation of Toxic Pb (II) Ions from Water

Asha H. Gedam, Prashil K. Narnaware and Vrushali Kinhikar

Additional information is available at the end of the chapter

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Abstract

An environmental pollution is the unfavorable alteration of surrounding toxicity due to heavy metals, organic pollutants, radioactive materials, pesticides, dyes, pigments, fatty/oil impurities and minerals that are responsible for crucial ecological and health concerns. The indiscriminate industrial and anthropological activities render water resources unsuitable for consumptions. Percolations of synthetic pollutants in water are responsible for detrimental effects on aquatic flora and fauna. Environmental contamination of water poses the major challenge to develop efficient water treatment techniques based on usage of biopolymers. Hence, chitosan (de-acetylated chitin: *β-(1 → 4) D-glucosamine*) biosorbent is preferred which is cheap, biodegradable, and biocompatible for the mitigation of few heavy metals from water. Chitosan's flexible skeleton was modified by doping few organic/inorganic moieties to yield biocomposites for adsorption of varied pollutants. In this chapter, the batch adsorption of toxic Pb (II) ions from water using graphite doped chitosan composite (GDCC) as an adsorbent is discussed. Maximum Pb (II) ions adsorption capacity was 6.711 mg/g (from Langmuir) at optimum pH 6 with dosage of 1 g/L in 120 min. Biosorption mechanism is emphasized in context with wastewater cleanup procedures.

Keywords: chitosan, graphite, GDCC, Pb (II) ions, water, adsorption

1. Introduction

Chitin (pronounced as Kite-in) is the precursor of chitosan (pronounced as Kite-o-san) was first discovered in 1811 by a Frenchman named Henri Braconnot as a result of extraction

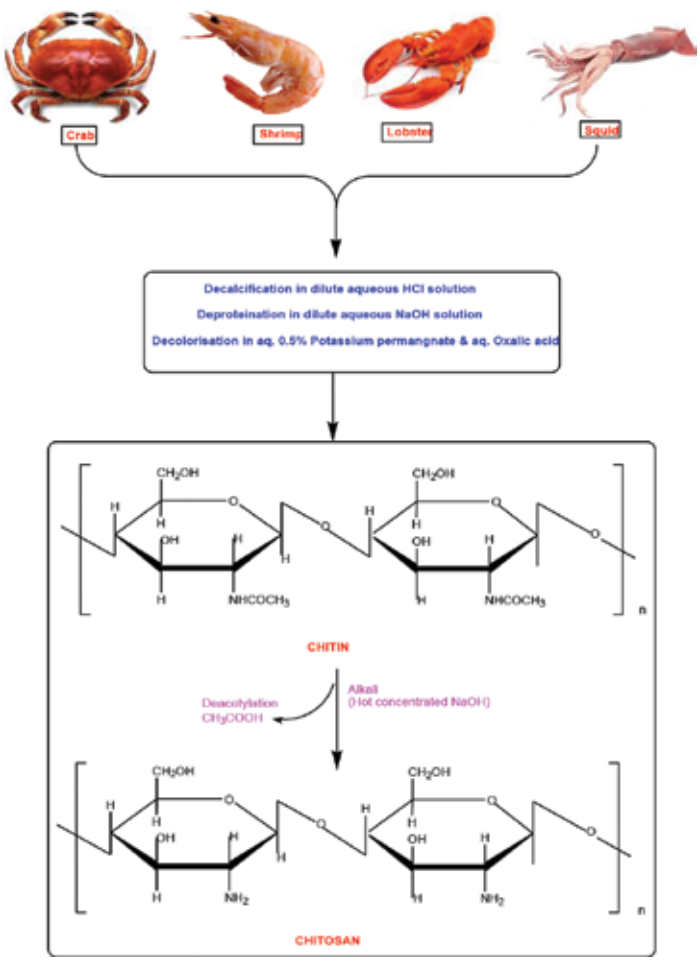

Figure 1. Chemical structures of chitin and chitosan.

from the fungus. Later on in the year 1820, it was derived from the skin of insects. In 1859, C. Roughet obtained "modified chitin" from chitin treated with alkaline sodium hydroxide solution. However, in 1894, F. Hoppe-Seyler treated chitin with different alkaline solution of potassium hydroxide at 180°C and the product called as Chitosan [1]. In 1902, Frankel and Kelly recognized the chemical composition of chitin and chitosan, and in the 1950s, several researchers such as H. Sponsler and W.H. Dore have determined their chemical structures through X-ray experiments.

Chitosan is a hydrophilic natural polymer produced by alkaline deacetylation of chitin, which is the most abundant biopolymer occurring in nature, after cellulose and an exoskeleton part of crustaceans such as shrimp, lobsters, and crab shells [2]. Chitin and chitosan are commercially produced in countries like India, Japan, Poland, Norway and Australia. Chitin and chitosan are nitrogenous polysaccharides that consist of acetyl glucosamine and glucos-

amine units. Chemically these two polymers are β-(1–4)-2-acetamido-2-deoxy-*D*-glucan and β-(1-4)-2-amino-2-deoxy-*D*-glucan, respectively (**Figure 1**). The difference between the chitin and chitosan lies in the degree of deacetylation (DD) and their solubility in 0.1 M dilute acidic medium. If the degree of deacetylation of chitin falls to 50%, then it becomes soluble in aqueous acidic media such as acetic acid, propionic acid, and so on. [3]. The insolubility of chitosan in water, alkaline medium and organic solvents is due to the presence of hydrogen bonds between its molecules; however, the protonation of amine group renders its solubility in acidic solutions [4]. The DD affects the adsorption capacity of the chitosan. High DD generally results from the presence of high amounts of amino groups, and it can increase the adsorption capacity of chitosan by protonation [5].

Chitin and chitosan possess molecular weight up to several million g/mol. Commercially available chitosan has an average molecular mass ranging from 3800 to 500,000 g/mol and its degree of *N*-acetylation is 2–40% [6]. Deacetylation of chitosan ensures the presence of free amino groups that can be easily protonated in an acidic environment, making chitosan as cationic polyelectrolyte (pKa ≈ 6.5) and water soluble below the pH of 6.5 [7]. It shows high affinity for water pollutants adsorption due to the presence of amine ($–NH_2$) and hydroxyl (–OH) functional groups that act as chelating sites. This integral amine functionality (primary, secondary and tertiary) acquires positive charge in acidic condition and thus become sorption site for anions. Chitin and chitosan are of commercial interest due to their high nitrogen content (7.21%) compared to synthetically substituted cellulose (1.25%) and their excellent properties such as biocompatibility, biodegradability, non-toxicity and adsorptive abilities. It was found that chitosan is highly selective with respect to the uptake of metal ions. It shows an uptake of transition and post-transition metal ions and does not allow the sorption of alkali and alkali earth metal ions from the aqueous solution [8]. These selective adsorption properties have been used for environmental cleanup viz. uptake of heavy metals ions, pesticides, dyes/pigments, radionuclide, and so on from the polluted water resources.

2. Chitosan modification

Although of its several broad spectrum advantages, it has severe limitations viz. lower chemical and mechanical stabilities (due to hydrophilic nature), high pH sensitivity, and solubility in most organic acids, non-porosity and low specific surface area. These inadequacies limit its usage in wastewater treatment applications. Thereby to overcome all of these pitfalls and to tailor it for the specific wastewater treatment application, several attempts for its physical and chemical modification to achieve their biocomposites/nanocomposites have been conducted. The physical and chemical modification of chitosan was adopted to derive the desired adsorbent's characteristics and to improve its adsorption kinetic parameters feasible for pollutants removal.

Physical modification of chitosan has been carried out by various techniques to obtain accomplished polymer as powders, beads, flakes, nanoparticles, hydrogels, films, fibers

membranes, sponge, honeycomb, and so on (**Figure 2**). Chitosan beads and fibers of various porosities can be prepared by neutralization methods (**Figure 3**) where chitosan is treated with acetic acid and mixture added drop wise to 1 M NaOH by using microsyringe [9].

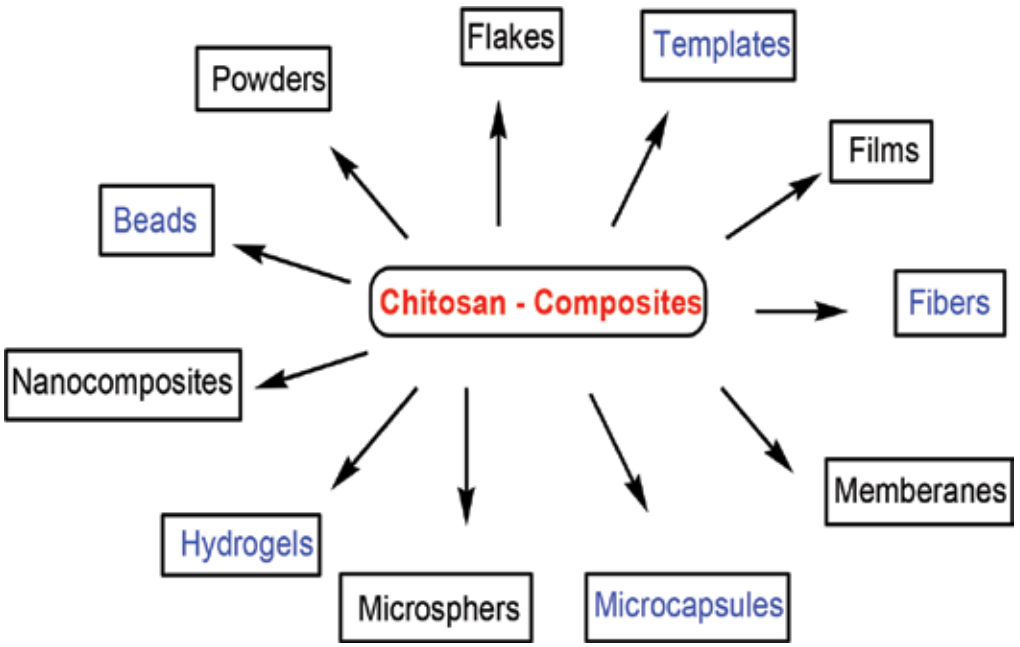

Figure 2. Various physical forms of chitosan bio-composites.

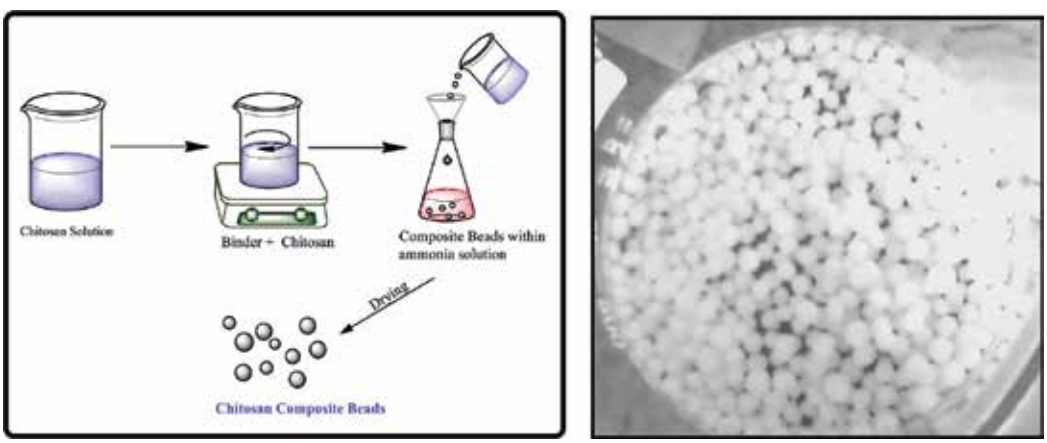

Figure 3. Synthesis of chitosan bio-composites beads.

Chitosan membranes can also be synthesized by treating chitosan with acetic acid where the solution is poured into a Petri dish and once the solvent evaporated, the membrane is neutralized with sodium hydroxide [10]. Moreover, chitosan sponges with different porosities can be prepared by freeze-drying techniques where chitosan solutions or gels are frozen followed by lyophilization [9]. One of the attractive ways of physical modification is to provide new desirable characters to chitosan and to synthesize chitosan-based biocomposites by mixing or blending of chitosan with the support or reinforcement matrix [11]. In blending, at least two polymers are mixed to obtain a new material with different physical properties [12]. At thermodynamic equilibrium, the two polymers of amorphous nature appear to be as a single phase or homogeneous on blending with a new set of improved properties from the individual components. The miscibility and compatibility between the blended polymers are decided by their mechanical and thermal properties [13]. The method of blending is effective in practical application due to its simplicity in operation and availability of various organic compounds and natural polymers. Blending permits the wide range of properties by union of both the components viz. chitosan and the reinforcement matrix to achieve physically and chemically stable biopolymers required for the specific applications.

Chemical modification is the application of various chemical treatments such as cross-linking, sulfonation, carboxymethylation, depolymerization, nitration, alkylation, sulfonation, phosphorylation, xanthations, Schiff's base formation, alkylation, acylation, hydroxylation and graft copolymerization. Various extensive novel chitosan derivatives can be obtained by chemical modifications. The chemical modification of chitosan has two main objectives: (1) to enhance the metal adsorption properties and (2) to improve the stability of chitosan in water or acidic medium. The chemical modification incorporates the various functional groups in the chitosan may involve the $-NH_2$ group at the C-2 position (specific reactions) or –OH groups at the C-3 and C-6 positions (nonspecific reactions) [3]. The various cross-linking reagents such as glyoxal, formaldehyde, glutaraldehyde, epichlorohydrin, ethylene glycol, diglycidyl ether and isocyanates are commonly used to modify the chitosan but are not preferred due to their toxicity. Cross-linking decreases the adsorption efficiency particularly in case of chemical reactions involving amino groups, but it provides mechanical strength and enhances the stability of chitosan against acidic and basic solutions [14].

Graft copolymerization is also the promising method that allows the covalent bonding between the grafted functional groups onto the chitosan backbone. The objectives of grafting new functional groups on chitosan are to alter the pH range and adsorption sites so that to enhance adsorption selectivity for the target pollutant.

Enzyme-modified chitosan is also one of the attractive methods of chitosan modification due to the reaction specificity. The alteration of the surface and rheological properties of chitosan using hexyloxyphenol which was grafted onto chitosan mediated by tyrosinase [15] has been studied for the modification of chitosan to achieve desired characteristics for the particular applications (**Figure 4**) .

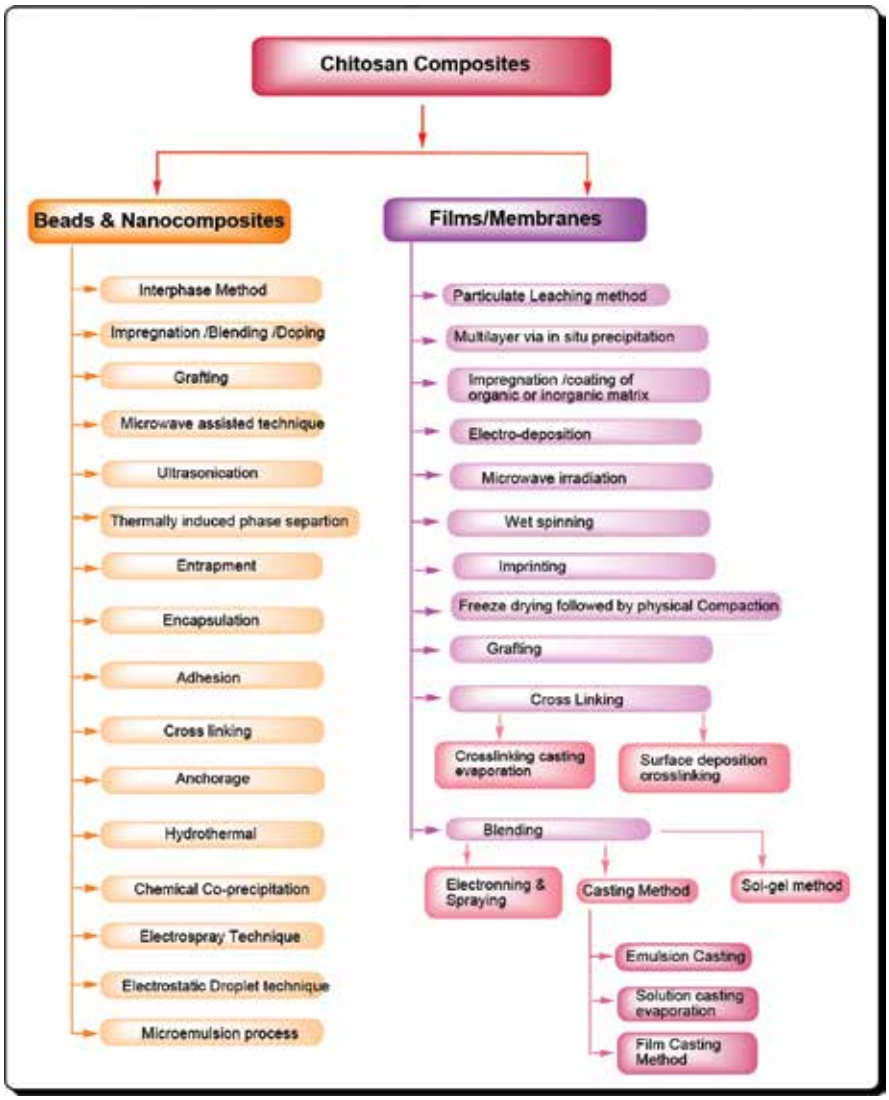

Figure 4. Various synthetic methods of chitosan biocomposites.

3. Applications of chitosan

Chitosan has wide and vast variety of applications ranging from biomedical and cosmetics products to agriculture and wastewater treatment [16]. The applications of chitosan in various fields and its specific properties responsible for specific applications are tabulated as follows (**Table 1**).

Applications of chitosan	Properties of chitosan
Cosmetics	Fungicidal and Fungistatic in nature. Facilitates the interaction with common integuments (skin covers) and hair. Chitin, chitosan and its derivatives offers uses in three areas of cosmetics: Hair, skin and oral care.
Paper industry	Chitosan molecules greatly resemble those of cellulose the main constituent of plant walls. It is used in the production of toilet paper, packaging/wrapping paper and cardboard.
Textile industry	Antistatic and soil repellant properties of chitin derivatives are used in textile industries.
Food processing	Chitosan used in food industry as it is nontoxic to warm blooded animals. Microcrystalline chitin shows good emulsifying properties, superior thickening and gelling agent for stabilizing foods.
Agriculture	It acts as plant growth enhancer.
Photography	Scratch resistance, optical and film forming property.
Chromatographic separation	The presence of free $-NH_2$ group and primary and secondary –OH groups enables its use as chromatographic support by using HPLC.
Solid-state batteries	Solubility in acetic acid facilitates ionic conductivity.
LED application	Dyes containing chitosan gel have been used as potential component in laser and other light emitting devices (LEDs).
Biomedical applications	Such as artificial skin, artificial kidney, wound dressing, drug delivery system and space filling implants. Chitosan has been found to have an accelerator effect on the tissue engineering process owing to its polycationic nature. Chitosan used for burn treatment since it can form tough water absorbent, biocompatible films and shows excellent oxygen permeability.
Opthalmology	It possesses all the properties required for an ideal contact lens, optical clarity, mechanical stability, sufficient optical correction, gas permeability, wettability and immunological compatibility.
Pharmaceutical application	Since chitin and chitosan do not cause any biological hazard and are inexpensive, these polymers might be suitable for use in the preparation of commercial drugs.

Table 1. Applications of chitosan and its composites in various fields.

4. Wastewater treatment application

The toxic heavy metal ions must be detained before its percolation into the water resources to protect the aquatic flora, fauna, human beings and consequently the environment.

To accomplish the increased stringent environmental regulations and maximum permissible limit of contaminants in water, a wide range of treatment technologies such as chemical precipitation, coagulation flocculation, flotation, ion exchange, membrane filtration, electrochemical treatment technologies, adsorption/bioadsorption [17], and so on are most frequently examined. Among the aforementioned technologies, adsorption has been preferred due to its flexible operation, generation of high-quality treated effluent and regeneration of

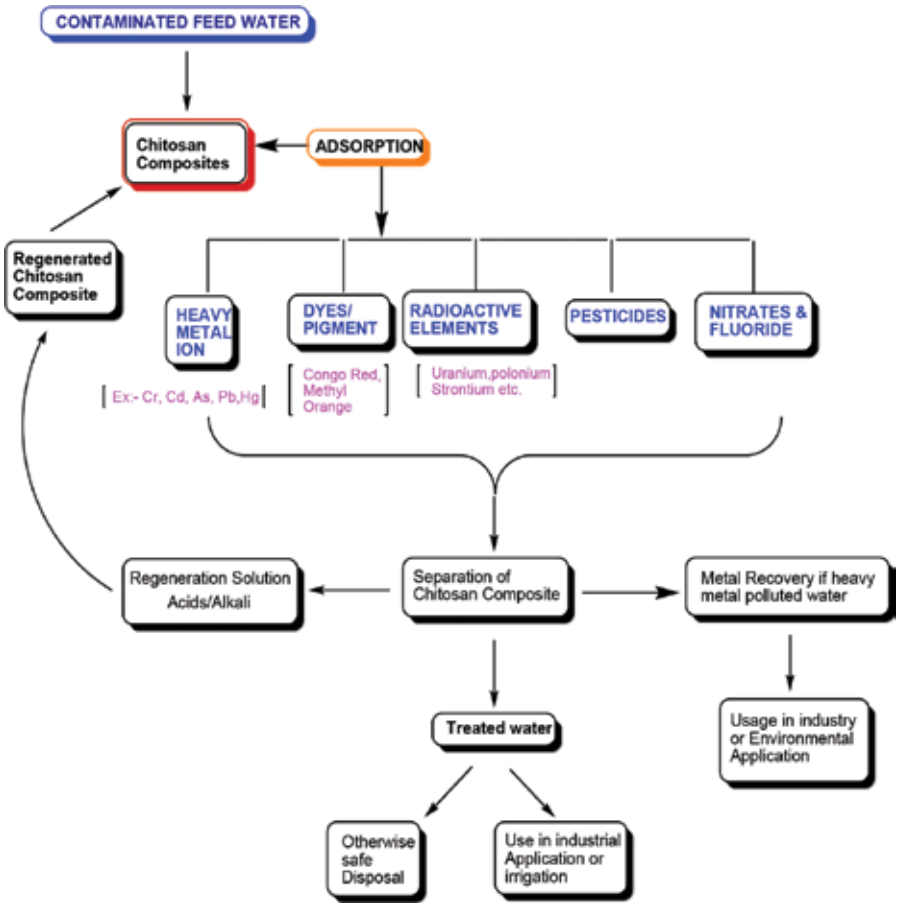

Figure 5. Schematic representation of chitosan composite/biocomposite in wastewater treatment application.

adsorbent by desorption. In chitosan, the integral $–NH_2$ and –OH functional groups acts as a chelating sites for the adsorption of various water pollutants viz. heavy metal ions, dyes/pigments, pesticides, and so on (**Figure 5**).

5. Heavy metal contamination status in India

The Central Pollution Control Board (**CPCB**) [18] carried out a major groundwater quality survey and the report recognized about 20 critical sites of ground water pollution in various states of India. CPCB found that industrial effluents are the primary and major cause for ground water pollution. The major heavy metals contamination sites including lead metal in Indian scenario are given in **Table 2**.

The heavy metal lead (Pb) was considered as father of all metals during Roman era. Much of its gratitude was due to its huge availability, consequently used in daily life by people across

Sr. No.	Area	Industrial activities	Groundwater quality problems
1	Digboi (Assam)	Oil refinery	Fe and Mn ions were more than permissible limit. Ni, Zn, Cd, Cr, Pb were also reported.
2	Howrah (West Bengal)	Foundries and Electroplating	Heavy metals viz. Pb, Cd, Cr were very high and Zn, Cu, were within limit. Hg, Fe, Mn and pesticides were also very high, CN and phenolic compounds in traces.
3	Botharam Patncheru (AP)	Pesticides, Pharmaceuticals	Phosphates, Hg, As, Cd, Fe, Mn and Pb were beyond limit, Pesticides, coliform, TDS, were also exceeded the desirable limit.
4	North Arcot (TN)	Tanneries and dyeing units	Hg, Cd, Pb and As were in traces. Zn, Cu, Cr, Fe and Mn beyond limit at several locations. Total Coli form and fecal Coli form were also on higher side.
5	Ratlam, Nagda (MP)	Distillery Dye, Pharmaceuticals	TDS, Hg, Pb were on higher side. Pesticides and fecal Coli forms were also present.
6	Mandi Gobindgarh (Punjab)	Wooden, chemicals, electro-plating and other steel metals units.	Pb, Cu, Cd exceeded the desirable limit of drinking water. Phenol compounds and cyanide were also present on higher side.
7	Parwanoo (HP)	Ancillary, fruit proceeding plant, air pesticides.	The presence of Cd, Pb, Fe, and Mn was observed on higher side. Pesticides and phenol were above the toxic limit.
8	Kala Amb (HP)	Paper mills	Heavy metals like Cd, Pb and Mn and Phenol compound were higher than the toxic limit. Pesticides, Coli forms were also present.
9	Pali (Rajasthan)	Textile, dyes	Pb and Zn, F, TDS and Cl found higher.
10	Jodhpur (Rajasthan)	Textile, steel, engineering foundry, chemicals, oil, pulses and rubber.	Heavy metals such as Fe, Cr, Mn, Pb were also on higher side. Na, TDS exceeded the limit.
11	Angul Talcher	Thermal power station, fertilizers, chemicals, mining activities.	Cr, Fe, Cd, Pb & F, NO_3 all were found in concentration level exceeding standards limits.

Table 2. Pb (II) ions contamination status in India.

all classes. The special attention needs to be given on its mitigation from contaminated water bodies. Lead was ranked second on the Comprehensive Environmental Response.

Compensation and Liability Act (1980) (CERCLA) Priority List of Hazardous Substances in 1999 and 2001 (after arsenic #1, and before mercury - #3). The priority list is prepared by the Agency for Toxic Substances and Disease Registry (ATSDR) and Environmental Protection Agency (EPA) and is based on the frequency of occurrence of particular contaminants at National Priorities List (NPL) sites and their potential threat to human health. Pb (II) ions can be found in effluents from battery recycling plants, lead mining and electronic assembly plants. Lead metal elucidates destructive effects almost on every organ

systems viz. nervous, blood circulation, reproductive, digestive, kidneys as become highly toxic and carcinogenic even at low concentration. World Health Organization (WHO) prescribed the maximum permissible limit (MPL) of lead metal in drinking water as 50 ppb initially during 1995 that was further decreased to 10 ppb in 2010. However, more recently, an EPA document recommended a zero lead value in a national primary drinking water standard [19].

In recent years, the chitosan blended biocomposites have been synthesized by impregnation with graphite [20], iodate [21] activated carbon of *Luffa cylindrica* [22], and so on and were utilized for Pb (II) ions mitigation from water. The choice of these materials was concerned with its high adsorption efficiency, safe and simple to use, easy to maintain, minimal production of residual mass, low capital cost and nontoxicity. The resultant adsorbents viz. graphite doped chitosan composite (GDCC), Iodate doped chitosan composite, and activated carbon of *Luffa cylindrica* doped chitosan biocomposite satisfy all these requirements during their usage as a bioadsorbents. In this chapter, the synthesis, characterization and batch adsorption of Pb (II) ions by using GDCC are explained.

6. Synthesis of graphite doped chitosan composite (GDCC)

Chitosan dissolved in acetic acid and heated at 50°C to obtain gel followed by the addition of powdered graphite in (1,1 w/w) ratio. Mixture was then agitated magnetically (800 rpm) at 27°C) for 5–6 h and dropped in aqueous ammonia to obtain beads. Finally, it was filtered, washed with double distilled water and dried in oven at 70–80°C. The GDCC was grounded, and the particle size recorded in range of 176–246 μm. (**Figure 6B**).

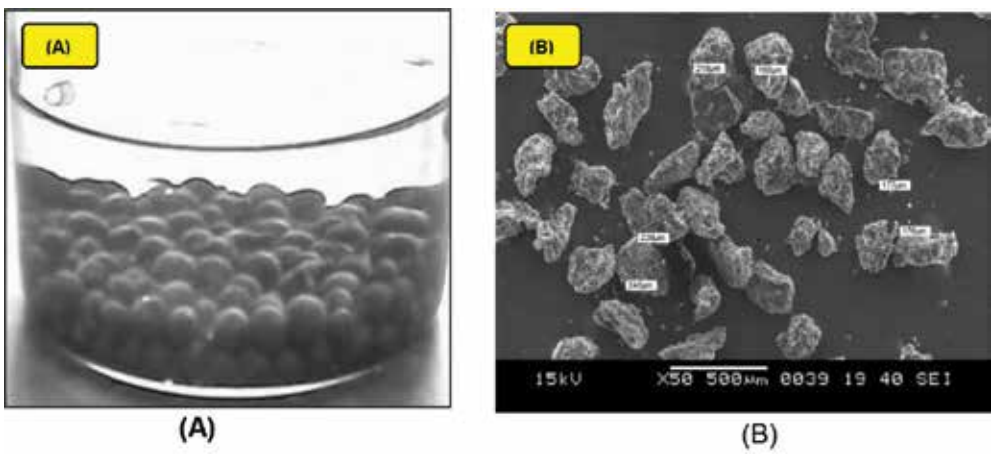

Figure 6. (A) GDCC beads and (B) SEM image of powdered GDCC, with particle size in range of 176–246 μm.

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7. Results and discussion

7.1. Physicochemical characterization of GDCC

The results of the proximate and elemental analysis of GDCC is shown in **Table 3**

7.1.1. TGA and DSC analysis of GDCC

Thermogravimetric/Differential Scanning Calorimetry (TGA/DSC) was used to evaluate the thermal stability and to determine the decomposition temperature of the adsorbents. TGA and DSC analysis of chitosan (CS), Graphite (Gr), and Graphite doped chitosan composite (GDCC) are shown in **Figure 7(A)** and **(B)** respectively.

From TGA curve, it was observed that CS showed two steps of degradation. The initial degradation occurred at around 30–100°C and displayed 5% weight loss. This degradation may correspond to the loss of adsorbed and bound water or moisture vaporization. Initial decomposition around 100°C can be attributed to the strong water adsorptive nature of CS. The second decomposition stage was at 270°C and continued up to 312°C with 46.28%

Adsorbent	Proximate analysis (%)				Elemental analysis (%)				
	Moisture	Volatile matter	Ash	Fixed Carbon	C	H	N	S	O
CS	5.3		6.2		38.5	7.8	7.1	0.19	46.45
GDCC	7.2	55	4	33.8	57.69	3.78	4.03	0.23	34.27

Table 3. Proximate and elemental analysis of GDCC.

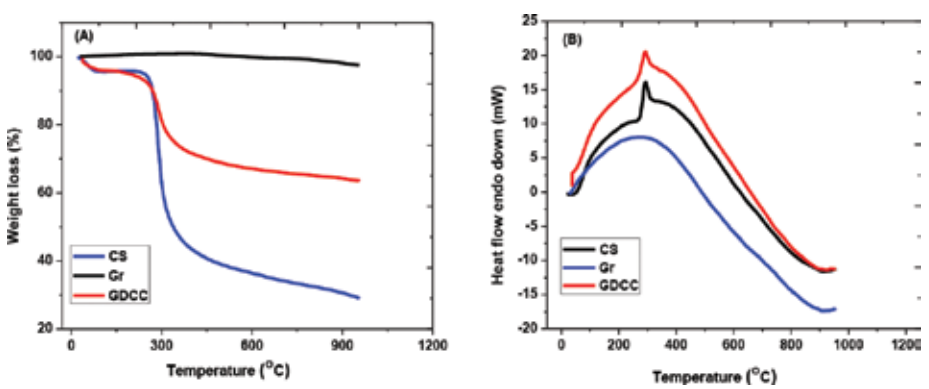

Figure 7. (A) Thermogravimetric analysis (B) differential scanning calorimetric analysis of chitosan (CS), graphite (Gr) and graphite doped chitosan composite (GDCC).

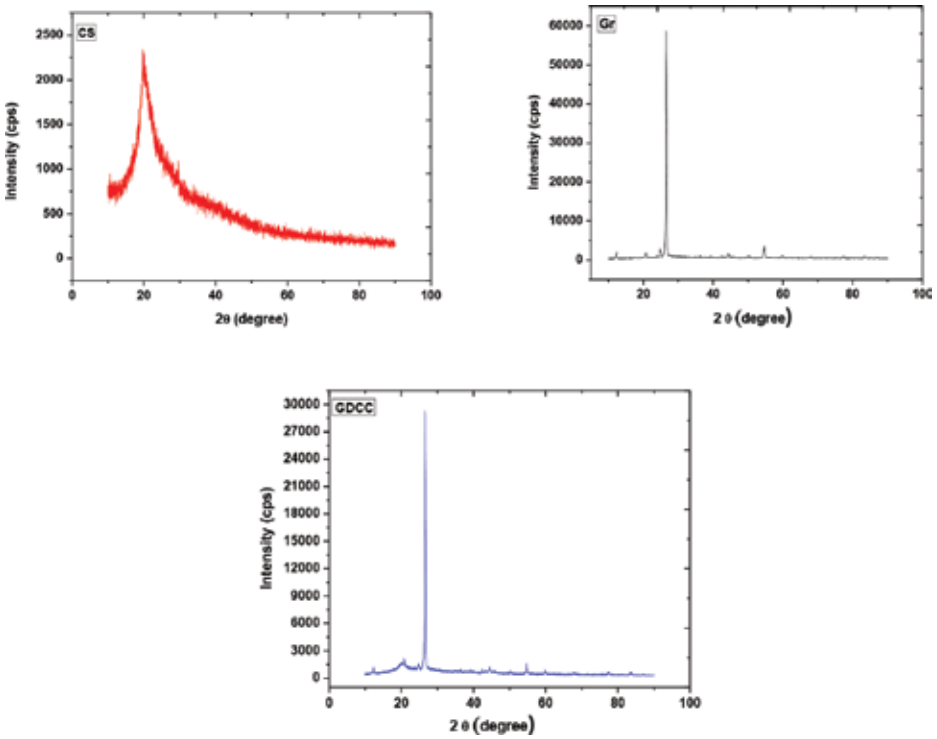

Figure 8. XRD analysis of chitosan (CS), powdered graphite (Gr) and GDCC.

weight loss. The temperature at which maximum degradation occurred was 288.35°C. At the end of 955.1°C, the total weight loss of CS was 70%. TGA analysis of powdered graphite shown high thermal stability up to 700°C and displayed only 2.5% weight loss at the end of 955°C.

TGA analysis of GDCC also exhibited two steps of degradation. First stage decomposition occurred between 38.01 and 200°C which showed about 5% weight loss due to evaporation of water. The second stage of decomposition showed a weight loss of 18.37% in the temperature range of 265.15–321.6°C. The temperature at which the maximum degradation occurred was 288.55°C. At the end of 955°C, the total weight loss was 35%. The TGA analysis revealed that with respect to powdered graphite, the GDCC became less thermally stable, whereas with respect to the CS, the composite became more thermally stable. These observations showed a good miscibility between CS and graphite to achieve GDCC or biocomposite.

The DSC curve of CS and GDCC both shows one exothermic peak at 292 and 291.37°C, respectively. For CS, the onset of exothermic peak was at 276.89°C and continued up to 311.49°C with $\Delta H = -149$ J/g and for GDCC the onset of exothermic peak was at 270.64°C and continued up to 311.86°C with $\Delta H = .-60.1211$ J/g.

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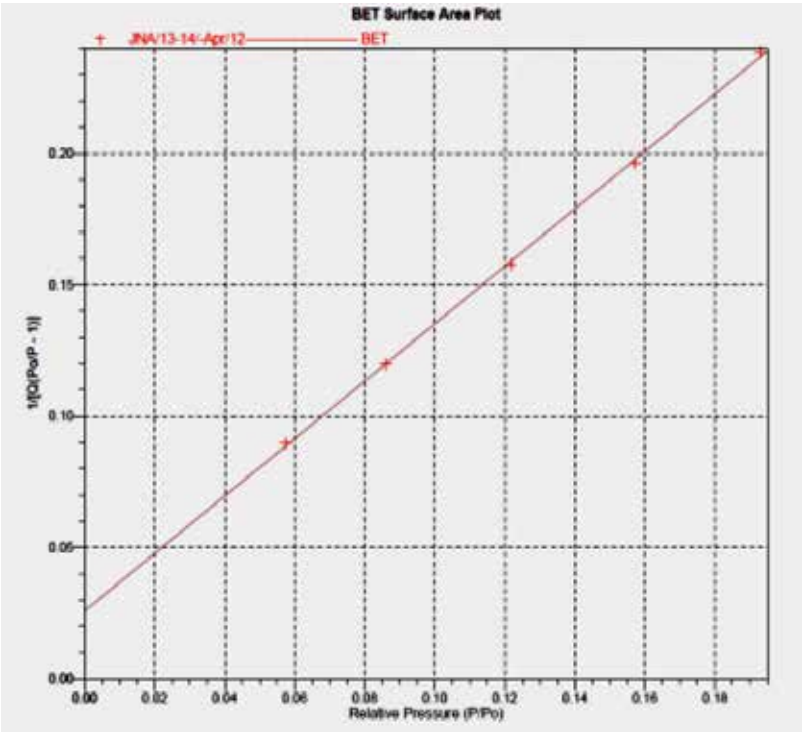

Figure 9. BET surface area plot of GDCC.

7.1.2. XRD analysis of GDCC

The XRD pattern of powdered graphite, chitosan and GDCC is shown in **Figure 8**. X-ray diffraction pattern of CS exhibited broad diffraction peak at 2θ = 20° with d-spacing of 4.2 Å is characteristic of semi crystalline chitosan [23]. The peaks are broadened due to amorphous nature of chitosan polymer. The diffraction peak appeared at 2θ = 26.5° which indicated d-spacing of about 3.35 Å is a characteristic of graphite peak [24]. The XRD pattern of the GDCC indicated the formation of homogeneous/single phase composite, and the peaks were obtained at 2θ value 26.5°. The broad peak at around 2θ = 20° which was due to CS decreased in intensity after doping with graphite which confirms that graphite is doped on the surface of chitosan. A predominant peak of graphite along with small peak of chitosan appeared in GDCC showed that the incorporation of graphite in matrix was successful and effectively provided a support to the chitosan.

7.1.3. BET surface area analysis of GDCC

The BET surface area plot of GDCC is shown in **Figure 9**. The BET surface area of GDCC adsorbent was 3.89 m^2/g, whereas for CS, it was 9.923 m^2/g. Thus, it was observed that the BET surface area of GDCC composite was decreased with respect to CS. During modification of

CS by graphite, the decreased surface area may be due to the blockage of internal porosities of CS by incorporated modifier, that is, powdered graphite to achieve GDCC composite. The adsorptive ability of GDCC for Pb (II) ions is good in spite of decreased BET surface area with respect to CS.

It is due to the participation of various functional groups such as –OH, C=O and $–NH_2$ on the adsorbents surface thereby adsorption occurred predominantly via chemisorption mechanism. Surface area is the physical parameter and the adsorptive capacity increases with increasing surface area for a pure physisorption process.

7.1.4. SEM analysis of GDCC before and after Pb (II) ions adsorption

The scanning electron microscopic images of chitosan and GDCC before and after Pb (II) ions adsorption are shown in **Figure 10(A-C)** respectively. **Figure 10(A)** revealed small amount of round voids and well-developed elongated bilobed porous structure in chitosan. The surface morphology of chitosan was drastically changed to flaky, smooth and porous nature with some voids/cavities after impregnation with graphite as in **Figure 10(B)**. The morphology of Pb (II) ions loaded GDCC exhibited accumulation of shiny, whitish, sharp needle shaped crystalline mass onto its surface due to the adsorption of Pb (II) ions (**Figure 10**C).

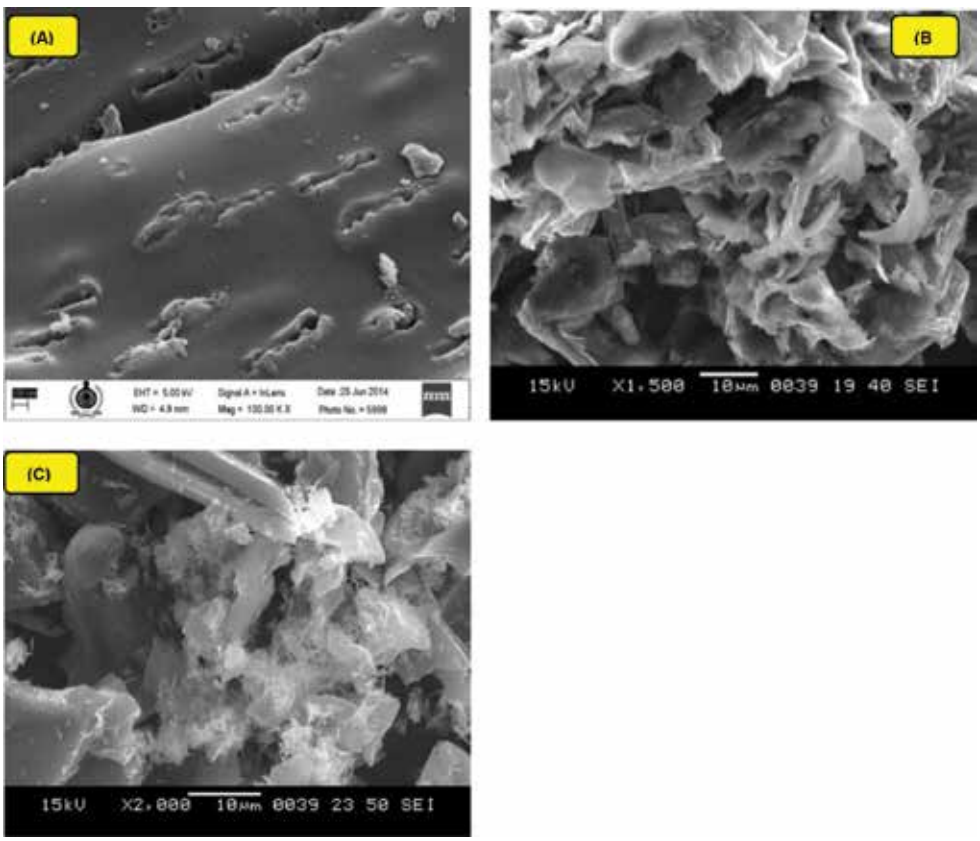

Figure 10. SEM image of (A) CS, (B) GDCC before adsorption, and (C) GDCC after adsorption.

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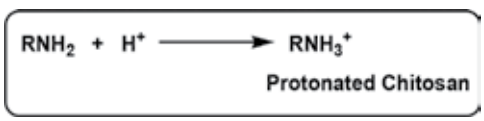
8. Adsorption mechanism

The bioadsorbents possess various functional groups like carboxyl, hydroxyl, amino, phosphate, and so on that can provide an active binding site for the adsorption of heavy metal ions. The mechanism of bioadsorption is quiet complicated due to assorted structure of the bioadsorbents. The factors that affect the efficient bioadsorption onto the surface of biosorbents are the availability of number of active binding sites, the affinity of pollutant for the bioadsorbent surface and the presence of variety of functional groups that can exhibit an acceptor-donor interaction with the heavy metal ions. The adsorption process is a combination of ion exchange, complexation, precipitation, and so on and greatly influenced by the solution pH. Similarly, in order to understand the adsorption mechanism, it is also necessary to determine the pH of point zero charge (pHpzc) of the adsorbent. pHpzc is of prime importance in the field of environmental science. It determines how easily and adsorbent adsorbs toxic ions. The difference between the initial pH (pHi) and final pH (pHf) values is plotted against initial pH (pHi). The point of intersection of the resulting curve at which difference between pH = 0 is noted as pH of point zero charge. The cationic adsorption is favored at pH > pHpzc while anionic adsorption is favored at pH < pHpzc [25] This is due to the fact that at low pH values, hydronium ions concentration increases that competes with cationic pollutants for the adsorption sites on the adsorbent. While at higher pH, hydroxide ions concentration increases and the adsorbent surface becomes negatively charged thereby increases the attraction between the cationic pollutants with the adsorbent surface. Thus, pH > pHpzc is favorable for the cationic adsorption. In anionic adsorption, solution pH should be less than the pH pzc so that the adsorbent surface becomes positively charged to enhance the anionic pollutants adsorption onto the adsorbent surface. The general adsorption mechanistic representation is shown in **Figure 11**.

On the basis of results obtained from the analytical and spectroscopic data, the schematic representation of Pb (II) ions adsorption mechanism onto GDCC is represented in scheme as below.

The amino group of chitosan plays a major role in removal of Pb (II) ions via adsorption as it acts as coordination site for metal ions. The amino group gets protonated in acidic medium due to reaction with H^+ ions and is chemically represented as below.



The FTIR analysis revealed the corresponding prominent changes of –NH bending vibrations after doping of graphite with chitosan indicated that –NH vibration is affected as a result of modification. Thus, the doping of graphite with chitosan results in formation of nonprotonated chitosan-graphite composite and is represented as follows.

The abovementioned relationship suggests that the acidic pH can enhance the complexation between chitosan and graphite.

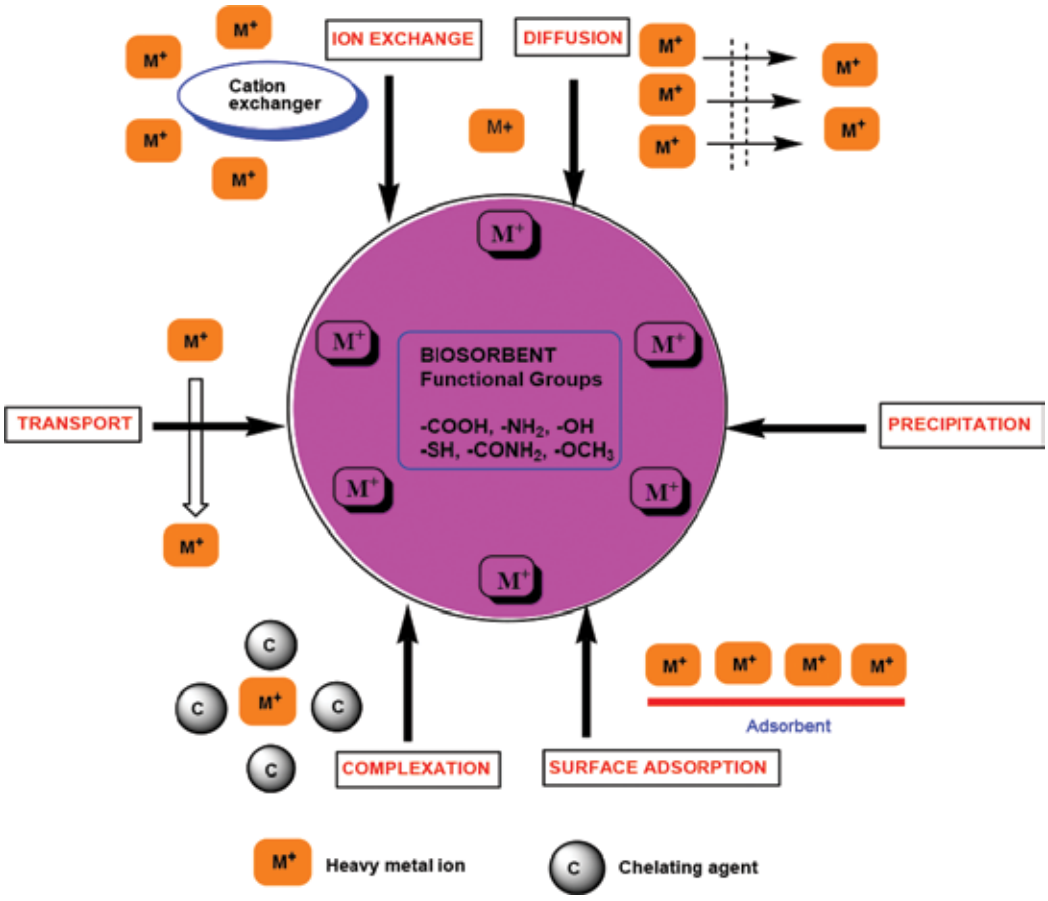


Figure 11. Plausible adsorption mechanism of heavy metal ions onto the adsorbent.

As chitosan acts as a chelating agent signifies nitrogen atom as the prominent adsorption site for Pb (II) ions adsorption. The chitosan-graphite complex binds with Pb (II) cation via the formation of coordination bond, and it is represented as follows:

$$(RNH_2\text{-Graphite}) + Pb^{2+} \longrightarrow Pb\text{-}(RNH_2\text{-Graphite})^{2+}$$
$$\text{OR}$$
$$(Pb\text{-GDCC})^{2+}$$

9. Adsorption kinetics

To study the mechanism and kinetics of Pb (II) ions adsorption, characteristic adsorption constants were determined using pseudo first order, pseudo second order and intraparticle diffusion models.

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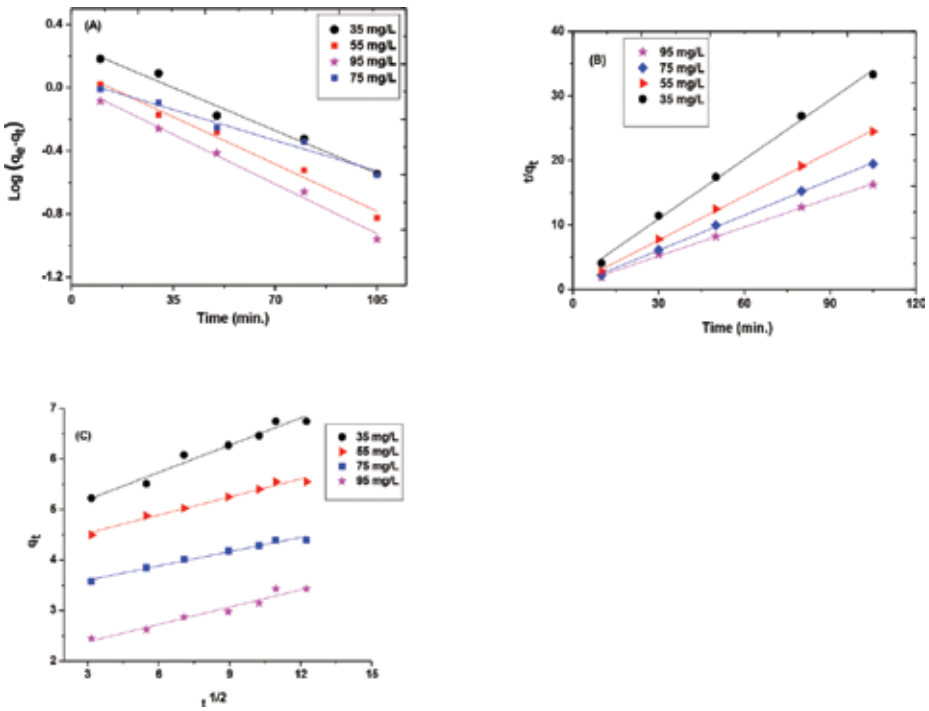

Figure 12. Linear plot of (A) pseudo first-order kinetics (B) pseudo second-order kinetics and (C) Weber-Morris Intraparticle diffusion model.

The Lagergren pseudo first-order plot, Ho presented Pseudo second-order kinetics plot and Weber Morris Intraparticle diffusion plot are shown in **Figure 12(A-C)**. Similarly, the kinetic parameters viz. rate constant (k_1 and k_2), equilibrium adsorption capacity (q_e) and correlation coefficient (R^2) are indicated in **Table 4**. Equilibrium adsorption capacity (q_e) and the pseudo first-order rate constant (k_1) can be obtained from the intercept and slope of plot between Log ($q_e - q_t$) against time t. Similarly, the equilibrium adsorption capacity (qe) and the pseudo-second-order rate constant K_2 were obtained from the slope and intercept of the plots of t/qt against t.

The linear correlation coefficient values of pseudo first order are comparatively lower than pseudo second-order kinetics mechanism. The calculated q_e values are much higher than the experimental q_e values for 35 to 95 mg/L Pb (II) ions concentration and thus does not represent the good fit of pseudo first order with the experimental adsorption data. Consequently, it can be concluded that the adsorption of Pb (II) ions onto GDCC is not better explained by the pseudo first-order kinetics mechanism. Results presented in the table clearly show that the correlation coefficient for pseudo second-order equation is higher than pseudo first order for all 35 to 95 mg/L concentrations of Pb (II) ions. Similarly, high k_2 of pseudo second order suggested that the metal could be rapidly sequestered by carbon functional groups, resulting in the system quickly reaching equilibrium. The calculated q_e values from pseudo second order are much closer and in good agreement with the experimental q_e values, which indicate that

Pb (II) ions (mg/L)	Pseudo-first-order			q_e (cal.)	Pseudo-second-order			Intraparticle diffusion model		
	K_1 (min^{-1})	q_e (mg/g)	R^2		K_2 (g/ mg^{-1} min^{-1})	q_e (mg/g)	R^2	$K_i t$ (mg/g $min^{0.5}$)	C (mg/g)	R^2
35	0.0115	1.1350	0.97	3.43	0.0545	3.2573	0.99	0.114	2.042	0.95
55	0.0207	1.0423	0.99	4.4	0.0628	4.4052	0.99	0.094	3.319	0.98
75	0.0184	1.3001	0.98	5.55	0.0505	5.5248	0.99	0.119	4.176	0.98
95	0.0161	1.8663	0.97	6.745	0.0324	6.7114	0.99	0.179	4.658	0.96

Table 4. Adsorption kinetics parameters for Pb(II) ions onto GDCC.

the adsorption of Pb(II) ions by GDCC follows pseudo second-order kinetics. The confirmation of pseudo second-order kinetics indicates that during the adsorption process, concentration of both adsorbent and adsorbate is involved in rate-determining step, which may be chemical adsorption or chemisorptions [26].

Intraparticle diffusion parameters are shown in **Table 4**. The intraparticle diffusion k_i values were obtained from the slope of a plot of q_t versus $t^{1/2}$. From figure, it follows that the correlation coefficient values are lower for varying Pb (II) ions concentration (35, 55, 75 and 95 mg/L) than pseudo second-order kinetics. Similarly, intraparticle diffusion plot is not linear, and the straight line does not pass through the origin, indicating that intraparticle diffusion was involved in adsorption but was not the only rate-controlling step.

10. Conclusion

The quality of water is an ever growing concern throughout the developing countries. The natural and manmade activities have a large impact on drinking water contamination that ultimately affects the human health, ecological balance and social and economic progress of countries. The chemical contamination due to heavy metal ions makes water unsuitable for drinking. Among the prominent chemical pollutants, arsenic, mercury and lead threatens health of billions of world population. It is very important for a rapidly developing country like India to be vigilant of these heavy metal problems and to ascertain preventive and remedial measures for their management. Sometimes modern and expensive remedial measures are problematic for a country like India, and hence the attempts have to be focused on the prevention and mitigation of the environmental pollutants. This chapter dealt with the mitigation of heavy metal Pb (II) ions from contaminated water using graphite doped chitosan composite (GDCC). Maximum Pb (II) ions adsorption capacity was 6.711 mg/g (from Langmuir) at optimum pH 6 with dosage of 1 g/L in 120 min. The choice of these materials was concerned with its good adsorption efficiency, safe and simple to use, easy to maintain, minimal production of residual mass, low capital cost and nontoxicity.

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Chapter 7

Sewage Polluted Water Treatment via Chitosan: A Review

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Additional information is available at the end of the chapter

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Abstract

Due to the increasing scarcity of water, wastewater treatment and water conditioning are one of the major future issues. Together with the need to apply highly accessible abundant materials and the demand to replace fossil-based chemicals with sustainable compounds from renewable resources, chitosan (CS) provides some of the solutions to obtain these goals and combines both, abundance and sustainability. Hence, the focus of this review is on the application of CS in wastewater treatment providing advantages and drawbacks in using CS in contrast to chitin. We herewith present the application of CS for coagulation/flocculation purposes, whether as native compound, as functionalized molecule or as blend, respectively, composite. The heavy metal, respectively, dye removal is an additional theme to be addressed in the body of the text. The third topic of this review contains the application of CS blends or composites in order to prepare membrane materials for water purification or conditioning. Together with a summary of the recent study, we discuss these findings and possible consequences for future works. In addition, we provide some theoretical background of the processes that CS is involved in and state some mechanistic insights.

Keywords: adsorption, anionic dye, blends, composites, coagulation/flocculation, heavy metal removal, membrane, wastewater treatment, water conditioning

1. Introduction

There are different kinds of sewages derived from industrial production, agriculture, or directly emerging from the households. As a consequence, the three sectors generate high volumes of wastewaters containing inorganic and organic compounds of every description: dyes, heavy metal ions, antibiotics, hormones, feces, colloids, and further contaminants with a

broad structural variety. The removal of these pollutants from wastewater and the conditioning of the water are thus of major interest for the water conservancy. The major parameters that need to be considered and measured in municipal wastewater are as follows: total solids, suspended solids, dissolved solids, total organic carbon, chemical oxygen demand, conductivity, alkalinity, pH, nitrogen, phosphorus, and sulfate content [1]. Special wastewaters, for example, from pharmaceutical or mining industry, require further quantifications such as hormone or heavy metal cation content. According to this, the methods applied for water conditioning mainly depend on the kind of pollution that needs to be separated. In general, processes to purify wastewater include physical, mechanical, chemical, and biological methods with various technologies ranging from adsorption, filtration, biodegradation, oxidation and reduction, UV irradiation, and coagulation/flocculation [2]. However, at the end, qualitative and quantitative criteria required must be met after purification, whereas sustainability as modern key performance is pushed into the foreground. A further aspect concerns the availability and the efficiency of the materials used during conditioning of the wastewater. Chitin, or especially the acid-soluble derivative CS, as the second abundant polysaccharide after cellulose, is one of the polymers to be applied in the wastewater meeting the two requirements. CS has the benefit that it originates from fishery waste and is biocompatible, biodegradable as well as nontoxic and has thus a versatile application portfolio [3]. In contrast to the most naturally occurring polysaccharides, which are either anionic or neutral, such as cellulose, starch, or alginate, CS is a polycation. The free amine groups, which are responsible for the polycationic character, bear nonbinding electrons providing donor properties suitable for coupling to electrophiles, for example, for the formation of imines or amides [4, 5].

One question always arises concerning the application in wastewater treatment: Rather use CS than chitin? Chitin is a glycosidic polymer that has a high degree of polymerization, forms numerous intra- and intermolecular hydrogen bonds, is semi-crystalline, and almost completely acetylated. Chitin manufacturing benefits from known chemical or biotechnological production routes starting at fishery waste that provides a cheap and renewable source. Furthermore, the purification of chitin results in calcium carbonate- or protein-rich side streams that can be further processed supporting economics of chitin production [6]. CS is a derivative of chitin with commonly lower molecular weight and lower acetylation degree, predominantly produced by the chemical conversion of chitin. The conversion reaction includes the application of high concentrated alkaline solution at increased temperature providing thus a nonsustainable process. The harsh conditions are prerequisite due to the fact that the acetamido groups are arranged in a trans-configuration with regard to the hydroxyl group at C3 [7]. The enzymatic deacetylation of chitin at mild conditions is nowadays the content of numerous extensive studies so that the development of prospective economic conversion processes is expected. The prospect of an economic production process contributes to the prediction that CS has a more promising future than chitin in the wastewater treatment [8]. Younes and Rinaudo accordingly stated that CS has a wider range of application areas in comparison to chitin [9]. This is due to the high availability and accessibility of the amine groups, the lower intermolecular forces, and the solubility in acidic aqueous media. Chitin takes advantage of the poor solubility resulting in decreased leaching and thus repeated application in all media. On the other hand, there are processes and functionalizations described increasing

CS stability in acidic media: CS is applicable as powder, flakes, and gel, such as membranes or beads. Gel preparation processes comprise freeze-drying, ionotropic gelation, neutralization, crosslinking, and solvent evaporation method. At least the latter four methods include concluding steps resulting in a more robust CS derivative, composite, or blend [10]. However, CS could be applied as a concentrated solution in acidic media, and this offers the opportunity for homogeneous modification reactions or heavy metal adsorption at the free amine functionalities. The bulky acetyl groups of the chitin provide steric hindrance disabling the proper approximation of reagents to the nitrogen. The inefficient adsorption of heavy metal ions by chitin was already demonstrated [11]. Further, the higher the deacetylation degree, as in CS, the higher the density of the available primary amine groups mainly responsible for the electrostatic interaction. The higher proportion of amorphous regions increases accessibility, sorption capacity and makes it furthermore suitable to act as a flexible linker of different colloids as required for the application in coagulation/flocculation techniques [12].

Flocculation/coagulation is an abundant, efficient, cheap and thus one of the most important processes for the treatment of effluents [13]. The removal of the suspended solids or colloids is mandatory in order to perform the succeeding purification steps. This is one of the processes carried out with inorganic metal salts and/or polyelectrolytes, as CS is. The majority of the CS publications and patents in wastewater treatment focuses on this field of application. Hence, section 2.1 of this chapter elucidates the CS usage in coagulation and flocculation. The specific removal of heavy metals or anionic dyes is a topic that needs to be considered in special effluents as derived from mining or textile industries. CS showed promising results within this field of application and is thus content of section 2.2 of this chapter. The last section has the application of chitin and CS in membrane materials for a theme. Membrane-assisted approaches become more and more important due to their efficiency, improved process controls, and the opportunity for a directed compounding of different materials. Within these applications, CS takes the role of not only a structural substance but also a functional compound as it is the case for adsorption as well as coagulation and flocculation.

2. Main body

2.1. Chitosan as coagulating and flocculating agent

Water quality is commonly diminished by the presence of colloids or smaller organic substances resulting in a high chemical oxygen demand (COD) and high turbidity. The removal of the majority of these compounds is predominantly performed via coagulation and flocculation. Though coagulation and flocculation are used interchangeably, they represent two distinct processes: coagulation is the act of destabilizing a suspension, whereas flocculation means either the spontaneous or polymer-induced formation of large agglomerates succeeding destabilization [14]. The technique transforms colloidal particles or solutes with settling times of years to flocculated or precipitated particles with settling times between seconds and hours. Furthermore, the moisture content of the resulting slurry from up to 99% can be reduced to 65–85% enabling succeeding processing [15]. In general, different organic and

inorganic coagulants and organic flocculants were used. Often, ferric chloride, lime, or alum as agents of choice are applied as the primary coagulant for the destabilization of suspensions due to their availability, efficacy, and their little cost [16]. However, residual concentrations of alum bear environmental risks to the whole aquatic biota [17, 18]. Synthetic polymeric flocculants, as polyacrylamide, are currently used based on the ability to improve flocculation despite their application is associated with lack of biodegradability and the release of degradation products [19]. A decrease or substitution of these reagents by the addition of biopolymers would be beneficial regarding sustainability, ecology, and health. One of the polymers focused on within the investigations was CS. An overview of the recent studies concerning the usage of CS for coagulation and flocculation purposes with original wastewaters rather than model solutions can be obtained from **Table 1**.

The experiments summarized in the table were carried out with different kinds of wastewaters and with native crab shell CS rather than the modified one. Economic viability would be greatly decreased if CS modification is essential to ensure a high efficiency of such a low-cost unit operation even if several investigations were performed with grafted or modified CS [27]. The results revealed that the CS could significantly reduce COD (>60%) and turbidity of the treated solutions (>80%) applied to all kinds of wastewaters and at different conditions. Regarding this, the authors stated that CS has approved to be efficient, concerning the coagulation of suspended matter in wastewater even at low temperatures and low doses [28].

Wastewater from	Effect	Notes	Refs.
Textile treatment	72.5% COD and 94.9% of nephelometric turbidity units (NTO) reduction	Optimum performance at pH 4–6 and 30 mg/L chitosan	[20]
Pulp and paper mill industry	90% turbidity reduction and 60% COD reduction	$FeCl_3$ as coagulant, CS as auxiliary, improves sedimentation and compaction, heterogeneous photocatalysis succeeded	[21]
Olive oil and wine production	80–94% turbidity reduction, 81–94% decrease of total suspended solids, and 73% COD decrease	Best performance at actual pH of wastewater (olive oil wastewater) or no significant influence of pH (wine production wastewater)	[22]
Catfish farming	>99% of microalgae removal efficiency, significant NTU reduction and 80% of the microalgae recovered	CS could be used for both removal recovery of biomass and the overall reduction of the microbial community	[23]
Cardboard industry	Turbidity lowered more than 85% and COD more than 80%	Higher efficiencies than polyaluminum chloride; additionally decreases heavy metal, removes colored compounds	[24]
Tequila industry	CS was the most efficient biopolymer removing 84% of COD	Catalytic oxidation succeeds coagulation/flocculation process	[25]
Rural domestic water treatment	Turbidity reduction of at maximum 99%	Unmodified chitin was used in comparison to alum and sago	[26]

Table 1. An overview about latest results obtained applying CS for coagulation/flocculation purposes with "real" effluents.

The independence of the CS flocculation efficacy from different environment conditions, as temperature, is mandatory since the proposed application is likely to be carried out in non-temperated outdoor sewage plants or in open aquatic systems. The application in aquatic systems for fish or shrimp farming reasons on the requirements to reduce the high nutrient loading and to prevent the accumulation of toxic substances. Synthetic polymers or inorganic compounds mentioned before are not adequate to perform this since they are commonly not biocompatible and biodegradable. For the same reason, CS was successfully applied in accelerating the sedimentation of microalgae. The authors reported differing flocculation efficiencies depending on the pH and dosage but approved that CS can be successfully applied to harvest microalgae [29]. Hence, the application of CS to induce biofloc formation with microorganisms for clarification purposes or reducing harmful nutrient contents would be suitable. This could on one hand increase the water quality in aquatic systems, on the other hand, the resulting flocs can be easily removed or uptaken by the marine animals [23, 30]. However, it can be expected that the growth of different bacterial strains is decreased due to the antibacterial characteristics of the CS constraining the application to eukaryotic microorganisms that are used for this process [31]. Since the antibacterial property is dependent on the amount of amine functionalities, chitin usage in aquatic systems is worth trying out. Native chitin was also successfully applied to decrease COD and turbidity of surface water, but it has to be considered that high concentrations of chitin (>0.1 g/L) were applied. At the same time, surface water contains a lower amount of contaminants in comparison to industrial or municipal effluents [26]. This reduces the amount of flocculant or "active" sites necessary, possibly enabling the application of chitin.

In contrary to the findings of the authors summarized in **Table 1**, it was proposed several years ago that the effectiveness of flocculants based on biopolymers is low compared to synthetic polymers [27]. It is also obvious that a combination of CS with other techniques seems to be favored rather than the direct application as flocculant [21]. The addition of CS could significantly lower the amount of coagulant required or enhance floc formation, approved for the flocculation of a model system with clay or bentonite, the conditioning of groundwater or for clarification of pulp and paper mill wastewater [21, 32, 33]. Such a process is exemplary provided for the synergistic action with Fe salts shown in **Figure 1**.

In **Figure 1**, ferric chloride is applied as a primary coagulant to destabilize the system, and the so-called perikinetic flocculation due to Brownian motion under vigorous stirring succeedingly occurs. This results in a formation of smaller flocs. The polyelectrolyte activity initiating a large floc formation (orthokinetic flocculation) is not only based on one mechanism. For cationic polymers, such as CS, two main mechanisms can be postulated which occur coincidently: (a) Bridging by the adsorption of one polymer to adjacent colloids (bridging model). This applies also to polymers bearing the same charge as the colloid. (b) Reduction of the electronic repulsion between adjacent colloids by electrostatic interaction, adsorption, and charge neutralization of a polymer with opposite charge (patch mechanism) [14]. The interaction of the CS with the colloids leads to the formation of flocs having low settling times. The performance of floc formation and turbidity removal is withal dependent from the CS dosage, thereby approving the contribution of the patch mechanism. First, increasing CS dosage fosters turbidity decrease; further CS addition concludes in a contrary effect based on the

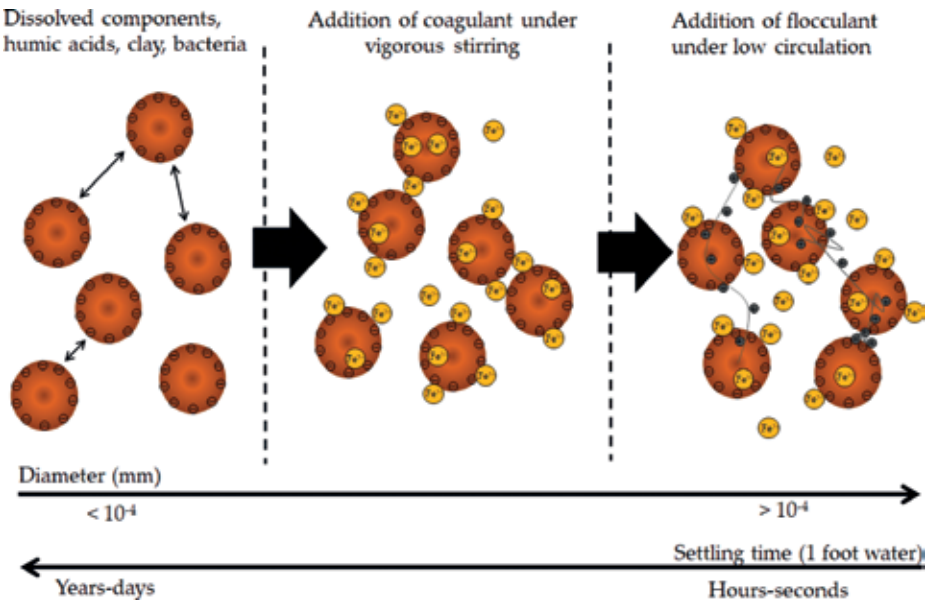

Figure 1. Mechanism for coagulation/flocculation of colloids or solutes with a ferric chloride-CS system. The mechanism is exemplarily shown for Fe^{3+} salts (yellow circles) as primary coagulant and CS (gray line) as flocculant auxiliary. The size relations are not representative.

repulsion of the excess CS adsorbed to the colloids [34]. The size or molecular weight of the polysaccharide provides a further influencing factor. Investigations revealed a higher efficacy of the turbidity removal with increasing CS molecular weight. This approves that bridging also contributes to the effect, but only in tap water, determining the importance of the ionic strength for the mechanism [14, 35]. Guibal et al. stated that the effect of the deacetylation degree was not very significant except at nearly neutral pH values and low ionic strength suspensions. This confirms that a variance analysis with only one independent factor to optimize the flocculation process with CS is not expressive without coincident consideration of other relevant parameters [36].

Based on the results, CS was approved to be an efficient flocculant auxiliary but needs to be applied at an optimum dosage and as a polymer bearing physical-chemical characteristics suitable for the application. Furthermore, the performance is greatly influenced by the pH of the reaction medium [37]. The pH dependency of the CS is linked to the charge density, as confirmed by several authors, providing a double-edged sword [38, 39]. The pH of an effluent can scarcely be adapted due to the commonly high volumes of wastewater generated. This applies not only to the application as flocculant but the more for the usage as adsorbent which is highly sensitive for pH changes.

2.2. Chitosan as sorbent

The coagulation/flocculation mechanism is also effective with regard to the removal of anionic compounds and positively charged heavy metal ions via solid–liquid separation. Besides the

destabilization of dispersions and the removal of the formed floccules, CS can also act as a specific adsorbent although both processes, coagulation/flocculation and adsorption, cannot be investigated separately and occur in general simultaneously. In this section, we focus on the adsorption of the mentioned contaminants present in the effluents. This is especially the case for effluents generated by metal finishing, textile dyeing, or board manufacturing, resulting in wastewaters with high concentrations of toxic heavy metal ions and anionic dyes. Native and derivatized CS demonstrated to separate both compounds with a high effectivity [40]. The capacity of the native CS to adsorb dyes or heavy metals ions is in general dependent from various parameters as deacetylation degree, the particle size, the physical state of the CS, the pH value, and the temperature [41–44]. According to different studies, deacetylation grade is the most relevant parameter and thus the primary amine groups [7]. It has to be stated that the total amount is not relevant but the accessible amount of amine groups is, depending on crystallinity and diffusional properties [45].

Native CS is able to interact with other compounds via free primary alcohol or free primary amine groups depending on the system conditions. In comparison to the application as flocculant, it is widely accepted to modify or combine the CS in order to modulate the stability, rigidity, and viscosity [46]. Crosslinking as one of the most prominent modification procedures prevents leaching of CS at acidic pH and gives additionally the opportunity to recycle, respectively, reuse, the resin [40]. Furthermore, modification is carried out to increase sorption capacity as well as selectivity to adsorb specific compounds as can be inferred from **Table 2**. There are two general modification processes described here for CS: the linkage to reactive molecules and thus the insertion of functional groups, named as grafting, or the crosslinking reactions to form a dense network of CS chains conferring stability to the resin [47]. For derivatized CSs, crosslinking method, crosslinking grade, and the kind of derivatization are crucial for the performance. There are several techniques, covalent and ionic, to crosslink the CS [48]. A third opportunity besides the crosslinking and grafting is the formation of composites or blends to combine the benefits of CS and other materials or better, to develop synergistic effects [40, 49]. Here, the type of compound used and the content greatly alter the functionality and efficiency.

Common to all experiments is that the majority of compounds applied in combination or used to derivatize CS are non-sustainable materials (polyacrylamide and epichlorhydrin). To become the benefits important and to pursue a holistic sustainable approach, materials from renewable resources have to be applied in combination with CS, focusing investigations concerning the removal efficiency with different effluents. All investigations, summarized in **Table 2**, were performed with aqueous solutions spiked with model substances. Although this is only a short overview of the current study, it is obvious that there is a lack of experiments carried out with "real" wastewaters as it is shown in **Table 1** for the coagulation/flocculation studies. Studies with model solutions are suitable for an estimation of the prospective potential and application field but cannot substitute the experiments with effluents. This bases on an activity and stability reduction that has to be expected in a complex matrix. However, results revealed the effective removal of heavy metal ions or dyes from model solutions. Especially the dye removal based on the polycationic character of CS seems to be promising indicated by the high removal efficiencies.

Substrate	Agents	Adsorption characteristics	Notes	Refs.
Synthetic heavy metal solutions	CS dianhydride (ChD)and ChD amine (ChDA)	>66% removal of Cu^{2+}, Pb^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, and Cr^{3+}	Binding constants from 10^4 to 10^5 M^{-1}; ChDA sedimentation velocity 20* higher than native CS	[50]
	Native CS, polyethylene-imine-CS	146 mg/g Cu^{2+} capacity	Desorption with alkaline EDTA; capacity of native CS lower	[51]
	Carboxymethyl CS-hemicellulose	Up to 909 mg/g Cd^{2+} and 333 mg/g Cu^{2+}	Desorption with EDTA; covalent bond via thermal crosslinking	[52]
	Glycine or chloroacetic acid reacted CS	280 mg/g Cu^{2+}, 99% removal of Cu^{2+} and Co^{2+}	Modification does not significantly enhance properties; pH 9 adsorption works best	[53]
Synthetic dye and heavy metal solution	Carrageenan/ CS-microspheres	212 mg/g for dyes and 20 mg/g for Cu^{2+}	Ampholytic microspheres with magnetic Fe_3O_4 core were applied	[54]
	CS-lignin composites	>95% removal and 111 mg/g for RBBR, >95% removal and 20 mg/g of Cr(VI)	The composites exhibit better performance than CS and lignin, no significant pH effect	[55]
Synthetic dye solutions	Carboxymethyl CS grafted polyacrylamide	>93% removal of dyes	Effective removal of anionic and cationic dyes also results in flocculation of dyes	[56]
	CS crosslinked with sulfonates and epichlorohydrin	>90% removal of dyes	Ampholytic character enables the removal of different dyes with one crosslinked CS	[57]
	CS/PVA-blends	130 mg/g capacity for RR	Desorption by pH increase, Langmuir model fits best	[49]
	Nano-ZnO/CS composite beads	76% removal, adsorption capacity of 190 mg/g for RB5	Langmuir model fits best, pH 4 suitable for adsorption	[58]

RBBR: Remazol Brilliant Blue R; RB5: Reactive Black 5; RR: Reactive Red.

Table 2. Abstract of the current research and results concerning the application of native and derivatized CS in dye and heavy metal removal.

2.2.1. Adsorption of dyes

Dyes as adsorbate are usually classified with regard to their charge, succeeding dissolution in water. There are cationic (basic) dyes, reactive (acidic dyes), and non-ionic (dispersed) dyes. The adsorption of anionic dyes is a property originally derived by native CS due to the cationic character at low pH values. Electrostatic interactions play thus the major role with regard to the adsorption of the dyes. The modification of the CS is commonly carried out to improve stability or to extend the adsorbate spectrum. Herrera-González et al., for example,

crosslinked the CS with epichlorohydrin and subsequently grafted the modified polymer with sulfonates resulting in the ability to capture positive-charged dyes [57]. In general, to capture a broader spectrum of dyes and to overcome the hurdle that they show inert properties, CS composites, as CS/bentonite, CS/montmorillonite, or CS/activated clay, were stated as promising materials. As an additional feature, the materials provide an increased stability at low pH values [40]. The compounds to form the composite resins implement new properties resulting in a variety of further interactions between dyes and adsorbent and thus in stronger bonds [55]. Bond strength between adsorbent and adsorbate can be assessed by thermodynamic measurements. The adsorption of reactive dyes with crosslinked CS or CS composites revealed enthalpic values from −53 to 46 kJ/mol. This determines that the enthalpy values are highly dependent on the crosslinking agent and the other compounds the CS is applied with. This is not the case for the Gibbs energy showing low negative values for all investigations and thus exhibiting a spontaneous reaction [58–60].

Rashid et al. bridged the gap between heavy metal removal and dye adsorption. They applied a mixed Fe^{3+}- and Cu^{2+}-CS complex for the efficient removal of Reactive Black 5 [61]. The separation of both, dyes and heavy metal ions, is the content of several investigations using composites. It has to be stated that the adsorption capacity of the materials is decreased (~20 mg/g) in respect to materials developed for the recovery of one compound class alone even if the simultaneous separation was not in the focus of these experiments. Hence, the adsorption of heavy metals should be focused using other CS grafts, blends, or composites.

2.2.2. Remediation of heavy metal pollutants/contaminants

Heavy metal contaminations constitute a severe risk for the environment and also for humans. Not least because they are at the top of the food chain, humans will inevitable uptake and accumulate heavy metals released. An adsorptive removal directly at the source of formation would thus be advantageous in minimizing exposition potential. Based on many studies, CS provides an adsorbent to accomplish this task.

Since the term "heavy metal" is inconsistently defined in the scientific literature [62], we refer to toxic metals with a high density and their oxyanions. The majority of investigations thus focuses on Al^{3+}, Cu^{2+}, Pb^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+}, and Fe^{3+}, demonstrating the successful application of CS for the removal of these metal ions. On the other hand, CS does not tightly adsorb alkali and alkaline earth metals according to the HSAB (hard soft acid base) principle [63].

As it is the case for the dye removal, the combination of CS with other compounds was in the scope of recent investigations: the hydroxyapatite/CS nanostructures were efficient concerning the removal of Pb^{2+} from wastewater, revealing a higher adsorption capacity than comparable sorbents [64]. The same applies to CS-tannic acid modified biopolymers being able to adsorb Pb^{2+} and Al^{3+} [65]. Blending lignin and CS provides a sustainable material for the removal of metal ions, whereas not only interpolymeric interactions exist but also synergistic effects to capture the adsorptive. The authors state several adsorption sites for one adsorptive based on the interactions derived from hydrogen bridge bonds [55]. All together is that the CS additionally provides a backbone for modifications with functional molecules, improving the chelation of metal ions. However, the results also indicate that the CS itself significantly contributes to the adsorption of these compounds [53].

Investigations approved the high affinity for different metal cations, as, for example, Fe^{3+} to CS by an equilibrium constant of 9.49×10^5 M^{-1} determining that the equilibrium is strongly shifted toward the CS-heavy metal complex [66]. These findings were confirmed by thermodynamic measurements revealing highly negative Gibbs free energy values (−37 kJ/mol) and negative enthalpic values (−41 kJ/mol) [67]. In contrast to that, Negm et al. claimed that the adsorption of copper and cobalt ions is endothermic and entropy-driven, likely reasoning on the different conditions and the functionalization of shrimp shell CS with glycine/chloroacetic acid greatly altering the sorption mechanism [53]. Summarizing the CS-metal ion equilibrium systems revealed that particularly the Langmuir isotherm was the isotherm of choice to analyze the equilibrium data in over 30 systems since they provided a very good fit to the data. However, the authors mentioned that there is a lack of comparable results with other isotherms [68].

Although the detailed mechanism of metal sequestration remains unclear, in general, the removal of metal ions by the action of CS can occur via coprecipitation, chelation, coordination of amine groups as well as ligand exchange or electrostatic interactions with protonated amine groups [69, 70]. The majority of the complexation and chelation studies were performed using Cu^{2+} which is particularly reasoned by the fact that copper ions provide the highest affinity towards CS (up to 1.2 mmol/g) [43]. According to the HSAB model of Pearson, nitrogen as the hard base is appropriate, donating electrons to a borderline acid such as Cu^{2+} [71].

In contrast to the removal of anionic dyes, it is stated that the adsorption of heavy metal ions decreases due to protonation. A chelation process would be efficient at increased pH values since the adsorption of different metal cations is mainly attributed to the unprotonated amine groups of the CS acting as ligands of the metal ion [72]. In the year 1986, the bridge model was one of the first trials to propose a coordination geometry emphasizing the relevance of the amine groups for adsorption [73]. Schlick suggested a square planar structure of the CS-Cu^{2+} complex with four nitrogen ligands derived from the primary amine groups of CS. An octahedral coordination geometry (coordination number: 6) is formed by the arrangement of axial water molecules (see **Figure 2a**) [74]. However, there are investigations suggesting the C3-hydroxyl group as further ligand for complexation substantiated by thermodynamic data that led to the development of refined models (**Figure 2b**) [67]. Further investigations supposed a neutral complex (see **Figure 2c**) consisting of two nitrogen ligands and two hydroxide ions coordinating the copper ion occurring at high Cu^{2+} loadings and pH values of >5.5 [75, 76]. The structures illustrated in **Figures 2a–c** can be described as "bridge model "since they involve the chelation of the Cu^{2+} by nitrogen atoms from different glucosamine units in an inter- or intramolecular fashion [77]. The structure depicted in **Figure 2d** is called the "pendant model "and is up to date the most prominent model describing the interaction between Cu^{2+} and CS supported by potentiometry and circular dichroism data [78]. The model developed by Ogawa et al. in the year 1993 based on X-ray studies assumes the chelation of different heavy metal cations by one amino group only [79]. On the other hand, several authors stated that at least a degree of polymerization of four is required to affect an efficient chelation, the consequence being that not only one glucosamine unit is responsible for chelation [80]. However, the truth probably lies somewhere in between these models based on the dependency of the heavy-metal ion-CS ratio and pH [75]. Some amine functions may be inaccessible for chelation, and others suffer from steric hindrance to form a regular coordination geometry. This is based on the fact that CS provides a natural polysaccharide with all its heterogeneities.

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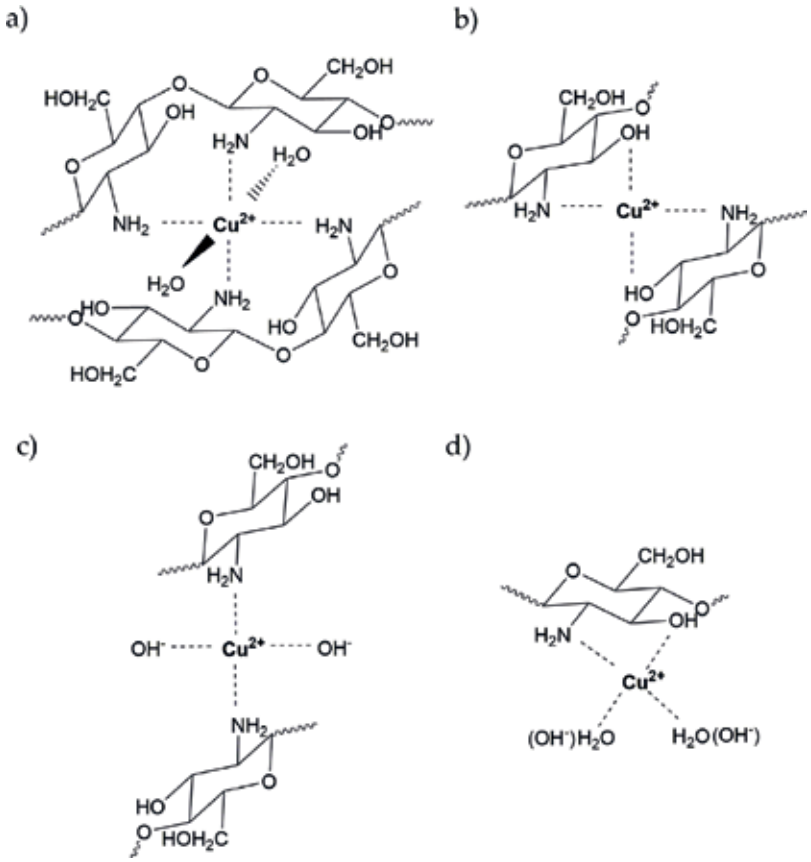

Figure 2. Different chelation mechanisms between CS and metal ions using the example of copper ions: (a) octahedral coordination geometry of the CS-Cu^{2+} complex with four amine and two axial water ligands; (b) Cu^{2+} chelation mechanism utilizing the C3-hydroxyl groups of two glucosamine units; (c) neutral complex with two nitrogen ligands and two hydroxide ions coordinating the Cu^{2+}; (d) "pendant model", chelation of Cu^{2+} by one amino group and the C3-hydroxyl group of one glucosamine unit.

The desorption of metal cations succeeding the adsorption onto CS is scarcely described in the studies. The desorption of the metal ions was either performed by the application of ammonium chloride, potassium iodide, or by EDTA [81]. Shifting pH represents the most suitable method for elution. For example, a pH decrease resulted in a 94% cadmium ion desorption, whereas 8.3 mM H^+ per gram of beads was adsorbed to substitute the bound metal ion [82].

Oxyanions like chromate or vanadate can be adsorbed by protonated amine groups at lower pH values due to electrostatic interactions resulting in an exothermic reaction [67, 83]. The simultaneous recovery of oxyanions and metal cations is described in a further study. In common, protonation reduces the adsorption capacity for metal cations but increases the effectivity of metal anion adsorption. The authors performing the experiments take advantage of the distribution of deprotonated and protonated amine groups in a pH range of 5–6. Deprotonated amine groups enable the formation of chelate complexes with Ni^{2+}, Cu^{2+}, and Fe^{2+}, the metal anions were attracted by electrostatic interaction [84]. The optimum pH removing majority of

metal anions by electrostatic attraction is in the range of 2 and 4. At lower pH values, competitive pressure by other anions derived from the acid for pH adjsutement for binding sites on CS is drastically increasing [77]. The decreased adsorption capacity of metal anions in the presence of, for example, high chloride, sulfate, or nitrate concentrations is based on the same effect. A further option to remove toxic oxyanions as selenite or arsenite was provided by Yamani et al. The group demonstrated that the chelation of copper ions leads to the formation of electron-accepting "anion" adsorption sites which did not exist previously, enabling the directed binding of these oxyanions. The bimetallic complex connected via oxygen linker offers the separation of both even in the presence of phosphate at high concentrations [76]. According to this, it was approved that Fe-crosslinked CS enables the adsorption of chromate. The adsorption mechanism is suggested to be a ligand exchange substituting an anion by the chromate in the coordination sphere of the iron ion, resulting in an uptake of Cr(VI) (295 mg/g at pH 4.8) [85].

Resuming the study and investigation results, CS is a valuable sorbent for dyes and heavy metals. However, an efficient simultaneous removal of both compound classes with native CS is unlikely due to the pH dependency of both processes. A successful removal can be expected if the solute containing media has a suitable pH for adsorption. CS solubilization can be prevented by crosslinking, functionalization, or blending, additionally resulting in an increased performance of the resin, widening pH range for optimum sorption and creating synergies between the compounds as for CS-functionalized membranes.

2.3. Chitosan-functionalized membranes

The previously mentioned wastewater treatments, adsorption and coagulation/flocculation, require the direct physical-chemical interaction of the effective agent with the contaminant in the effluent. The intention is to remove the compounds from the bulk solution to achieve effluents for further processing steps. By way of contrast, membrane-assisted applications are commonly applied to enable the purification or conditioning of water with regard to physical rather than chemical properties. The composition of the membrane and the quantity of the materials contained are of great importance to provide selectivity for membrane permeation. In common use, membrane materials consist of synthetic polymers and their composites or blends. Green and sustainable compounds as membrane components are highly demanded for well-known reasons. According to Dobosz et al., biopolymers could additionally reduce biofouling which is crucial for the lifetime, increasing the time span between sanitization cycles of the membrane [86]. Especially CS with its antibacterial activity is thus predestined for the production of membranes sensitive to fouling. Carboxymethyl CS membranes, for example, were applied during a 6-week protein separation process within which no fouling or deterioration in the membrane flux was recorded [87]. Weng et al. approved the antimicrobial activity of a cellulose/CS membrane against *Escherichia coli* in disc diffusion experiments [88]. Studies concerning CS-coated polyacrylonitrile hollow fiber membranes approved the antimicrobial and antibiofouling effect in respect to Gram-positive and Gram-negative bacteria [89, 90]. Another aspect to consider is the hydrophilicity of the membranes, which is a major requirement in water-conditioning applications to obtain membrane permeability. CS provides a high hydrophilicity allowing especially water from aqueous solutions to permeate. Together with a high salt rejection efficiency, this is also the relevant property for the main application fields of CS membranes which can be obtained from **Table 3**.

As can be inferred from the table, CS is content of reverse osmosis (RO), forward osmosis (FO) and nanofiltration membranes. Nanofiltration membranes differ from the other mentioned in the ability to separate particles in the size of 2–5 nm and thus enable permeation of minerals, which would be separated by osmosis membranes. In contrast to FO, RO processes work against the osmotic potential demanding membranes produced to resist high pressure. Both together have the need for semi-permeable membranes revealing high salt rejection grades and high water permeability, approving high efficiencies at moderate costs.

Research and innovation activities concerning the utilization of CS in the three membrane processes are mainly rooted in the countries in North Africa and the Arabian Peninsula. This originates from the access to seawater, resulting in a high availability of crustacean-derived chitin/CS and water deficiency leading to an increased demand for conditioning of water. Seawater is scarce, whereas saltwater is ubiquitous. Hence, in the majority of studies, synthetic monovalent salt solutions were applied, and the results revealed that removal by the CS-containing membranes is

Application	Membrane material	Effect/notes	Refs.
Reverse osmosis (RO) with NaCl solutions	Polyamide-6/CS	Salt rejection increases with the addition of CS as additive up to 52%	[91]
	CS crosslinked graphene oxide (GO)/titania hybrid lamellar membrane	Rejection rate is ~30% at 7.2–14.3 LMH/bar	[92]
	Thin film composite RO membranes covalently linked to CS	>90% salt rejection at LMH/bar >3	[93]
Forward (FO) and pressure-retarded RO with NaCl and Na_2SO_4 solutions	Polymerization of CS with trimesoyl chloride on the surface of SPES/PES support layer	95% NaCl rejection with up to 4.6 LMH/bar permeability, higher CS concentration revealed lower water permeabilities, but higher salt rejections	[94]
FO with synthetic salt and sucrose solution	Membrane consisting of layer-by-layer assembly of CS and GO nanosheets on a sulfonated polyethersulfone (SPES)/PES support layer	>90% Na_2SO_4 rejection at 3 LMH/bar, 2–4 orders of magnitude higher water flux compared to polyamide membrane on sulfonated PES/PES support	[95]
Nanofiltration with synthetic mineral saltwater	CS crosslinked buckypaper membranes	Rejection rates: 80–95% $MgCl_2$, 21–63% for NaCl, 18–37% $MgSO_4$ and 6–14% for Na_2SO_4; water permeability of 0.2–0.9 LMH/bar	[96]
Nanofiltration, retention of basic, neutral and acidic dyes	Polyurethane foam membrane filled with humic acid-CS crosslinked gels	Rejection efficiency >60% for all dye types, >99% for the anionic dyes, adsorption of dyes at the membrane	[97]
Nanofiltration, synthetic emulsion and dye solution	Coating layer with CS and silver nanoparticles, alginate nanofibers as midlayer and nonwoven as mechanical support	Nanoparticle retention >98%, oil removal >93%	[98]
Nanofiltration, humic acid retention	Carboxymethyl CS was blended with polyvinylidene fluoride	>97% humic acid retention; low irreversible fouling, good permeability, durability and stability	[99]

Table 3. An overview of the application fields of chitinous membrane materials and the thereof obtained results.

successfully carried out but with swaying rejection rates (30–90%) [91–93]. Separation efficiencies of nanofiltration membranes are significantly higher considering low-molecular weight organic compounds (>60%) grounding on larger molecular weight differences between the molecules to be separated and thus higher selectivity. Hence, it is not necessary to build high-density networks, which can be produced by utilizing the amine functions of CS as anchor points for modifications. In common, CS is not applied as native but as crosslinked polymer embedded in a matrix or coated on a support layer in order to introduce and combine the advantages of several compounds, or to compensate their weaknesses. Researchers report on the incorporation of modern and innovative components in CS, such as metal-organic frameworks, developing synergies of the two materials, resulting in $MgCl_2$ rejection efficiencies of 93% [100]. As already mentioned, the hydrophilicity of the membrane is the relevant factor for the water flux commonly determined by measuring the contact angle. CS coating of membranes indicated a significant higher water flux than the native membranes and thus resulted in a decreased pressure and energy demand in the process [101].

Swelling of CS is one of the properties to be compensated for the adequate application in membrane technology. As swelling is tantamount to a high water content, this greatly affects the water permeability as well as the mechanical strength of the membrane. Investigations revealed that the ability to swell must be controlled to create membranes that enable a selective separation of water and salt whatever, simultaneously guaranteeing a high water flux [102]. Decreasing the swelling of CS-based membranes means to constrain the movement of the CS chains especially in solvents in which the CS can be solubilized [103]. Further properties affecting the swelling behavior are the pH of the medium and the resulting electrostatic repulsion of CS chains at low pH values [104]. Crosslinking is thus a suitable tool to control this phenomenon as several researchers reported a significant decrease in swelling by crosslinking or modification of the CS up to 300% [96, 105].

Finally, CS seems to be a promising material with regard to the application as a membrane component. Its antibacterial activity in combination with the functionality of the amine groups provides a suitable tool to prevent fouling and coincidently adapts the network to the substrates to be filtrated. The challenges to be mastered are the reduction of swelling of these membranes while approving a high water permeability predominantly in drinking water purification. In addition, further investigations concerning osmosis membranes have to test real saltwater samples not lacking all other natural occurring compounds than sodium chloride as is the case for the synthetic model solutions.

3. Conclusion/summary

Within this publication, we reviewed the purification of effluents with native and modified CS as well as the application of CS-containing membranes for filtration purposes. Crosslinking, derivatization, and the production of composites or blends with other natural and synthetic polymers as well as low-molecular weight compounds are the main type of application described in the study rather than the usage of native CS. It seems to be appropriate to introduce new functionalities, to prevent leaching, or to foster the beneficial properties. Due to these manifold-positive properties in combination with other compounds, the preconditions are favorable for the implementation of CS in wastewater treatment. CS represents a compound to be effective as coagulant/flocculant in

the native state, whereas the addition as flocculant auxiliary decreasing the amount of inorganic coagulants required for the process seems to be promising. The good overall performance adsorbing heavy metal ions and dyes is stated in several investigations, enabling CS to be applied in the treatment of special wastewaters, such as textile and mining effluents. The simultaneous adsorption of both is limited due to the necessity to adjust the pH in order to protonate/deprotonate the CS, effectively removing the single compounds, respectively. Investigations concerning CS-containing membranes showed that a biopolysaccharide could also contribute to more sophisticated water conditioning processes. The water permeability and the selectivity have to be evaluated especially considering the swelling behavior. It can be assumed that CS can also be implemented to produce switchable membranes, which means membranes altering the properties due to pH shifts. However, the investigations concerning the application of CS and its derivatives suffer from several drawbacks not adequately addressed in the past, aggravating the market accessibility (1) cost factors were not considered; (2) experiments limited to lab scale; (3) only batch experiments were carried out; (4) mechanical strength should be increased; (5) studies have to be performed with actual wastewaters; (6) regeneration of the materials was not investigated; (7) swelling behavior of CS needs to be limited, and (8) the heterogeneity of different CS batches is not considered, yet [47, 106]. The heterogeneity particularly derives from the origin of the CS, the crab shells being exposed to varying environmental conditions. Not only the CS derived from fishery waste can be applied for wastewater treatment, but also the CS isolated from fungi or insects for the provision of more homogeneous batches. Since the production of fungal biomass or insect-based protein is already industrially established, a higher quantity and quality of fungal- or insect-based CS can be assumed and could be applied in a prospective effluent purification. For example, Adnan et al. successfully applied commercial fungal CS to purify a synthetic kaoline solution and palm oil mill effluent. They stated that in contrast to crab shell and shrimp shell CS, the fungal CS is available all over the year. Further, it has a narrow molecular weight based on the controlled production process, opposing the argument that the heterogeneity of CS limits its application. The authors assume therefore that prospective works will focus on fungal- and insect-based CS, increasing the opportunity to develop profitable products with improved properties [107].

Conflict of interest

The authors declare that there is no conflict of interest.

Appendices and nomenclature

CS	chitosan
FO	forward osmosis
LMH	liters per m^2 per hour
RO	reverse osmosis

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Chapter 8

Chitosan-Based Green and Sustainable Corrosion Inhibitors for Carbon Steel

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Additional information is available at the end of the chapter

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Abstract

Development of non-toxic and environmental friendly corrosion inhibitors is highly desirable owing to the increasing demands of "green chemistry" throughout the world. In view of these several forms of green corrosion inhibitors such as drugs or medicines, plant extracts, ionic liquids and synthetic inhibitors derived from multicomponent reactions (MCRs) and mechanochemical mixing are being employed. Nowadays, MCRs in association with microwave and ultrasound irradiations represent one of the best green strategies. Natural polysaccharides particularly chitosan derivatives gained substantial advancement. Chitosan and its several derivatives have been employed effective as corrosion inhibitors for metals and alloys in various aggressive media. The present chapter features the collection of major works that have been published on the inhibition effect of chitosan and its derivatives. The utilization of the chitosan and its derivatives as effective corrosion inhibitors is based on the fact that they contain several polar functional groups such as amino (-NH_2), hydroxyl (-OH) and acetyl (-$COCH_3$) groups that effectively bind with metallic surface and behave as adsorption centers.

Keywords: chitosan, chitin, green corrosion inhibitors, aggressive solution, mixed-type inhibitors

1. Introduction

Alloys steel such as carbon steel and mild steel have been extensively utilized as construction materials for several purposes because of their high mechanical strength and cost-effective behaviors. However, they are highly reactive and undergo corrosion when exposed to the

environment particularly in acid treatment processes like acid cleaning, acid descaling, acid pickling and oil well acidification. Therefore, these cleaning processes require application of some external additive to avoid the corrosive dissolution of metallic materials. The external added chemical species are known as corrosion inhibitors. It is important to mention that the difference in the mild steel and carbon steel is the amount of carbon. Mild steel has relatively small amount of carbon ranging from 0.16 to 0.30%. Carbon steel contains larger amount of carbon, generally ranging from 0.30% to more than 2% [1, 2]. Among several available methods of corrosion protection, the utilization of organic compounds is one of the most appropriate and cost-effective methods. The extensive utilization of the organic compounds as corrosion inhibitors is also attributed due to their high effectiveness and ease of application. These compounds adsorb and form a corrosion protective barrier by transferring their non-bonding and π-electrons into the metallic d-orbitals. The electron transferring (Adsorption) ability of these compounds influences by several factors such as electronic structure of the compound, nature of metal and corrosive environment, surrounding temperature, presence of impurities, exposure duration etc. [3, 4]. The polar functional groups of heteroatoms (N, O, and S) such as –CN, -OH, $-NH_2$, $-OCH_3$, -COOH, $-CONH_2$, $-COOC_2H_5$ etc. and double and triple bonds behave as adsorption centers [3, 4]. It is reported that these polar functional groups easily undergo protonation in strong acidic medium like 1 M HCl and exist in their cationic form. On the other hands, metallic surface becomes negatively charge due to the adsorption of counter ions of electrolyte (chloride ion in HCl). These two oppositively charged species attracted each other through electrostatic force of attraction (physisorption mechanism). In the later stage of the adsorption phenomenon neutral heteroatoms transfer their unshared electron pairs to the empty d-orbitals of the surface metallic atoms to form coordinate bonds that results in to the chemical adsorption. Recently, the growing natural awareness and severe environmental guidelines demand application of the compounds for different purposes that have been originated from natural and biological resources. The chemical synthesis of the organic compounds is not only expensive but also causes discharge of several toxic chemicals into the surrounding environment that can have several adverse effects on living beings. The increasing demands of "green and sustainable chemistry" throughout the world, forces to the scientists working in the field of corrosion chemistry to grow highly desirable "green and sustainable corrosion inhibitors" either by deriving them from natural resources or by synthesizing them using suitably modifying the available synthetic methods. In last two decades, use of multicomponent reactions (MCRs), chemical reactions catalyzed by energy efficient microwave and ultrasound irradiations, plant extracts, chemical medicines (drugs), ionic liquids etc. toward "green and sustainable corrosion inhibition" have gained significant milestone in this direction.

In recent decades, the use of carbohydrates and their derivatives as metallic corrosion inhibitors has been a growing effort to decrease the environmental pollution [3, 4]. Natural availability, biosynthesis using greenhouse (CO_2) gas, biodegradability, biocompatibility, and high solubility in aqueous media make the carbohydrates as "green" chemicals for variety of chemical transformations [5–7]. The carbohydrates act as inhibitor for protein glycosylation activities, medicines for bacterial infections (antibiotics), viral infections (antiviral), neuronal proliferation, cancer metastasis and apoptosis [8–10]. Additionally, carbohydrate derivatives

(including chitosan) are extensively used as detergent, food, and cosmetics, sweetening agents, cloths, paper, lumber and other variety of other purposes [10–12]. Chitosan is polymeric form of deacetylated chitin with a variety of properties such as immunological activities, low toxicity, wound healing and biodegradability [13, 14]. The chemical structure of chitosan is shown is shown in **Figure 1**.

Similar to most of the carbohydrates, chitosan is rich in functional groups (hydroxyl and amino) it would be a potential inhibitor for metallic corrosion [15, 16]. The amount of amino group in chitosan is determines by degree of deacetylation. Chitosan and its derivatives are important materials having several industrial and biological applications. These materials are gaining attention in food, biomedical, agricultural, environmental and pharmaceutical industries because of their non-toxic, environmental-friendly, non-allergenic and biocompatible nature. Their diverse biological applications include anti-hypertensive, anti-oxidant, anti-diabetic, anti-coagulant, anti-inflammatory, anti-microbial, anti-obesity, anti-cancer and neuro-protective properties [17, 18]. The hydroxyl (-OH) group at 6-position and amino ($-NH_2$) group at 2-position are the most chemically reactive sites of chitosan for modification procedures. Chitin is natural source of chitosan which is mainly distributed in the shells of crabs and shrimp, in the cell of fungi and cuticles of insects [13, 14]. The chitosan can be derived by N-deacetylation of chitosan using several deacetylation agents. The chitosan and its derivatives are being utilized for variety of purposes because of their biocompatibility, non-toxic nature, high biodegradability, high 1 wound healing behavior and immunological activity, etc. [19–23].

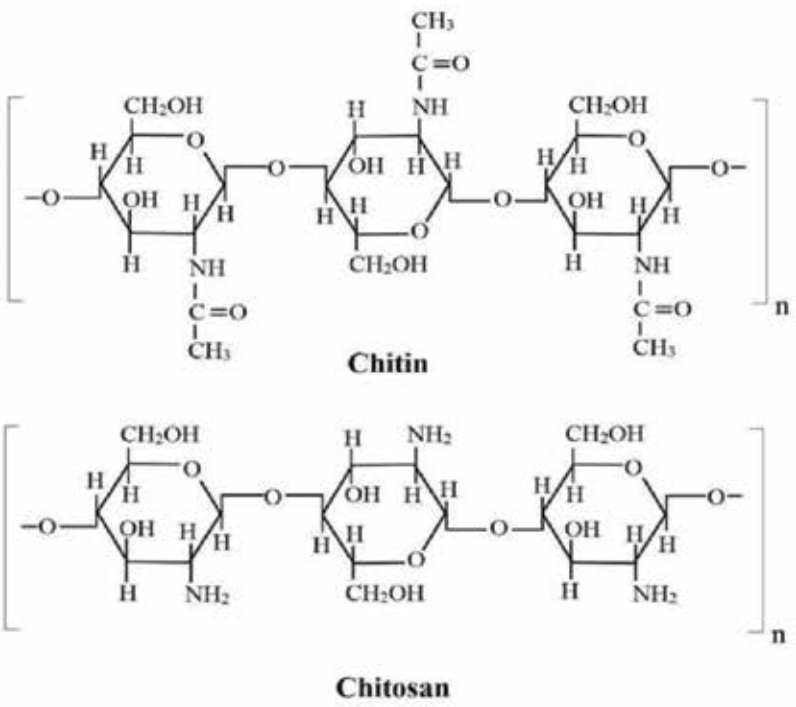

Figure 1. Chemical structure of chitin and chitosan.

2. Main body

Because of their green and environmental friendly nature chitosan and its derivatives are being utilized as effective corrosion inhibitors for metals and alloys for several electrolytic media including HCl, H_2SO_4 and NaCl etc. The corrosion inhibition property of chitosan and its derivatives in correlation with other commonly employed organic and inorganic corrosion inhibitors are presented in **Table 1**. Abd-El-Nabey et al. [24] demonstrated the inhibition properties of chitosan in 0.1 M HCl using potentiodynamic polarization (PDP) and electrochemical impedance spectroscopic (EIS) methods. Chitosan showed the optimum inhibition efficiency of 90% at 0.028 g/L concentration. EIS study revealed that chitosan adsorbs at metal/HCl interface and behaved as interface corrosion inhibitors. PDP study showed that chitosan behaved as mixed type corrosion inhibitor.

The inhibition property of the chitosan on mild steel corrosion in 0.1 M HCl has also been investigated using gravimetric, PDP, EIS, scanning electron microscopy SEM and UV-visible methods [34]. At 60°C temperature chitosan showed 96% inhibition efficiency which drops to 93% on increasing temperature 70°C [34]. Chitosan acted as mixed type corrosion inhibitors as observed by PDP study. Adsorption of the chitosan on mild steel surface obeyed the Langmuir adsorption isotherm. EIS study showed the chitosan acted as interface corrosion inhibitor that is retards the corrosion process by adsorbing on the metal/ electrolyte interface [35–37]. The inhibition property of chitosan for corrosion of copper in 0.5 M HCl has also been studied weight loss, PDP, EIS and electrochemical frequency modulation (EFM) measurements [38]. Chitosan acts as mixed type inhibitor and its adsorption obeyed the Langmuir adsorption isotherm. The high protection ability of the chitosan forced the people working in the field of corrosion to develop and use of chitosan derivatives as corrosion inhibitors. Cheng and his coworkers [13] demonstrated the inhibition property of carboxymenthylchitosan (CM-chitosan) as ecofriendly corrosion inhibitors for mild steel in 1 M HCl using weight loss, EIS and PDP techniques. The structure of CM-chitosan is shown in **Figure 2**.

The CM-chitosan showed maximum protection ability of 93% at 200 mg/L concentration. Adsorption of the CM-chitosan on mild steel surface obeyed Langmuir adsorption isotherm. PDP study suggested that CM-chitosan acted as mixed type corrosion inhibitor. In other study [14], these authors studied the effect of cupric (Cu^{2+}) ions on corrosion inhibition property of CM-chitosan toward acidic dissolution of mild steel in 1 M HCl. Results showed that CM-chitosan+Cu^{2+} showed better protection ability much more effectively than the inhibiting action of each additive separately. In continuation of this type of works, acetyl thiourea chitosan polymer (ATUCS) was synthesized and investigated as effective inhibitor for mild steel in aerated 0.5 M H_2SO_4 solution using EIS, PDP and SEM methods [19]. The chemical synthesis of ATUCS is shown in **Figure 3**.

Results showed that ATUCS acted as interface corrosion inhibitor and its adsorption on mild steel surface obeyed the Langmuir adsorption isotherm. The ATUCS acted as mixed type corrosion inhibitor. Two formaldehyde based chitosan derivatives based on thiosemicarbazide (TSFCS) and thiocarbohydrazide (TCFCS) (**Figure 4**) were synthesized and investigated as effective corrosion inhibitors for heavy metals [21]. TCFCS behaved as mixed type corrosion inhibitor and showed maximum efficiency of 92% at 60 mg/L concentration.

Type of inhibitors	Name of inhibitor	Nature of metal and electrolyte	Adsorption behavior	Highest efficiency and concentration	Ref.
Organic inhibitors	2, 2′-bis(benzimidazole)	Mild steel/ 1 M HCl	Mixed type/ Langmuir adsorption isotherm	97.8% at 10^{-4} M	[25]
	2, 5-bis (4-methoxyphenyl)-1,3,4-oxadiazole	Mild steel/ 1 M HCl	Mixed type/ Langmuir adsorption isotherm	96.19% at × 10^{-4} M	[26]
	Pyridine-2-thiol (P2T) and 2- Pyridyl disulfide (2PD)	Mild steel/in flow HCl	Mixed type/ Langmuir adsorption isotherm	More than 98% 200 mg/L	[27]
	4,4-dimethyloxazolidine-2-thione (DMT)	Mild steel/ 1 M HCl	Mixed type/ Langmuir adsorption isotherm	82% at 4×10^{-3} M	[28]
	2-mercapto benzimidazole (2MBI)	Mild steel/ 1 M HCl	Mixed type/ Langmuir adsorption isotherm	98% at 10^{-3} M	[29]
	1,3-dioctadecylimidazolium bromide and N-octadecylpyridinium bromide	Mild steel/ 1 M H_2SO_4	Mixed type/ Langmuir adsorption isotherm	82% and & 88% at 100 ppm	[30]
	Tryptamine	Mild steel/ 0.5 M H_2SO_4	Mixed type/ Langmuir adsorption isotherm	97% at 500 ppm	[31]
	N-Phenyl oxalic dihydrazide (POD-H) and oxalic N-phenylhydr-azide N′-phenylthiosemicarbazide (OPHPT)	Mild steel/ 1 M HCl	Mixed type/ Langmuir adsorption isotherm	92% for OPH-PT and 79% for PODH	[32]
	Cysteine	Copper/1 M HCl	Cathodic type/ Langmuir adsorption isotherm	84.13% at 18 mM	[33]
Chitosan based inhibitors	Chitosan	Al, Mild steel/ 0.1 M HCl	Mixed type	More than 90% at 0.028	[24, 34]

Table 1. Corrosion inhibition efficiencies of some common reported organic and chitosan based corrosion inhibitors in aggressive solution.

The new compounds were characterized and studied by Fourier transform infrared spectroscopy, elemental analysis, thermal gravity analysis and differential scanning calorimetry, and their surface morphologies were determined via scanning electron microscopy. The inhibition effect of two chitosan derivatives namely 2-N,N-diethylbenzene ammonium chloride N-oxoethyl chitosan (compound I), and 12-ammonium chloride N-oxododecan chitosan

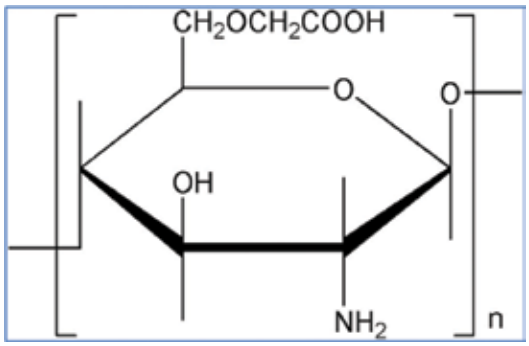

Figure 2. Chemical structure of carboxymenthylchitosan (CM-chitosan).

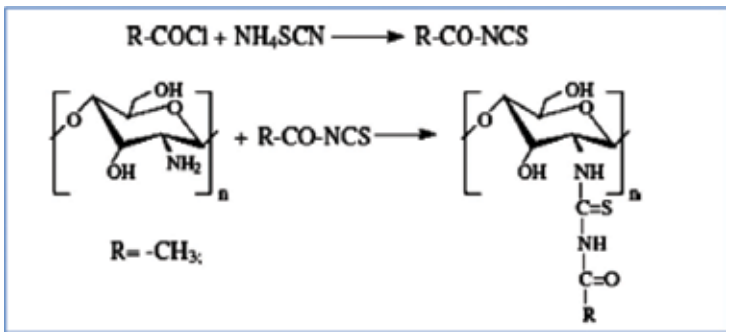

Figure 3. Synthetic scheme for ATUCS.

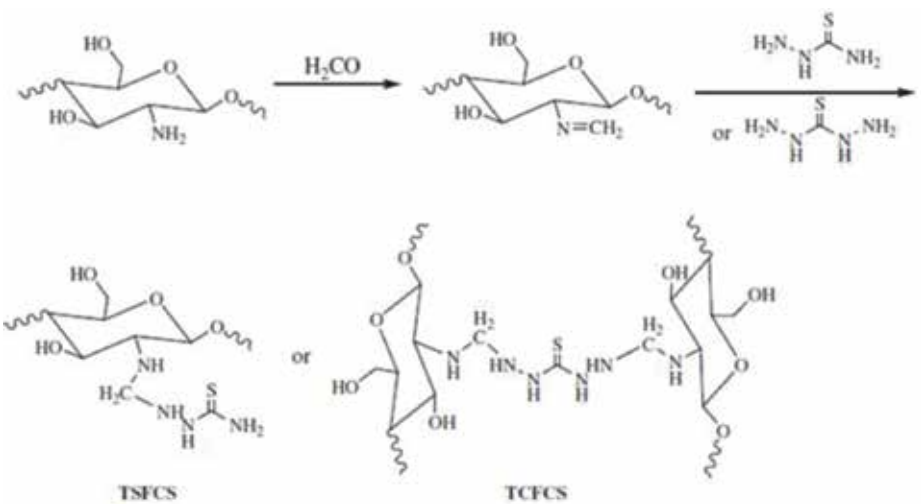

Figure 4. Synthetic scheme for TSFCS and TCFCS.

(compound II) on carbon steel corrosion in 1 M HCl using weigh loss method has been reported [15]. Along with the antibacterial property these compounds showed good corrosion inhibition efficiency toward carbon steel corrosion in acidic medium. The authors claimed that functionalization of the chitosan into compound I and II causes significant change in the physiochemical properties. The enhanced solubility in the polar testing solution (1 M HCl) due to presence of polar amino (-NH_2) and several hydroxyl (-OH) groups the functionalized chitosan molecules adsorb efficiently on the metallic surface and showed good corrosion inhibition efficiency. These compounds inhibit corrosion by adsorption mechanism and their adsorption of compound I and compound II obeyed the Langmuir adsorption isotherm. Compound I showed highest inhibition efficiency among the tested compounds. These authors also observed that the antibacterial activity of chitosan for Enterococcus faecalis, *Escherichia coli*, *Staphylococcus aureus*, and Candida albicans is higher than for its derivatives. Menaka and Subhashini [16] investigated the inhibition effect of chitosan thiophenecarboxaldehyde Schiff base, synthesized by a condensation reaction of the carbonyl group of thiophene 2-carboxaldehyde and free amino groups of chitosan on mild steel in 1 M HCl solution using weight loss, EIS, PDP, EDX, SEM and AFM methods. The synthesized Schiff's base was characterized by UV-visible spectroscopy method. After 12 hrs immersion time, investigated SB showed 92% inhibition efficiency. PDP study showed that SB behaved as mixed corrosion inhibitor and its adsorption on mild steel surface obeyed the Temkin adsorption isotherm. Wan and coworkers [39] synthesized carboxymethylhydroxypropyl chitosan (CHPCS) containing both carboxymethyl and hydroxypropyl groups was investigated as a corrosion inhibitor for mild steel in 1.0 M HCl solution using weight loss, open circuit potential (OCP), potentiodynamic polarization and EIS techniques. The CHPCS showed maximum inhibition efficiency of 95.3% at 1000 ppm concentration. CHPCS acts as mixed type corrosion inhibitor and its adsorption obeys the Langmuir adsorption isotherm. Further, inhibition effect of polyamine grafted chitosan copolymer for Q235 carbon steel in 5% HCl at 25°C [40] and β-Cyclodextrin modified natural chitosan for carbon steel in 0.5 M HCl [41] reported in other studies. Chauhan et al. [42] demonstrated the effect of two functionalized chitosan derivatives namely Chitosan-Thiosemicarbazide (CS-TS) and Chitosan-Thiocarbohydrazide (CS-TCH) as inhibitors for mild steel corrosion in 1 M HCl. The investigation was performed using gravimetric, electrochemical (PDP and EIS), AFM, DFT and MD simulation methods. The authors observed that CS-TCH is better corrosion inhibitors as compared to the CS-TS and showed maximum efficiency of 93.2% at 200 mgL^{-1}, concentration. Adsorption of the CS-TS and CS-TCH on the metallic surface obeyed the Langmuir adsorption isotherm. Increase in the polarization resistance (R_p) values for inhibited case revealed that charge transfer from metallic surface to electrolytic solution become difficult owing to the formation of protective film by the CS-TS and CS-TCH molecules. The inhibition effect of two tested chitosan based corrosion inhibitors are shown in **Table 2**. In another study our research group [43], investigated the effect of chitosan as corrosion inhibitor for mild steel in 1 M sulfamic in combination with potassium iodide (KI) using weight loss, electrochemical and surface techniques. Results of the analysis show that presence of KI in the corrosive medium caused significant enhancement in the inhibitive performance of the chitosan. At 200 ppm concentration chitosan showed inhibition performance of 73.8% while in the presence of 5 ppm concentration of KI, inhibition efficiency of chitosan enhanced to 90%. Under both conditions, chitosan acts as mixed type

Inhibitors	Inhibitor Conc. (mg L^{-1})	C_R (mg cm^{-2} h^{-1})	Surface coverage (θ)	η%
Blank		12.27	—	—
CS-TS	40	5.36	0.5631	56.31
	60	4.99	0.5932	59.32
	80	4.66	0.6201	62.01
	100	4.04	0.6707	67.07
	120	4.03	0.7727	77.27
	140	2.42	0.8027	80.27
	160	1.67	0.8636	86.36
	180	1.47	0.8800	88.00
	200	1.39	0.8864	88.64
CS-TCH	40	5.24	0.5731	57.31
	60	4.75	0.6132	61.32
	80	4.48	0.6352	63.52
	100	3.93	0.6800	68.00
	120	3.53	0.7935	79.35
	140	2.07	0.8315	83.15
	160	1.42	0.8843	88.43
	180	1.23	0.9000	90.00
	200	1.17	0.9043	90.43

Table 2. Weight loss parameters obtained for mild steel in 1 M HCl in the absence and presence of different concentrations of CS-TS and CS-TCH.

corrosion inhibitor and its adsorption obeyed the Langmuir adsorption isotherm. Chitosan acts by adsorbing and blocking the active sites present on the metallic surface. The formation of inhibitive film by chitosan molecule is supported by SEM and AFM analyses.

Besides the use of chitosan and its derivatives as solution phase corrosion inhibitors, few organic and inorganic composites of chitosan have also been used as coating materials for protection of their dissolution in aggressive environments. Pang and Zhitomirsky [44] coated 316 L stainless steel hydroxyapatite-chitosan and characterized them using X-ray diffraction (XRD), thermo-gravimetric and differential thermal analysis, scanning and transmission electron microscopy, PDP and EIS methods. Electrochemical investigations showed that the obtained coatings provide the corrosion protection of the 316L stainless steel substrates. The heat treatment of the coating at

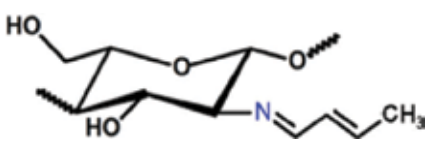

Figure 5. Chemical structure of Ch-Cr-SB.

140°C resulted in an improved corrosion protection. The crotonaldehyde based chitosan Schiff's base derivative designated as Ch-Cr-SB (**Figure 5**) was synthesized and coated on the surface of AZ91E alloy for its anticorrosive behavior [45].

The protection abilities of chitosan and chitosan derived SB was compared in the present study. The electrochemical corrosion behavior has been also studied for Ch-Cr-SB in aerated 3% NaCl solution containing different concentrations of Schiff's base, in the range from 0.03 to 0.075 mM, using different. Results showed that presence of these inhibitors in the corrosive 3% NaCl solution decreases the rate of corrosion. The chitosan electrochemically deposited over the metallic surface from the solution of chitosan in acetic acid. In order to further improve the effectiveness of coating, coated samples were further treated with formaldehyde solution. The coating of chitosan on mild steel surface was measured by FTIR, SEM, PDP and EIS methods [46]. The coating samples showed improvement in the protection ability up to 98.1% at 0.5 M H_2SO_4. A significant increase in the charge transfer resistance was observed for coated mild steel surfaces. The electro-deposition of Zn-chitosan composite coating on mild steel and its corrosion studies has been reported in 3.5% NaCl [47], Self-healing protective coatings with chitosan based pre-layer reservoir [48], chitosan/diclofenac coatings for medical grade stainless steel [49], copper modified chitosan inhibition of AA-2024 corrosion [50], 2-Mercaptobenzothiazole (MBT) based functionalized chitosan-based coatings for active corrosion protection of AA2024 alloy [51], chitosan/Silver nanoparticles composite on St37 steel corrosion in a 15% HCl solution [52], self-healing protective coatings chitosan doped with cerium nitrate for inhibition of aluminum alloy 2024 [53] and Poly(itaconic acid)-modified chitosan for inhibition of aluminum corrosion [54] and other composites materials have also been investigated as effective chitosan based coatings.

3. Conclusions

Chitosan and its derivatives are important class of natural/bio-polymers that have several biological and industrial applications. They are used as anti-oxidant, anti-hypertensive, anti-inflammatory, anti- diabetic, anti-coagulant, anti-obesity, anti-microbial, anti-cancer and neuro-protective agents. Their extensive use and demands based on the facts that these compounds are non-toxic, environmental-friendly, biocompatible, commercially availability and non-allergenic behavior. In view of the above it is concluded that chitosan and its derivatives are "green and sustainable materials" to be used for their various applications. Present chapter deals with the collections of reports available on the corrosion inhibition properties of chitosan and its various derivatives. The ongoing discussion showed that chitosan based compounds represent a green and sustainable class of corrosion inhibitors and can be successfully employed at the place of traditional toxic corrosion inhibitors. These inhibitors can be derived either from biological systems or can be synthesized in laboratories from the hydrolysis of chitin and further functionalization. Chitosan and its derivatives act as efficient solution phase inhibitors for mild steel, carbon steel, copper and aluminum. Generally, there adsorption on metallic surface obeyed the Langmuir adsorption isotherm. PDP study revealed that chitosan and its derivatives behaved as mixed type corrosion

inhibitors. Through electrochemical impedance spectroscopic measurement it can be derived that chitosan and its derivatives behaved as interface type of corrosion inhibitors that is they adsorb at the interface of metal and electrolytic solution. Chitosan and its derivatives have also been used in coating for protection of metallic and alloys dissolution in aggressive media like NaCl and HCl solution.

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Chapter 9

Overview of Electrospinned Chitosan Nanofiber Composites for Wound Dressings

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Additional information is available at the end of the chapter

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Abstract

Chitosan has a medical application because of its natural origin and properties of biodegradability, biocompatibility, nontoxicity, and antimicrobial capacity. Electrospinning produces non-woven nanofibers to wound dressing with high specific surface area and small pores. These properties are favorable for absorption of exudates and prevent the penetration of bacteria, thus promoting wound healing. For this reason, chitosan blends are used to produce nanofiber dressings, and the characterization of the structural, mechanical, and biological properties is very promising for further studies. Nowadays, the researchers are seeking for biomaterials that provide modern dressings with many qualities, which are designed to promote wound healing. In this chapter, the electrospinning parameters that affect the nanofiber properties based on chitosan to prepare wound dressings are highlighted.

Keywords: electrospinning, chitosan, dressings, nanofibers, biomaterials

1. Introduction

Chitin is a polymer composed of *N*-acetylglucosamine that has been recognized as one of the most abundant natural polysaccharides and has been found in crustaceans, fungi, and insects [1]. To improve its solubility in water, it is partially deacetylated and converted into chitosan, which is a linear polymer of glucosamine and *N*-acetylglucosamine [2]. Due to its properties, chitosan has many applications in food preservation, medicine, biotechnology, agriculture, and water treatment. Among the properties of interest in biomedicine are its biodegradability, biocompatibility, nontoxicity, and high antimicrobial activity [3]. Thus, chitosan preparations have

been used as gels, microparticles, films, and coating agents. Medical products based on chitosan have been studied as dietary supplements, wound healing agents, hemostatic devices, and drug delivery [4].

The appropriate selection of dressings depends on the characteristics of the wound and its mode of action. The purposes of the dressings are to facilitate the healing process, control symptoms, achieve an esthetic healing, keep the wound moist, absorb exudate without leakage, prevent infection, avoid trauma when dressing needs to be changed, not present toxic skin irritation, and maintain gaseous exchange [5]. Currently, it is necessary to find these conditions in a single product, with chitosan and other biomaterials being the most studied [6].

The electrospinning process is a technique that emerged in the 1970s with the purpose of producing fibers with a size smaller than 100 nm called nanofibers [7, 8]. Specifically, the electrospinning system is recognized for its ability to produce nanofibers from a wide variety of polymers of natural or synthetic origin and natural proteins [9].

The electrospinning system faces several problems during the elaboration of nanofibers for biomedical applications, particularly, the optimization of the process conditions for each polymer [10]. This chapter presents a detailed review of the influence of electrospinning parameters on the properties of the biocomposites of chitosan in mixture with other polymers to produce dressings, as well as some in vitro and in vivo assays of their application as wound dressings.

2. Electrospinning technique for production of nanofibers

The electrospinning process is a recognized system for obtaining polymer fibers by creating an electric field. To do this, a jet of the polymer is injected through a charged needle, and the solvent where the polymer is dissolved evaporates, thus allowing the deposition of solid polymer fibers on the collector. As a result, the electrospinning system equipment consists of a high voltage source, syringe pump, a syringe loaded with the polymer solution, and a collector (**Figure 1**).

During the process, high-voltage loads are applied to the polymer solution that is injected. The increase in the electric field formed causes the repulsion interactions between the same charges and attraction between the oppositely charges in the liquid. Whereas the collector that is grounded exerts forces of attraction on the injected drop, once the electric field increases, it will reach a point where the electrostatic forces reach the equilibrium with the surface tension, deforming the cone to a drop, which it is called Taylor cone [11].

Many polymers and operating parameters have been reported for the electrospinning process. The electrospinning system is affected by polymer characteristics such as concentration, viscosity, molecular weight, and surface tension. Other parameters that influence are the components and processing parameters of the system, specifically the applied voltage, injection flow, distance between the needle and the tip, and the type of collector used [12], presented in **Table 1**.

2.1. Characteristics of chitosan polymer for electrospinning nanofiber process

According to Kai et al. [12], electrospinning requires a very concentrated polymer solution. The low concentrated solutions cause the formation of weak chains, as well as insufficient electrostatic

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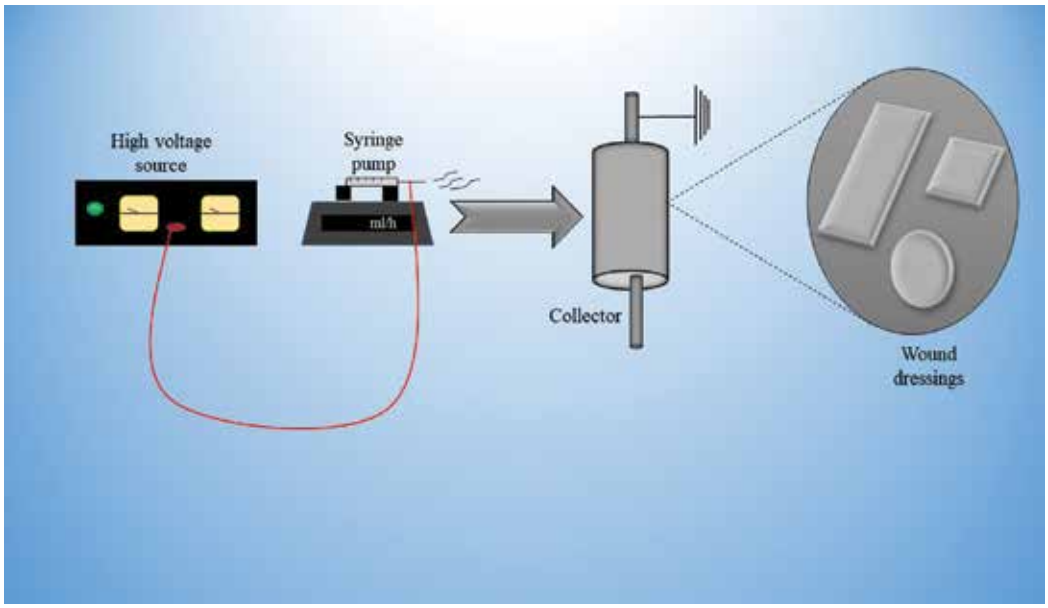

Figure 1. Components of the electrospinning system.

repulsive forces, giving rise to the appearance of droplets in the collector and the incomplete formation of the fibers.

Surface tension and viscosity are parameters of the polymer solution that mainly affect the diameter of the fiber [10]. These authors agree with Sill and von Recum [11], who mentioned that if the solution is very diluted the polymeric fiber will break into droplets before reaching the collector due to surface tension. When the solution is very concentrated does not complete the fiber formation is not obtained due to the high viscosity, which makes difficult the flow during injection.

The molecular weight of the polymer is another parameter that affects electrospinning. Low molecular mass polymers lead to bead formation, while high molecular weight polymers produce fibers with larger diameter [12]. Likewise, Cui et al. [24] found that to produce pectin nanofibers by electrospinning the molecular weight of the polymer should be in the range of 200–950 kDa.

2.2. Components and processing parameters of the electrospinning technique

Haider et al. [25] reported that the critical amount of applied voltage varies according to the polymer. Also, the formation of nanofibers with small diameters is due to the increase in the applied voltage, attributed to the stretching of the Taylor cone, and the formation of the jet (**Figure 2**). However, the increase in the critical voltage value forms beads in the nanofibers, which is caused by the decrease in the size of the Taylor cone and an increase in the flow velocity.

One of the electrospinning parameters with greater influence on the mechanical properties is the type of collector. During the electrospinning process, the fibers are deposited in a lower potential electrode known as a rotating or static collector. Thus, the fibers can be guided through the electric field formed between the tip of the needle loaded with the polymer solution and the high voltage source [26].

Polymer	Electrospinning parameters	Collector	References
PVA	AV (14.5–17.5 kV)	Stainless steel collector	[13]
	TCD (125 mm)		
	FR (6 μL/min)		
CS/PVA	AV (18 kV)		[14]
	TDC (15 cm)		
	FR (0.35 ml/h)		
CS/HA	AV (15 kV)	Rectangular 6 × 2 cm aluminum collecting plate	[15]
	TCD (15 cm)		
	FR (1.2 ml/h)		
PVA/SA	AV (15 kV)		[16]
	TCD (15 cm)		
CS/PVA	AV (15 kV)	Copper plate wrapped with aluminum foil	[17]
	TCD (15 cm)		
SA/PEO	AV (20 kV)		[18]
	TCD (20 cm)		
CS/PEO	AV (15–35 kV)	Aluminum foil attached to a drum collector	[19]
	TCD (15 cm)		
	FR (0.1–2 mL/h)		
SA/PVA	AV (17 kV)	Aluminum target	[20]
	TCD (5 cm)		
	FR (0.2 ml/h)		
SA/PVA	AV (17 kV)	Rectangular nickel collector with a glass microscope slide taped to its surface	[21]
	TCD (10 cm)		
	FR (2 μl/min)		
Ge	AV (12 kV)	Stainless steel mandrel (30 cm length and 5 cm in diameter) and rotational speeds (1700, 2400, 3100, 3800, and 4500 rpm)	[22]
	TCD (10 cm)		
	FR (0.003 ml/min)		
PL/CS	AV (5 kV)		[23]
	TCD (12.5 cm)		
	FR (0.6 ml h^{-1})		

PVA, Poly(vinyl alcohol); CS, chitosan; HA, hydroxyapatite; PEO, poly(ethylene oxide); SA, sodium alginate; PL, polycaprolactone; Ge, gelatin; AV, applied voltage; TCD, tip-to-collector distance; FR, flow rate

Table 1. Polymers and parameters used in electrospinning.

The collectors of the first electrospinning equipment lacked movement, but over time they have been modified in order to improve the alignment of the fibers and modify their surface. The use of rotating cylindrical collectors has helped in the alignment of the fibers. At the same

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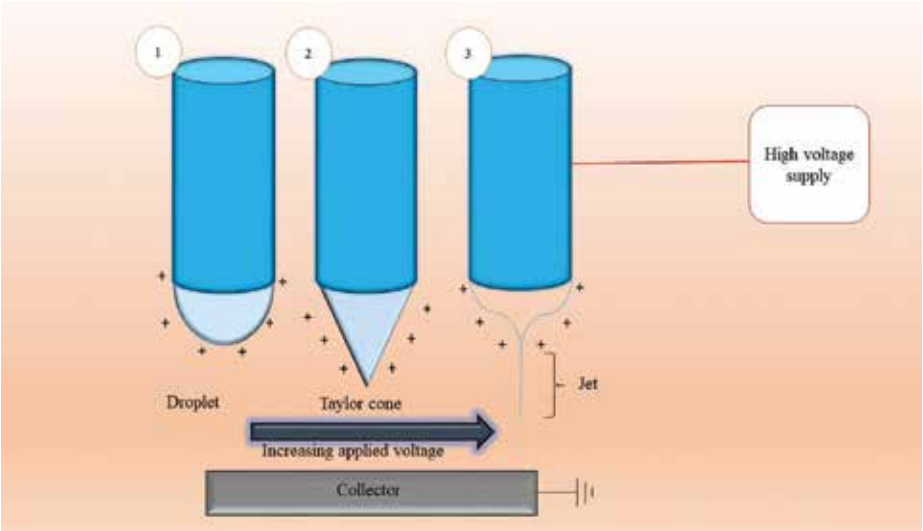

Figure 2. Taylor cone formation stages during voltage application.

time, Huang et al. [27] used a thin circular collector with a sharp edge and found that with this modification electric field is concentrated, favoring the alignment and collection of the fibers.

The collectors can be adjusted to various specifications from a stationary plate to a rotating cylinder. When a static collector is used, the fibers will have a random order, while aligned fibers will be generated with the rotary collector [11].

Huang et al. [27] used collectors with movement to prepare gelatin hydrofibers. They confirmed that the use of rotating collectors of cylindrical shape with high speed orientates the nanofibers. In addition, they reported that when the collector speed is lower than the alignment speed, the hydrofibers will have a random order, while high speed causes instability in the injection jet, affecting the surface morphology of the fibers.

In addition, the position of the collector influences the morphology of the fibrous materials. Haider et al. [25] reported that the diameter increases at short distances between the needle and the collector, while the diameter decreases at long distances between the needle and the collector. Also, Sill and von Recum [11] explained that the distance between the needle and the collector affects the continuous and complete formation of the fibers. Small distances are not enough to achieve the evaporation of the solvent before being deposited in the collector, resulting in the formation of loose and weak fibers that tend to stick together and make it difficult to remove from the collector.

With the intention of providing greater guidance in continuous nanofibers, Dabirian et al. [28] incorporated two injection syringes with different charges and positioned in a triangular form with respect to the initial needle of the process. Both syringes are attracted to each other, and the nanofibers are twisted and collected continuously in the form of a yarn.

3. Characteristics parameters of chitosan biopolymer

Chitosan is a linear polysaccharide consisting of *N*-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN), which is produced by alkaline deacetylation of crustacean chitin [29]. It is soluble in dilute acid solutions of acetic, lactic, malic, formic, or succinic acid. It is polycationic at pH 6 and easily interacts with negatively charged molecules such as proteins, fatty acids, anionic polysaccharides, bile acids, and phospholipids and, therefore, can be used as blended solutions [30].

This polysaccharide has antibacterial, antifungal, mucoadhesive, analgesic, hemostatic, nontoxicity, biodegradable, and biocompatible activity (**Figure 3**) [31]. These biological properties have favored its topical application and implantation. Another recent medical application is controlled drug delivery systems [32]. Baldrick [33] reported the possible oral use of chitosan nanoparticles with drug carrier, as gel beads of chitosan can be degraded between 14 and 28 days after being implanted. Mansouri et al. [34] propose to chitosan as a candidate to be used in gene therapy due to its ability to bind and form complexes with DNA by the electrostatic attraction created.

Chitosan has been shown to be a nontoxic polymer, the reason why the Food and Drug Administration (FDA) has approved its use in wound dressings [35]. Likewise, in the clinical reports with chitosan-based biomaterials, inflammation or allergic reactions have not been reported [2]. Waibel et al. [36] detailed the first study of chitosan as bandages in soldiers with shellfish allergy, where all soldiers tolerate the chitosan bandage without reaction.

A very important property is biodegradation, which can be chemical or enzymatic degradation. Chemical degradation refers to acid-catalyzed degradation, such as the gastric juices of the stomach. Likewise, chitosan can be degraded by enzymes, which are responsible for

Figure 3. Promising properties of chitosan for medical use.

hydrolyzing the bonds between glucosamine-glucosamine, glucosamine-*N*-acetyl-glucosamine, and *N*-acetyl-glucosamine-*N*-acetyl-glucosamine [35]. In addition, the degradation rate of chitosan depends mainly on the molecular weight and the degree of deacetylation. However, the degradation of chitosan is not fully understood.

Ng et al. [37] reported a new emerging technology that can provide 3D nanostructures named bioprinting and highlighting for chitosan, due to its poor printability. They prepared a polyelectrolyte complex blending chitosan and gelatin. The hydrogels were customized and fabricated considering the wound size.

Due to its cationic properties, the chitin and its derivatives have demonstrated that are a potent elicitor in low concentrations. Thus can activate defense mechanisms against pathogen nematodes. Also, in plants can be used to control herbivorous insect and viral diseases [38].

Biocompatibility according to Baldrick [33] is attributed to glucosamine, which is the most abundant component in chitosan and produced in the human body from glucose. Also, it is responsible for producing glycosaminoglycan which forms a cartilage tissue in the body. Chitosan by its positive charge can bind to free fatty acids and bile salt from dietary lipid during the absorption in the gut.

The hemostatic and antimicrobial activity of chitosan is related to its polycationic nature. Therefore, it is involved in the agglutination of red cells that stimulates the formation of clots [39]. The antimicrobial activity is attributed to the electrostatic interactions between the polycationic structure of chitosan and the anionic components of the surface of microorganisms [40].

Xia et al. [41] mentioned that chitosan has free amino groups which are able to neutralize gastric juices and form a protective barrier in the stomach. On this basis, they have proposed it as a safe material with biomedical applications in the treatment of peptic ulcers, for wound healing.

In the preparation of materials for wound healing, chitosan has been used to develop nanofibers by the electrospinning system [29]. Nevertheless, according to Pakravan et al. [19], chitosan has limited electrospinnability, due to its polycationic nature in solution and rigid chemical structure. However, it is possible by blending with other polymers due to the formation of hydrogen bonds.

De Vrieze et al. [42] reported that a strong solvent such as concentrated acetic acid is necessary for chitosan electrospinning, and Geng et al. [3] demonstrated that the concentration of the solvent decreases the surface tension of the solution generating stable jet stream during electrospinning. Also, Kriegel et al. [43] reported that the increase in acetic acid concentration affects surface tension with the appearance of beads in chitosan/poly(ethylene oxide) nanofibers.

4. Myriad nanocomposites for wound dressings

The development of biocomposites from biodegradable polymers has attracted great interest due to their capacity to completely degrade and not produce toxic effects [44]. Also, the main challenge in science of biomaterials and tissue engineering is to create matrices either

of natural origin, synthetic, or blends that are suitable for the development of medical prototypes with improved properties.

In biomedical area, they are interested in developing polymers as biomaterials, because more complex structures need to be achieved on the requirements for their different applications [45].

According to the National Institute of Health (NIH), biomaterials are defined as "any substance (other than a drug) or combination of substances synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system which treats, augments, or replaces tissue, organ, or function of the body " [46].

4.1. Natural and synthetic polymeric nanofibers

In biomedicine, the natural polymeric nanofibers are polysaccharides, collagen, keratin, silk, tubulin, actin, cellulose, chitin [47]. Likewise, there is great variety polymers such as poly(lactic acid-co-glycolic acid) (PLGA), poly(lactic acid) (PLLA), polycaprolactone (PCL), poly(ethylene oxide), (PEO), and poly(vinyl alcohol) (PVA). All these have been used in wound dressings [7, 45], as shown in **Table 1**.

4.2. Wound dressing theory

Traditional dressings used on wound treatment were made from natural or synthetic materials. In the past, the main function of the dressings was to keep the wound dry through the evaporation of the exudates and to avoid the introduction of dangerous microorganisms to the wound [48]. However, over time this idea has been modified, according to Newman et al. [49]; nowadays, an ideal wound dressing must have the objective of providing an optimum moisture, accelerate the healing process, absorb large amounts of exudates, and prevent tissue maceration around the wound, which would cause a second injury. Also, Caló and Khutoryanskiy [50] reported that the dressings are designed with the purpose of maintaining a moist environment between the wound and the dressing, favoring the healing of wounds. The healing process is a dynamic process which allows a complex sequence of events, which include homeostasis, inflammation, proliferation, and remodeling [25].

A wound is defined as a defect or a break in the skin resulting from trauma or medical/physiological conditions. Traditionally, wounds can be classified depending on the number of layers of skin and affected area. When only the epidermis is damaged, the wound is superficial. If, epidermis and deeper layers are affected, is named partial thickness. Moreover, when the subcutaneous fat and deeper tissue have been affected, the wound is named full thickness [50].

On the other hand, Zahedi et al. [51] mentioned that the dressings can be classified from other aspects. Therefore, these authors classified them into three groups called passive, interactive, and bioactive dressings. Passive dressings are ordinarily used for common wound coverage such as cotton gauze. Interactive dressings are characterized for being transparent and permeable. Bioactive dressings have the advantage that requires long periods to be removed and are made from biopolymers such as collagen, chitosan, alginate, and elastin. In the **Figure 4** are presented commercial dressing such as hydrocolloids, alginates, collagens and hydrofibers.

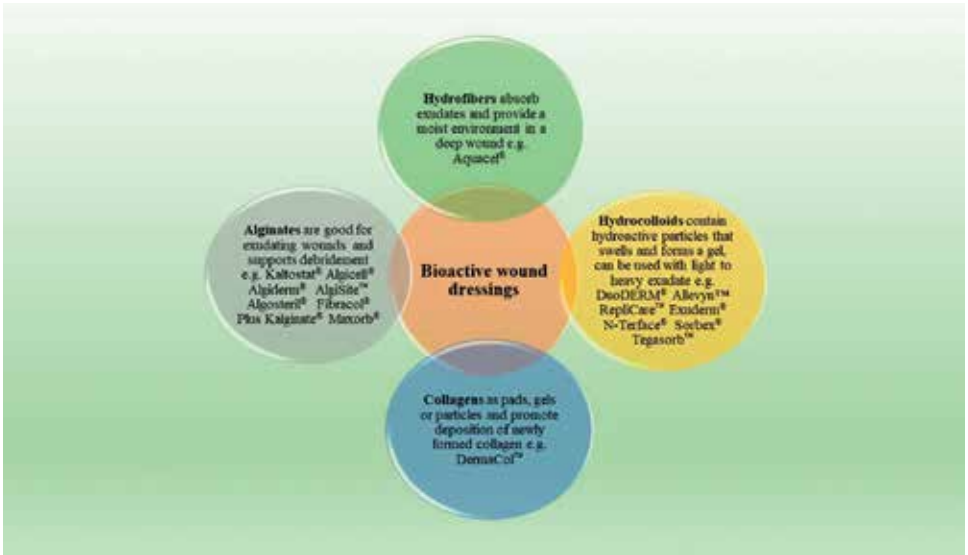

Figure 4. Commercial products of bioactive products.

According to Newsom et al. [52], hydrogels are effective for necrotic wounds because they hydrate wounds and reduce postoperative pain. However, they have disadvantages in wounds with large amounts of exudates and hemorrhages; that is why the use of alginates is recommended. Hydrocolloids promote a moist environment, autolytic tissue debridement, also as protection against microorganisms and are used in postoperative wounds. Hydrofibers are recognized for accelerating the wound healing process and improving the appearance of the final healing.

This wide variety of modern dressings has the advantage of creating an optimal environment that allows the proliferation of epithelial cells and improves the treatment of wounds [48].

5. Properties of electrospinned chitosan nanofibers/composites for wound dressings

The properties of the dressings are optimized through the modification of the electrospinning parameters. Most studies mentioned that the composition of the polymer solution such as solvent, concentration, viscosity, and surface tension affects the morphological structure and mechanical properties of nanofibers [12].

5.1. Superficial morphology

The structural morphology is visually evaluated with scanning electron microscopy (SEM), which also allows the measurement of the diameter of the nanofibers.

According to Bhardwaj and Kundu [8], the viscosity of the polymer solution determines the size and surface morphology of the nanofibers. Also, Bhattarai et al. [53] indicated that electrical conductivity is a physical property that modifies the final diameter of nanofibers.

Haider et al. [54] mentioned that the changes in the surface of nanofibers are attributed to the parameters of electrospinning and concentration of the solution and solvent (trifluoroacetic acid). Haider et al. [25] mentioned that selection of the solvent is very important for the formation of fibers with smooth and bead-free surface formation, considering the solvent capacity to dissolve the polymer and moderate boiling temperature. Also, Geng et al. [3] mentioned that a higher concentration of acetic acid decreases the surface tension and increases the charge density of the jet, improving the morphology of the nanofiber.

Pakravan et al. [19] prepared chitosan nanofibers with poly(ethylene oxide) and found that chitosan solutions, due to the polycationic nature, are more conductive compared to poly(ethylene oxide). Specifically, poly(ethylene oxide) reduces the protonation of the chitosan and avoid the formation of hydrogen bonds between amino groups of chitosan and other groups. The diameters of the chitosan and poly(polyethylene) oxide nanofibers are in the range of 60 to 120 nm.

Similarly, Bhattarai et al. [53] worked with chitosan and poly(ethylene oxide). They reported cylindrical nanofibers with a diameter of 75 nm and mentioned that viscosity is the parameter that modifies the electrospinning and the morphology of the nanofiber. They also indicated that the viscosity is attributed to the interactions between the chains of both polymers.

Lu et al. [55] prepared nanofibers of poly(ethylene oxide) with a low concentration of sodium alginate, showing smooth surfaces with a diameter of 250 nm; this was achieved by increasing the viscosity and decreasing the electrical conductivity of the solutions. Likewise, Shalumon et al. [20] prepared sodium alginate nanofibers blended with poly(vinyl alcohol) adding zinc oxide nanoparticles. The nanoparticles increased the electrical conductivity improving the electrospinning process to produce 190–240 nm fibers with smooth and uniform surface. **Table 2** shows the diameter of the nanofibers prepared by electrospinning with various polymers.

5.2. Porosity and pore distribution

The porosity of biomaterials such as nanofibers is advantageous for their application in wound healing, because they provide more structural space to enhance cell seeding, in addition to facilitating cell proliferation and migration [61]. It also improves oxygen exchange, nutrient delivery, and absorption of exudates. In addition, small pores in dressing nanofibers reduce wound infections and dehydration during the healing process [12].

The porosity is analyzed with water vapor transmission rate (WVTR). Archana et al. [62] mentioned that an ideal dressing should maintain water loss from the skin at an adequate rate, between 2000 and 2500 g^{-2} day^{-1}, indicating that higher values dry wounds quickly and retard healing, finding that for a chitosan dressing with pectin and nanosized titanium dioxide (TiO_2) particles the WVTR was from 1950 to 2050 g^{-2} day^{-1}.

However, Ziabari et al. [63] mentioned that there is no specific literature to measure the size of the pores and their distribution along the fiber. The techniques currently used are indirect and not very precise. Therefore, image analysis techniques are used, and these tools are more precise and direct. Coimbra et al. [64] used scanning electron microscopy (SEM) to observe the morphology of the chitosan/pectin wound dressing, which formed porous shapes from sheetlike to fibrous-like structures. Additionally, Ninan et al. [65] used micro computed

Polymeric solution	Average diameter (nm)	References
CS/PVA	279.843	[14]
CS/HA	227.8 ± 154.3	[15]
PVA/SA	500–50	[16]
CS/PVA	75–400	[17]
SA/PEO	130 ± 51	[18]
CS/PEO	60–120	[19]
SA/PVA	190–240	[20]
SA/PVA	250 ± 30	[21]
CS/PEO	40	[53]
SA/PEO	250	[54]
CMCTS/CS/PEO	50–300	[56]
COL/CS	434–691	[57]
CMC/HA	15–35	[58]
CA/PVA	98.1–191.5	[59]
PEO/CS/PC/O	86	[60]

PVA, poly(vinyl alcohol); CS, chitosan; HA, hydroxiapatitha; SA, sodium alginate; PEO, poly(ethylene oxide); PL, polycaprolactone; PC, poly(ε-caprolactone); O, olive oil; CA, calcium alginate; CMCTS, carboxymethyl chitosan; COL, collagen.

Table 2. Diameter of nanofibers by electrospinning.

tomography (micro-CT) in pectin/carboxymethylcellulose/microfibrillated cellulose dressings (15–280 μm) obtaining 3D images and the quantity of the number of pixels in the pore image. Kumar et al. [66] measured the porosity of the chitosan/pectin/calcium carbonate ($CaCO_3$) dressings based on the empty spaces present (41.8%) as a fraction of the total volume by liquid displacement method. Also, Liuyun et al. [67] used liquid displacement method to measure porosity (77.8%) and pore diameter, between 100 and 500 μm, in nanohydroxyapatite/chitosan/carboxymethylcellulose dressings. Besides, these authors reported the presence of interconnected pores that favor the administration of nutrients.

Recently, Sarhan et al. [68] reported the use of an automatic analyzer called mercury porosimetry to measure the size of the pores; however, it only measures sizes in the range of 0.0018–400 μm, finding that in chitosan nanofibers/honey/PVA the diameter of the pores is 140 μm.

5.3. Water absorption capacity

One of the properties of dressings is the ability to absorb exudates in the wound and provide a moist environment. However, it affects the efficiency of oxygen and nutrient transfer [62].

According to Ninan et al. [65], the swelling analysis and the water uptake provide information about the ability of the dressing to transport the nutrients inside the pores, in addition to avoiding dehydration of the wound.

Choi et al. [69] mentioned that the hydrophilicity of chitosan is due to a modification in its structure, which improves its solubility in water at physiological pH. Archana et al. [62] evaluated the swelling of the dressings of chitosan/pectin and TiO_2 in phosphate-buffered sodium (PBS), finding the highest values (1215%) at pH 2.0 and the lowest (900%) at pH 7.0, due to the osmotic effect of the dressing due to the absence of ionized amino groups.

Liuyun et al. [67] reported the loss in weight, 30% in 30 days, by degradation of the dressings of chitosan/nanohydroxyapatite/carboxymethylcellulose, caused by intermolecular interaction between the components of the system and the microstructure, which favors the growth of the cells for the regeneration of the bone tissue.

Zarghami et al. [60] reported that cross-linking agents modify nanofibers and their swelling behavior. For chitosan nanofibers with poly(ethylene oxide) cross-linked with glutaraldehyde vapors, the swelling decreases from 300 to 75%, due to the aldimine bonds (—CH═N—) formed between the free amino groups of chitosan and the aldehydes of glutaraldehyde. This increases the diffusivity resistance of water and decreases its water absorption capacity.

Duan et al. [70] also used glutaraldehyde vapors as a cross-linking agent, causing a decrease in swelling from 328.7 to 146.2% and shrinkage of poly(lactide-co-glycolide)/chitosan/PVA dressings. Similarly, Chen et al. [71] used glutaraldehyde vapors to reduce the water solubility of chitosan nanofibers with collagen and poly(ethylene oxide).

5.4. Mechanical properties

The mechanical properties are evaluated through Young's modulus, tensile strength, and elongation at break by tensile tests with a universal mechanical testing machine [8]. Parameters such as the composition of the polymer solution, the interaction between its components, and the cross-linking agents affect the mechanical properties of fibrous materials during electrospinning [55].

In some studies, it has been reported that obtaining nanofibers has great advantages for the development of medical prototypes, and the precise measurement of their mechanical properties is very important, especially in dressings which must be able to withstand the forces exerted by the growing tissue or during physiological and biomechanical activities [8].

The composition of the mixture influences the mechanical behavior of the fiber. Chitosan is a fragile and rigid polymer, while poly(lactic acid) (PLA) is resistant; it is possible to blend it to form nanofibers, which show greater tensile strength at rupture with decreases in elongation at break.

Archana et al. [62] studied the chitosan and pectin blend with nanoparticles of titanium dioxide (TiO_2). They found that by increasing the pectin content and the presence of the nanoparticles, the resistance increases significantly compared to those with a lower proportion of pectin and without the presence of TiO_2. Cui et al. [24] reported that pectin nanofibers with poly(ethylene oxide) (PEO) were rigid with values of Young's modulus (192.3 MPa) and tensile strength (14.6 MPa); this is attributed to the orientation of the polymer chains of pectin during electrospinning.

Cross-linking agents have been used with the intention of improving or adding properties; some of the most used cross-linking agents in electrospinning systems are glutaraldehyde vapors [60, 70, 71] and genipin [15].

Shalumon et al. [20] proposed cross-linking agents with glutaraldehyde vapors because it has less or no toxic effect. Chen et al. [71] elaborated by electrospinning nanofibers of chitosan/collagen/poly(ethylene oxide) cross-linked with glutaraldehyde vapors reported Young's modulus increase from 0.29 to 0.65 MPa and the decrease in tensile stress and strain.

Nanofibers of poly(lactic-co-glycolic acid)/chitosan/PVA cross-linked with vapors of glutaraldehyde succeeded to increase the tensile strength up to 3.8 MPa and Young's modulus tension up to 106.2 MPa; this is due to the union between the components of the structure [70].

Another cross-linking agent is genipin, which is extracted from the gardenia fruit (*Gardenia jasminoides*). This has been used in hydroxyapatite fibers with chitosan and was found to increase up to four to five times the stiffness with respect to non-cross-linked fibers [15].

5.5. Antimicrobial prophylaxis

Microbiological assays are evaluated through the inhibition provided by a biomaterial in the presence of microorganisms [61]. **Table 3** shows the microorganisms studied in microbiological assays with nanofibers. According to Heunis et al. [72], *Staphylococcus aureus* is the most prevalent microorganism in skin infections.

Infections in wounds are common, because the microorganisms damage the injured tissues causing adverse reactions to the immune system such as inflammation and tissue damage, retarding the healing process [65]. The main pathogenic bacteria that set the wound healing process at risk are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, some *Proteus*, *Clostridium*, and coliform species [48].

Study	Microorganism	Inhibitory effect	References
Fabrication of PEO/chitosan/PCL/olive oil nanofibrous scaffolds for wound dressing applications	*Escherichia coli*, *Staphylococcus aureus*	Superior against *E. coli*	[61]
Evaluation of chitosan nano-dressing for wound healing: characterization, in vitro and in vivo studies	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*	Superior against *B. subtilis*	[63]
The effect of increasing honey concentration on the properties of the honey/poly(vinyl alcohol)/chitosan nanofibers	*Staphylococcus aureus* *Escherichia coli*	Enhanced against *S. aureus* and *E. coli*	[68]
Chitosan-/polyurethane-blended fiber sheets containing silver sulfadiazine for use as an antimicrobial wound dressing	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA)	Superior against MRSA	[73]
Fabrication of an antibacterial non-woven mat of a poly(lactic acid)/chitosan blend by electrospinning	*Escherichia coli*, *Staphylococcus aureus*	Enhanced incorporating Ag nanoparticles	[74]

Table 3. Antimicrobial assay that involves chitosan nanofibers.

The antimicrobial activity of chitosan is of broad spectrum and has been shown to be effective against Gram-positive and Gram-negative bacteria and many filamentous fungi and yeasts [40]. According to Kumar et al. [75], the cationic amino groups of chitosan have the ability to bind to the anionic groups of the microorganisms by inhibiting the presence of *Escherichia coli*, *Fusarium*, *Alternaria*, and *Helminthosporium*. Sarhan et al. [68] attributed this to the polycationic nature of chitosan, which allows it to interact with bacterial membranes that are negatively charged, favoring the loss of permeability, causing cell disruption and subsequently death.

The inhibitory effect in dressings is evaluated by several techniques. Au et al. [74] used the optical density technique in a chitosan/polylactic acid/silver nanofiber blend, mentioning that bacterial cells are opaque and when they propagate in solutions they become turbid; therefore, lower optical density indicates greater antibacterial activity.

Other authors used the bacterial disk inhibition method. Archana et al. [62] reported that in nanofiber dressings of chitosan/pectin/TiO_2 the antibacterial activity is excellent; in addition large surface areas facilitate microbial adsorption and accelerate the antimicrobial activity rate. Sarhan et al. [68] report efficiency against *E. coli* in nanofibers of chitosan and honey; the inhibitory effect due to the polycationic nature of chitosan is potentiated with honey due to its acidity, high sugar content, and hydrogen peroxide production capacity. Additionally, Zarghami et al. [60] found greater efficiency against *E. coli*, Gram-negative in nanofibers of chitosan with poly(ethylene oxide).

The use of metal nanoparticles had been shown to have high antimicrobial activity against bacteria, viruses, and other microorganisms [76]. Lee et al. [73] reported higher efficiency against *S. aureus* in chitosan/polyurethane nanofibers with silver sulfadiazine. Silver is known for its great antimicrobial properties and its use for pharmaceutical applications. Its antimicrobial activity is due to the interaction between silver particles with the bacterial cell, penetrating its wall and causing damage and cell death to components (DNA) [77].

6. Cross-linked copolymers for electrospinned chitosan biocomposites/nanofibers

6.1. Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is a synthetic polymer soluble in water and with excellent chemical resistance. It is recognized mainly for its non-toxicity, biodegradability, and biocompatibility, and it is used in the biomedical area. Since the 1950s it has been commercialized for the formation of highly hydrophilic fibers [17]. It is known that PVA nanofibers dissolve instantaneously in water. That is the main reason why cross-linking is recommended. Destaye et al. [13] cross-linked PVA nanofibers with glutaraldehyde vapors and reported that the concentration of glutaraldehyde increases water retention capacity and the swelling of nanofibers improves their mechanical properties.

For biomedicine, the chitosan nanofibers with PVA have been elaborated by electrospinning because they are more favorable for cell culture compared with only PVA [17]. Recently, chitosan

nanofibers blended with PVA are being studied as dressings for the treatment of diabetic foot ulcers. Ahmed et al. [30] demonstrated that nanosized pores protect damaged tissue from bacteria, while high porosity increases fluid absorption and promoting wound healing.

6.2. Sodium alginate

Sodium alginate is a nontoxic polysaccharide with applications in the food and pharmaceutical products. The natural sources are all species of brown seaweed. It is a water-soluble salt of alginic acid. It consists of two uronic acids, β-D-mannuronic acid (M) and α-L-guluronic acid (G), in β-(1–4) union. These acids form homopolymeric blocks M-M or G-G and blocks with an alternating sequence of M-G blocks [78].

The alginate is used in medicine to prepare wound dressings, Tehrani et al. [79]. Fan et al. [80] reported that calcium alginate fibers interact with wound exudates forming a gel, resulting on ion exchange between fiber calcium and sodium ions from exudates. Furthermore, this polymer has been used for the treatment of different wounds due to its biocompatibility properties [81].

Pure sodium alginate solutions cannot be processed by electrospinning because of their high viscosity. But it is possible using organic solvents or with water-soluble synthetic polymer blend such as poly(vinyl alcohol) (PVA) and poly(ethylene oxide) [82]. Islam et al. [16] reported that blend of sodium alginate with PVA can be processed by electrospinning and forms ultrafine nanofibers with uniform surface.

Yu et al. [83] elaborated wound dressings with chitosan/alginate/collagen and hydroxyapatite with potential application in bone tissue engineering due to a suitable structure for cell development. Similarly, Jeong et al. [84] from a polyelectrolyte blend with chitosan/PEO obtained nanofibers for wound dressings, demonstrating their cell promotion and potential use as a dressing.

6.3. Carboxymethylcellulose

Carboxymethylcellulose is a semisynthetic natural polymer obtained by carboxymethylation of cellulose with properties such as biocompatibility, low toxicity, and low degradation rate [85]. Likewise, Ninan et al. [65] attribute their water solubility to their composition from the β-(1 → 4) glucopyranose residues. Its use in biomedicine as a biocompatible material has been proven [86]. Besides, Chen and Fan [87] studied their efficiency in clinical studies with humans and animals, finding that postoperative damages such as abdominal adhesions are reduced.

Chitosan blends with carboxymethylcellulose are possible because it is an anionic polymer with similar structure to chitosan allowing strong ionic cross-linking between them [67].

Fouda et al. [56] elaborated antimicrobial dressings for biological use from chitosan and carboxymethylcellulose blend with silver nanoparticles. Also, Ninan et al. [65] prepared wound dressings from a mixture of pectin, carboxymethylcellulose, and microfibrillated cellulose for skin wound treatment.

6.4. Collagen

Collagen is one of the proteins of the extracellular matrix more abundant in mammals. It is recognized for being reabsorbable with excellent biocompatibility and the ability to promote tissue regeneration [83]. The extracellular matrices in tissues are nanofiber structures that act as wound dressing to attach cells in the tissue, control tissue structure, and regulate the cell phenotype [88].

The main challenge for tissue engineering dressings is to design and create biodegradable matrices that can mimic the composition and structure of extracellular matrices [89]. Cross-linking and blending with biomolecules or synthetic materials have been used to improve the stability of collagen dressings [83]. According to Chen et al. [87], collagen is widely used as a biomaterial in the medical and pharmaceutical field. Collagen and chitosan are blended mimic the components of the extracellular matrix. Chen et al. [90] mentioned that electrospinning using a suitable solvent such as the mixture of 1,1,1,3,3,3 hexafluoro-2-propanol with trifluoroacetic acid to make dressings with application in tissue engineering is possible. Also, Yin et al. [91] used these solvents in electrospinning of collagen/chitosan/poly(L-lactide-co-ε-caprolactone) blend to produce dressings with application in vascular graft.

7. In vivo and in vitro assays

In vivo and in vitro assays evaluate the response of a biomaterial in experimental models or simulations. Biodegradable biomaterials have the characteristic of being completely degraded by the body's enzymes when the support is no longer necessary [55].

Table 4 shows the different in vitro and in vivo tests performed with biomaterials. Archana et al. [62] indicated that the dressings of chitosan/pectin/TiO_2 induce 1.14% of hemolysis of erythrocytes; for that reason they are highly hemocompatible when accepting values up to 5%.

Gautam et al. [92] reported high proliferation, adhesion, and cell morphology in polycaprolactone/elastin/chitosan dressings. This is due to the synergistic effect between the carboxyl group of polycaprolactone, gelatin amino group, and hydroxyl group of chitosan. In addition, in vitro degradation was almost complete after 8 weeks.

Likewise, Kumar et al. [75] in chitosan/pectin/calcium carbonate nanofibers reported that the dressings are biodegradable, because the pectin-chitin matrix gradually degraded up to 60% in 21 days.

Duan et al. [70] reported high cellular viability, because the poly(lactic acid-co-glycolic acid)/chitosan/PVA nanofibers promote attachment and proliferation of fibroblasts; in addition their morphology changed from round to elongated with little cellular activity. Likewise, Coimbra et al. [64] reported cellular growth and proliferation with a typical cellular form of pectin/chitosan dressings, thus demonstrating their biocompatibility and non-toxicity. On the other hand, Mendes et al. [93] evaluated the cellular metabolism and integrity of the membrane in fibroblasts in chitosan/phospholipid nanofibers by reporting biocompatible dressings.

Study	Assessment	Results	References
Application of chitosan/PVA nanofiber as a potential wound dressing for streptozotocin-induced diabetic rats	In vivo (adult Wistar rats)	Good adherence and promote tissue bonding	[14]
Electrospinning of carboxymethyl chitin/poly(vinyl alcohol) nanofibrous scaffolds for tissue engineering applications	In vitro (human mesenchymal stem cells)	High cell adhesion and proliferation	[20]
Evaluation of chitosan nano-dressing for wound healing: characterization, in vitro and in vivo studies	In vitro (mouse fibroblast (NIH 3 T3 and L929)) In vivo (adult male albino rats)	Induces blood clotting Fast wound healing Surface of the wound was covered with new epithelium	[62]
Preparation and chemical and biological characterization of a pectin/chitosan polyelectrolyte complex scaffold for possible bone tissue engineering applications	In vitro (human osteoblast cells (CRL-11372))	Cells adhered and proliferated	[64]
Drug delivery and tissue engineering applications of biocompatible pectin-chitin/nano-$CaCO_3$ composite scaffolds	In vitro (NIH3T3, MG63, and L929 cells)	Efficient cell adhesion and proliferation	[66]
A nanofibrous composite membrane of PLGA-chitosan/PVA prepared by electrospinning	In vitro (rabbit dermal fibroblast from rabbit back skin)	High fibroblast viability	[70]
Fabrication and characterization of PCL/gelatin/chitosan ternary nanofibrous composite scaffold for tissue engineering applications	In vitro (mouse fibroblast cells (L929))	High fibroblast growth and proliferation	[92]
Hybrid electrospun chitosan-phospholipids nanofibers for transdermal drug delivery	In vitro (L929 cells)	Biocompatible nanofibers	[93]

Table 4. In vitro and in vivo assays with chitosan nanofibers.

In vivo assays with animal experimental models on chitosan/PVA nanofibers reported cases of completely healed wounds. This has been attributed to protection of the injured tissue against bacteria that can infect damaged scar tissue by the pores of dressing; in addition the process of fluid absorption and healing was increased after 10 days [14]. Archana et al. [62] achieved wound closure after 16 days with chitosan/pectin/TiO_2 dressings, which were adhered, did not dissolve upon contact with the wound, and had easy removal.

8. Conclusion

Many technological advances continue to exploit the properties offered by polymeric materials as biocomposites. One of the more innovative advances is electrospinning. This technique is simple, low cost, and effective for the development of nanofibers. These polymeric nanocomposites are used as biomaterials for the preparation of dressings.

The surface properties of the nanofibers show the influence of the electrospinning parameters, from their diameter, pore size, porosity, ability to absorb the liquids of the wounds, and their ability to allow gas exchange in the wound, while the mechanical properties are influenced by the composition of the polymer solution. All this provides information about the ability of dressings to adapt to wounds and their ease to manipulate. In addition, it is well known that blending polymers to produce biocomposites is an effective method to improve the performance of materials.

Chitosan is a polycationic polymer widely studied because it has biological properties that make it a good candidate to be used for wound dressings. Thus, the microbiological assays show the capacity of the chitosan dressings against the main microorganisms present in the skin and wounds, demonstrating their efficiency. In vitro tests, through the simulation of wound conditions, reveal their behavior in the body. In contrast, in vivo tests expose the response of the dressing in animal experimental models. However, clinical study reports in human models are required to learn the precise behavior of the biomaterial and their response in the wound.

This chapter exhaustively reviews the literature that discusses the preparation of chitosan dressings by electrospinning and its blending with other polymers, as well as the biological properties that determine its potential medical use in the healing of cutaneous wounds.

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Conflicts of interests

The authors declare no conflicts of interest.

Summary

As a summary, electrospinned chitosan nanocomposites are promising biomedical wound dressings.

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Chapter 10

Chitosan and Xyloglucan-Based Hydrogels: An Overview of Synthetic and Functional Utility

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Additional information is available at the end of the chapter

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Abstract

The development of new strategies for wound healing has resulted in the design of biomedical devices using polymers of natural origin. Hydrogels are biomaterials formed by three-dimensional polymeric networks that can retain large amounts of water or biological fluids, and smooth texture similar to living tissue. Chitosan is a linear polysaccharide, (1-4)-2-amino-2deoxy-ß-D-glucan, which has desirable features such as biocompatibility, non-toxicity, hemostasis and antibacterial character. Xyloglucans have different applications in tissue engineering for their physicochemical properties, biocompatibility and control of cell expansion. Hydrogels had been made of homogeneous mixtures prepared of chitosan and purified xyloglucan, followed by a freeze-drying process to develop a flexible and porous structure. Additionally, their mechanical properties such as porosity, solubility, biodegradation, and the antibacterial activity of the hydrogels are studied. The results suggest that the incorporation of xyloglucan favors the characteristics from chitosan-based hydrogels, providing a promising alternative for application in biomaterials with antimicrobial activity.

Keywords: chitosan, chitin, xyloglucan, hemicellulose, hydrogels, biocomposites, biomaterials, polysaccharide

1. Chitosan

1.1. Chemical properties and production

Chitosan is an amino-polysaccharide of natural origin. This polymer consists of a linear chain of repeating monomers of D-glucosamine and N-acetyl-D-glucosamine, whose contents and

sequence are variable [1]. The amino groups allow specific chemical reactions and confer very important functional properties [2]. It is usually, produced by the partial deacetylation of chitin, a linear polymer of N-acetyl-2-amino-2-deoxy-D-glucopyranose linked with β-(1-4) bonds [3].

It is estimated that approximately 10 million tons of chitin, can be synthesized in nature every year [4]. Chitin and its derivatives are renewable, biocompatible, non-toxic, biodegradable and have biological properties such as anti-cancer, antioxidant, antimicrobial and anticoagulant [5]. It is mainly found in the exoskeleton of crustaceans such as shrimp and crab with contents from 58 to 85%, which are the most important source of chitin for commercial use due to their availability as waste produced during its industrial processing. These residues in turn constitute to one of the main problems of these industries for society because of its negative impact on the environment [5, 6]. Also, chitin is a structural component of the cell wall of fungi such as *Aspergillus niger* and *Mucor rouxii* with up to 45% [7]. Some marine invertebrates contain from 3 to 28% of chitin. In squid pens, 31–49% of chitin has been reported [8]. Moreover, in a recent investigation it has been reported that insect larvae and imagos are an alternative source of isomorphic α-chitin, in a range of 20–60% [4, 9], authors reported 14% of α-chitin [10] in grasshoppers (*Dociostaurus maroccanus*).

Chitin exists in three major polymorphic forms, α, β and γ-chitin. These differ in the arrangement of the chains within the crystalline regions [11]. α-chitin is the most stable and abundant form [5]. It possess a compact rhombic structure, due to the antiparallel chain that favors the formation of interlaminar hydrogen bonds [12] between the hydroxyl and carbonyl groups [13]. Conversely, the β-chitin structure is monoclinic with a parallel arrangement that inhibits the formation of interlaminar hydrogen bonds. In some studies, it have been reported that β-chitin has a higher solubility, reactivity and affinity to polar solvents than α-chitin [14, 15]. γ-chitin, is a combination of the α- and β-chitin configurations, has been found in the stomach of squid and in the buds of beetles. Squid pens, extracellular fibers of diatoms and spines of annelids are sources of β-chitin [5], while α-chitin is isolated from the exoskeletons of crustaceans, particularly shrimp and crab [15].

Traditionally, the extraction of chitin from exoskeletons of crustaceans consists of a treatment with hydrochloric acid in order to remove inorganic components such as calcium carbonate and calcium phosphate. This is followed by an alkaline treatment with NaOH to solubilize the proteins and remove some pigments such as melanin and carotenoids [16] temperature control and concentration of NaOH are crucial to achieve a satisfactory result. In addition to the chemical methods for obtaining chitin, biological methods involving the use of microorganisms [17] and enzymatic hydrolysis [18] have been reported.

One of the limitations in the use of chitin on a large scale is its insolubility in water, due to which water-soluble derivatives are produced, and chitosan being the most important of them [5]. Once the decalcification and deproteinization steps are completed, chitosan is obtained by alkaline deacetylation of chitin using a saturated solution of NaOH 45% [19, 20]. The deacetylated form of chitosan in acidic solutions offers the advantage to be efficiently processed as powder, pastes, gel, membranes, sponges, beads, microparticles, nanoparticles and nanofibers [21]. Some methodologies for the production of chitosan by chitin deacetylation are presented in **Table 1**.

Reference	Sources of chitin	Conditions/chemical method	Properties
[11]	Crab shells	NaOH 12 M Under nitrogen atmosphere 110°C (2–3 h)	—
[25]	Shrimp shells	NaOH 50%, ratio 1/50 g mL^{-1} Under nitrogen atmosphere, 100°C (9 h)	DD = 80%
[26]	Shellfish	NaOH 47%, ratio 1/10 g mL^{-1} Under nitrogen atmosphere 110°C (30 min)	DA = 26.9 ± 0.8%
[13]	Prawn shells	NaOH 40% High intensity ultrasound irradiation	DA = < 32% 1×10^5 g $mol^{-1} < M_v < 2 \times 10^5$ g mol^{-1}
[27]	Shrimp shells	NaOH 50%, ratio 1/20 g mL^{-1} Intensity 350 W (8 min)	DD = 82.73%, Mw = $2.3 \times 10^{-18} \pm 137$ g mol^{-1}
[28]	Squid pens	NaOH 40%, ratio 1/10 g mL^{-1} Ultrasound irradiation, 60°C (50 min)	DA = 36.7%, $M_v = 10.3 \pm 0.3 \times 10^5$ g mol^{-1}, $M_w = 12.6 \pm 0.4 \times 10^5$ g mol^{-1}
[29]	Lobster by-products	NaOH 50%, ratio 1/10 g mL^{-1} 120°C (4 h)	DD = 71.59%
		Enzyme/biological method	
[30]	—	Deacetylase chitin from *Colletotrichum lindemuthianu* Reaction performed at 45°C	—
[31]	Shrimp shells	Deacetylase chitin from *Mucor rouxii* Reaction performed at 50°C	F_A = 0.582, 0.400 y 0.188
[32]	Shrimp shells	Deacetylase chitin from *Pichia pastoris* Reaction performed at 50°C (60 min)	DA = 33%
[33]	Crab and shrimp shells	Chitinase isolated from the stomach of *Parapristipoma trilineatum* Reaction performed at 37°C (2 h)	
[34]	—	Deacetylase chitin from *Absidia orchidis vela coerulea* Reaction performed at 50°C (250 h)	—
[35]	—	Steam explosion (SE) High pressure (1 Mpa), at 180°C	DA = 3.7% Reduction of crystallinity index 11.28%
[12]	Shrimp shells and squid pens	Steam explosion (SE) (9 Kg/cm^2), at 179°C	DD = 42.9% (α-chitin) DD = 43.7% (β- chitin)

DA, degree of acetylation; DD, deacetylation degree; Mw, weight average molecular weight; Mv, viscosity average molecular weight; FA, fraction of acetylated units.

Table 1. Chemical and biological methods for the chitin deacetylation.

Chemical method for the preparation of chitosan provides a degree of deacetylation of 85–93%, products with a wide range of molecular weight are obtained [22]. Studies have shown that the enzymatic conversion offers a degree of deacetylation up to 97%, and could generate new polymers with different characteristics. Steam explosion is a hydrothermal method to deacetylate chitin, where the biomass is treated with saturated steam at high pressure and temperature for minutes, followed by an explosive decompression; during the process, molecular interactions are broken by thermo-mechanical forces [12]. It has been proposed to differ between chitin and chitosan based on their solubility in acid solutions, that is, if chitosan is soluble and chitin is insoluble [16].

Chitosan oligomers are short fragments of chitosan composed by the same units and glycosidic bonds, commonly oligomers are obtained by chemical or enzymatic methods [23]. They are named according to the number of sugar rings in their chemical structure (dimer, tetramer and hexamer). Compared with conventional chitosan and its derivatives, chitosan oligomers have relatively lower molecular weight and attributed remarkable characteristics of water solubility [24].

The confection of chitosan with respect to the degree of polymerization, polydispersity, degree of acetylation, molecular weight and acetyl group distribution provides tools to manipulate functions and properties in regard to their biological effects and/or applications [18, 23]. These parameters are important to examine the relation of structural units between N-acetyl glucosamine and glucosamine, for example, in the case of N-acetylation degree, the molecular weight depends on the source of obtention and the deacetylation conditions [16] during the conversion process, that is, temperature, time of exposure and alkali concentration.

1.2. Biological properties

Chitosan is one of the most widely used natural biopolymers due to its high biocompatibility, biodegradability, non- toxicity, bioadhesivity, antigenic capacity and hemostasis [23, 36–38]. In the materials science, biocompatibility is defined as the absence of cytotoxicity of a biomaterial and its biofunctionality that allows it to support cell-biomaterial interactions [39]. The evaluation of the biocompatibility of the implantable systems requires an understanding of the inflammatory and curative responses of each material. Inflammation, scarring and response to foreign bodies are tissue responses to injury [40]. Bioadhesivity refers to the ability of the polymer to adhere to hard or soft tissues [41]. It adheres to epithelial tissues and the mucous coating present on the surface of tissues [37]. Clinically, when the chitosan biocomposites come in contact with a wound, it adheres to covering the site of the lesion and attracts the red blood cells, forming a seal that prevents further bleeding [42]. Chitosan hemostatic mechanism involves agglutination of blood cells, possibly due to its intrinsic polycationic properties and non-specific binding to cell membrane [43]. Research has led the addition of new formulations to adapt the biocomposites to the needs of the injury. Researchers studied the gelatin-chitosan interaction for sponge formulation as a hemostatic agent [4, 44]. Chitosan-based hydrogel sheets with honey and gelatin have been made as a coating for burn wounds [45]. Authors reported the development of chitosan-agarose hydrogels for tissue engineering application [46]. Chitosan biocomposites treated with sodium hydroxide (NaOH) and sodium tripolyphosphate ($Na_5P_3O_{10}$) for hemostatic use have been developed [42]. Phosphate incorporation as a precoagulant and silver nanoparticles as antimicrobial agent into biocomposite-based chitosan has resulted in the blood clotting acceleration, platelet adhesion and significantly absorb more blood than chitosan biocomposites [47].

Researchers defined biodegradation as an event that takes place through the action of enzymes and/or chemical decomposition associated with living organisms and their secretion products [48]. The final result is a loss of structural integrity and radical decrease of molecular weight [4, 49].

The biodegradable chitosan effect is attributed to lysozyme, an existing enzyme in several plants and in the human body [4, 21, 50], which is produced by macrophages during wound healing [51]. It is known as a glycoside hydrolase that possess the ability to slowly hydrolyze the β-(1-4) N-acetylmuramic acid and N-acetyl-D-glucosamine bonds or between N-acetyl-D-glucosamine residues [52, 53] of chitosan membranes. Moreover, promotes tissue granulation, increases the expression of collagen among other components of the extracellular matrix and accelerates wound healing [54, 55]. Additionally, it acts by enhancing the proliferation and migration functions of inflammatory cells such as polymorphonuclear leukocytes (PMN), macrophages and fibroblasts [56] at the site of injury [6, 57]. The N-acetylglucosamine of chitin and chitosan are the major components of dermal tissue, and is essential for the wound repair; in addition, its positive surface charge allows it to be a support for cellular development and promotes blood clotting [58, 59].

1.3. Antimicrobial activity

An ideal antimicrobial polymer must be economically and simply synthesized, stable in long term, soluble in water or neutral medium, should not be decomposed or emit toxic products, must possess bactericidal activity against to a broad spectrum of pathogenic microorganisms in brief times of contact [16]. Chitosan has been shown to have advantages over other disinfectants, due its high antimicrobial capacity, a broad spectrum of activity and higher mortality rate [14, 60]. In medicine, wounds caused by burns are highly susceptible to infection by skin deterioration that acts as a barrier against microorganisms [54]. Researchers report that biocomposites with more than 0.025% of chitosan inhibits the growth of *Escherichia coli, Fusarium, Alternaria* and *Helminthosporium* [21]. Studies about the antimicrobial activity of chitosan, honey and gelatin hydrogels as possible coatings for burn injuries reported antibacterial efficiency against *S. aureus* and *E. coli* [45]. Chitosan-gelatin composites have presented similar inhibitory activity against Gram-positive and Gram-negative microorganisms [61]. PVA addition to chitosan solutions for nanofibers productions with multiple applications reported bacteriostatic activity against *E. coli* [62]. According to investigations, the use of chitosan sponges for diabetic foot ulcers treatment prevents polymicrobial infection and decrease the risk of amputation [63].

Chitosan it is a potent antimicrobial agent of cationic nature at pH below 6.3 [62]. An antimicrobial agent is one that eliminates microorganisms or inhibits its growth [21]. Some hypotheses indicate that chitosan could interact with anionic groups on the cell surface of microorganisms increasing the permeability of the membranes, facilitating the scape of proteins and other intracellular constituents of the microorganisms [61]. Another mechanism involves the formation of chitosan, chelates with trace elements or nutrients, resulting in the enzymatic activity inhibition [64] due to the chitosan-DNA interaction that modifies the synthesis of RNA messenger [7]. The antimicrobial effects are regulated by intrinsic factors including the type of chitosan, degree of polymerization, the source, chemical composition of substrates (e.g., moisture and/or water activity) and environmental conditions [21]. Research on antimicrobial properties of chitosan films with different deacetylation degree (DD) and molecular weight, against Gram-positive and Gram-negative bacteria demonstrated that the inactivation step of the bacteria increases with the increase in deacetylation degree of the biopolymer; however, the bacteriostatic and bactericidal mechanism of action is not fully known [65].

1.4. Structural properties

At neutral or basic pH, chitosan contains free amino groups and is insoluble in water, while in acidic pH it is soluble in water, due to the protonation of its amino groups [21]. Chitosan exhibits unique polycationic characteristics, chelating properties and film forming abilities, due to the presence of amino and hydroxyl active groups [64]. It is widely used to prepare natural hydrogels, however, they generally lack mechanical stability unless they are cross-linked and/or reinforced by suitable compounds [66]. By definition, hydrogels are polymer networks having hydrophilic properties [67, 68]. They have been used in the pharmaceutical and biomedical area for wound care, as drug releasers, organ and tissue transplantation [69]. The incorporation of therapeutic agents into the hydrogel formulations has been used in order to facilitate many healing processes, particularly in burn wounds [70].

Hydrogels are defined as three-dimensional, hydrophilic networks capable of swelling and absorbing large amounts of water or biological fluids [71], when deposited in aqueous solutions. Hydrogels containing more than 95% water are called as "superabsorbents" and have a high biocompatibility due to their high degree of water retention [72], which is due to its high water content, porosity and soft consistency that are very similar to the natural living tissues [73].

Physical hydrogels are the result of environmental changes (temperature, pH, molecular arrangements and supramolecular interactions), which have the advantage of forming gels under mild conditions, without the use of organic solvents, while chemical hydrogels can be produced by radical polymerization, chemical reactions and/or enzymatic reticulation that also possess better mechanical properties; however, they need solvents and/or toxic crosslinkers [74].

The cross-linking of chitosan can be achieved with the implementation of chemicals products such as epichlorohydrin or glutaraldehyde to enhance their stability in acid solutions [75]. Researchers studied the combination of two cross-linking methods, ionic with CaSO4 and covalent with genipin for the preparation of chitosan-based hydrogel films [76]. They found that ionic and covalent cross-linking exhibit differences in mechanical characterization (strength and maximum load). Investigators designed thermosensitive hydrogel-based chitosan and its derivatives, without the addition of chemical cross-linking agents [77], showed that it may be a viable alternative as a vehicle for the release of injectable drugs. Further studies on the behavior of chitosan-agarose hydrogels as a biocomposite for tissue regeneration [78], determined that the hydrogel provides an adequate environment for healing, that is, meets the criteria for an ideal wound dressing. Chitosan cross-linking with poly(alginic acid) for the manufacture of nanohydrogels (30–80 nm) with ability to remove aqueous metals, reported excellent absorption abilities for Cr (IV) removal [79].

The structures formed in the chitosan hydrogels are: (a) chitosan cross-linked with itself, that is, without the need for any additives, the process that is based on the neutralization of the amino groups of chitosan and thus the inhibition of repulsion between the chains of the polymer [73, 80]. (b) Hybrid polymer network consists of the mixing of two polymer solutions, which commonly use the same solvent, with or without the addition of cross-linking agents [81]. (c) Semi-interpenetrating polymer network is formed when a linear, biological or synthetic polymer is trapped within a polymer matrix [71] and (d) ionic cross-linking are considered as biocompatible have a non-permanent network formed by reversible bonds and have a

greater sensitivity to swelling in pH changes [50]. Depending on the nature of the cross-linker, the main interactions that form the network are ionic or covalent bonds [21].

1.5. Other uses

In addition to the biomedical and pharmaceutical area, the multiple properties of chitosan have made it a polymer of interest for food preservation, agriculture and water treatment, among others (**Figure 1**).

It has been used in water treatment as a flocculating polymer [66]. Chitosan has high efficiency in the removal of organic pollutants, suspended solids and metal ions in comparison with commercial chemical flocculants [82, 83]. In addition, its potential use as a natural coagulant in the hybrid membrane coagulation-nanofiltration process for water treatment has been studied [84], also the manufacture of hollow fiber nanocomposites in the removal of chemical compounds from water [85]. Chitosan even has been applied as a simple films for the selective removal of mercury in multimetal solutions [86].

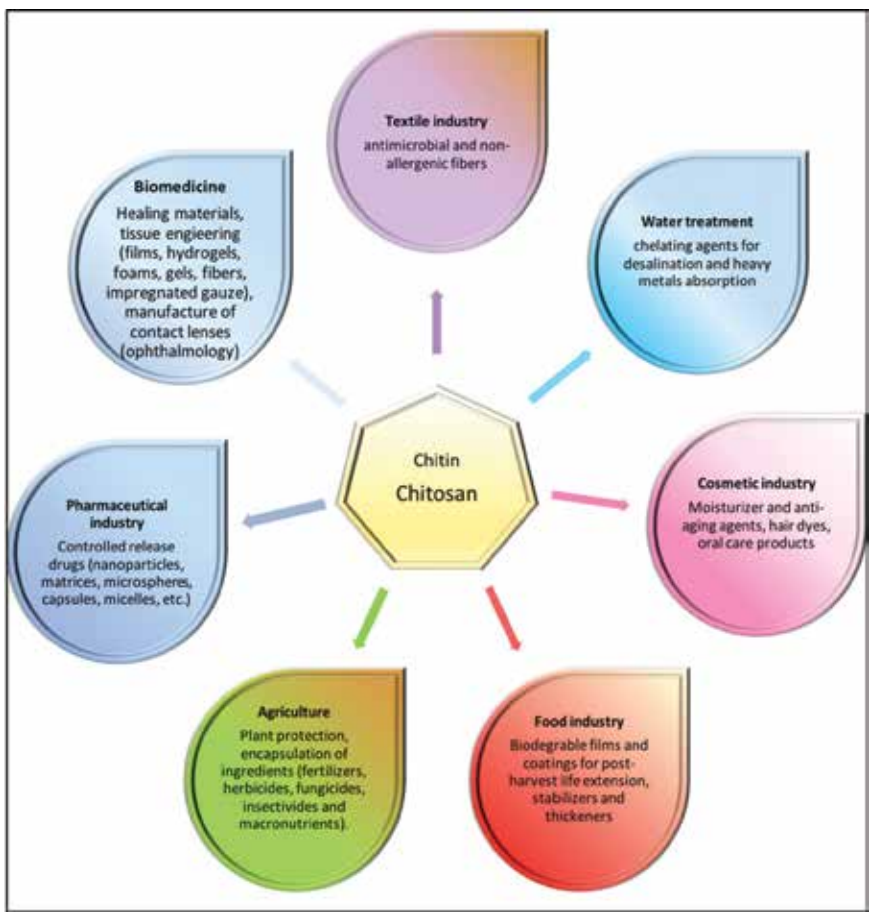

Figure 1. Potential applications of chitin and chitosan.

During food production, the packaging is an important part to ensure its integrity, in this sense, the food industry has put special interest in the application of materials with antimicrobial capacity. Edible coatings can be applied in liquid form while edible films are made as solid sheets and can be used to wrap food products, its application improves the quality and extends the shelf life of slightly elaborated products [87–89].

Panczyk et al. [65] evaluated the antimicrobial properties of chitosan-gelatin films, and found that *Pseudomonas fluorescens* and *Listeria innocua* were more sensitive to chitosan than *Escherichia coli* and *Staphylococcus aureus.* Studies have reported effective antimicrobial properties against *Listeria monocytogenes* in chitosan films added with plasticizers [19] (Sorbitol and glycerol) for active packaging use. Similar results were reported by Coma *et al*. [90] in chitosan solutions for the production of edible films. Leceta *et al*. [91] reported a bacteriostatic behavior of high and low molecular weight chitosan films against *E. coli* and *L. plantarum*, which cause the decomposition of food. Bourtoom *et al*. [92] investigated the mixture of chitosan and rice starch for the elaboration of edible films as an alternative to commercial packaging materials. Its applications have been studied as a cholesterol-lowering agent and its application as an agent for weight reduction [16].

Chitosan has been shown to stimulate plant growth and promote tolerance to abiotic and biotic stress in various horticultural products [93]. It has been studied that the application of chitosan, controlled the release of agrochemicals and genetic materials, and they function as a reservoir of protection for active ingredients [94].

In the cosmetic industry, it has been incorporated in the elaboration of shampoos, conditioners and hair coloring agents. Additionally, in deodorants, and for moisturizing the skin, it could compete with hyaluronic acid [18]. It can also be used for the formulation sunscreens, minimize acne problems, and reduce static electricity of hair, among others [37].

The textile industry has made use of biodegradable polymers for the manufacture of towels, filters and geotextiles for the control of erosion and landscaping [48]. The lack of commercial chitosan-based products could be attributed to the several challenges when working with it [95].

2. Xyloglucan

2.1. Chemical structure and sources

Hemicelluloses are branched polymers, and the main monomers found are D-glucose, D-mannose, D-xylose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid and D-galacturonic acid [96]. Together with lignin, they form the microfibrils surrounding cellulose [95].

Xyloglucans (XG) are structural polysaccharides and the major components of hemicellulose [97]. They are localized in the middle lamellar and gelatinous layer of the primary cell walls of the superior plants [98, 99]. XG are structurally related to cellulose, as it is associated in a non-covalently form within the cell walls of plants [100].

Xyloglucans or generally called galactoxyloglucans, possess a main chain identical to cellulose, is a glucose polymer linked by β-(1-4) bonds, with side residues of xylose linked by bonds 1–6 along the backbone. The major structural differences of the polysaccharide occur when galactosyl and fucosyl-galactosyl residues are added to the xylose residues in dicotyledons, as well as less residues of xylosyl in monocotyledons [96, 99].

Xyloglucans can be extracted from different species as *Copaifera langsdorffii, Hymenaea courbaril and Tamarindus indica.* Some investigations indicate that they are composed by the same monomers, nonetheless, their proportion and distribution result in a fine structure, which varies according to the species and even within the same species [101]. The XG commercialized on a large scale is extracted from the tamarind seeds [102]. **Table 2** describes the morphological characteristics of plants that produce seeds as a source of xyloglucan as well as their potential applications.

An alternative source of xyloglucan is chia seed (*Salvia hispanica L.*). This ancient seed is native from the central-west Mexico region to the north of Guatemala, where it was consumed by the Aztecs; however, at present it has spread to other regions. The seed has essential fatty acids such as linoleic acid (ω-6) and α-linoleic acid (ω-3), additionally, it is a source of phenolic compounds that provide various effects as antioxidant, antitumor, antithrombotic and anti-inflammatory [103]. It possess high fiber content formed of natural sugars [104].

Source	Plant description/cultivation area	Xyloglucan content	Applications
Chia seed (*Salvia hispanica* L.)	Seeds are 2 mm long, with a diameter 1.5 mm/from the region center-west of Mexico to north of Guatemala	4–6% [105]	Edible films [106]; pharmacology industry and nanocomposites [107]; drug delivery excipients for site-specific release and transdermal drug delivery agents [105]
Seed kernel of tamarind (*Tamarindus indica* L.)	Adult tree (20–30 m, with a trunk diameter (1.5–2 m), Seeds are 1.6 cm long/India, Africa, Pakistan, Bangladesh, Nigeria and most of the tropical countries.	Abundant deposits of xyloglucan [108]	Drug delivery [98]; stabilizer, binder and gelling agent [109]; food thickener, sizing agent in textile, paper and jute industries [110]
Seeds of *Copaifera langsdorfii*	Adult tree (up to 35 m), seeds are 1.5 cm long/forest and savanna populations	40% [111]	Potential use in the pharmaceutical, food or cosmetic industries [112]
Seed of *Hymenaea courbaril* L. (Jatobá)	Adult tree produces an average of 10 kg of seed. Seeds are 1.5 cm long with a diameter of 2.5 cm/Neotropical region of the world	40–50% [113, 114]	Useful as partial substitute for agar in culture media for micropropagation of apples [115]
Seed of *Guibourtia hymenaefolia*	Adult tree (10–18 m, with diameters 40–70 cm) produce approximately 1400 seeds per kg/Congo, Equatorial Africa, Nigeria, Cameroon Gabon and other tropical regions	54.20%	Food technologies and biotechnological processes, pharmaceutical and medical industries [116]
Seed of *Detarium senegalense*	Small tree (5–7 m high)/mainly found in West Africa, Chand and Sudan	Abundant deposits of xyloglucan [108]	Pharmaceutical (for controlling drug release) and food industries [117]

Table 2. Description of plants as xyloglucan sources and its applications.

When the seed is placed in contact with water, the gum or mucilage is secreted by chia seed (**Figure 2**), covering it with a transparent halo [118]. This mucilage represents 5–6% of the total weight of the seed, and is described as an anionic heteropolysaccharide with a high molecular weight (800–2000 kDa) [106]. Structurally xyloglucan is constituted by xylose monomers linked to glucose monomers in a 2:1 ratio [105]. A tentative polysaccharide structure is a tetrasaccharide with the main chain composed by (1-4)-β-D-xylopyranosyl-(1-4)-α-D-glucopyranosyl-(1-4)-β-D-xylopyranosyl units with 4-O-methyl-α-D-glucuronic acid ramifications in the O-2 position of β-D-xylopyranosyl main chain [119].

Hymenaea courbaril L. (legume tree) seeds store xyloglucan, which has a major chain of β- (1-4) glucose units, with some ramifications α-(1-6) xylanopiranosyl or β-(1-2)-D-galactopyranosyl-α-(1-6)-D-xylaropiranosyl [113].

Aspen wood, is a source of xylan composed by a linear chain β-(1-4), linked to a D-xylose with a 4-O-methyl-α-D-glucuronic acid replacing the 2-position of approximately every 8 xylose units [120]. Various sources of hemicellulose and the monomers that compose them are presented in **Table 3**. Aspen wood hemicellulose fractions contain mainly xylose monomers and in a less proportion arabinose and glucose , in comparation with abedul wood commercial xylan [121].

The monosaccharides composition from three different biomasses studied by liquid chromatography, reported a composition of arabinose: galactose: glucose: mannose: xylose (2.2: 1.4: 1.3: 4.7:13.5) for pine wood, arabinose: galactose: glucose: mannose: xylose (3.1: 0.8: 1.0: 0.2: 21.8) for switchgrass (*Panicum virgatum*) and for coastal bermudagrass, the composition was arabinose: galactose: glucose: xylose with a 4.4: 1.9: 0.8: 22.0 proportion, respectively [66].

Glucose, galacturonic acid and arabinose are the principal monomers found in quinoa (*Chenopodium quinoa W.)* and amaranth (*Amaranthus caudatus L.*) identified by gas chromatography [100]. In almond gum, the main hemicellulose monomers identify by gas chromatography are galactose, arabinose, xylose, mannose, rhamnose and glucuronic acid with 45:26:7:10:1:11 molar ratio, respectively [123].

The composition of hemicellulose monomers studied by acid hydrolysis and HPLC analysis in three fractions (apical, middle and basal) of *Neolamarckia Cadamba* [125], reported that xylose is

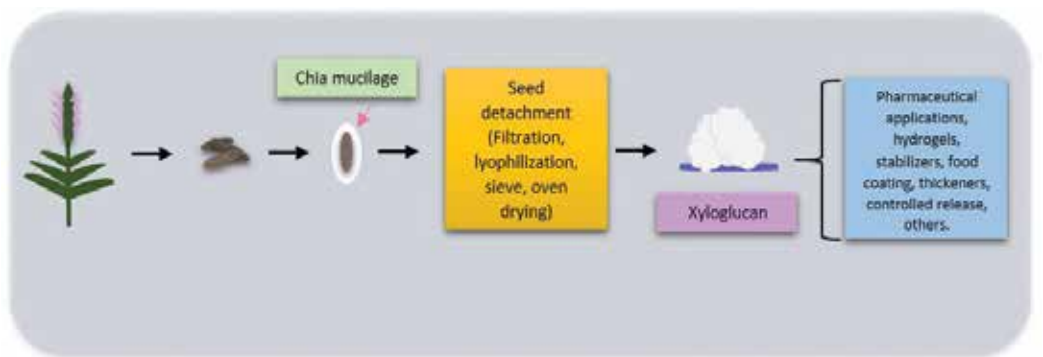

Figure 2. Extraction and potential uses of xyloglucan from chia (*Salvia hispanica L.*).

Authors	Source	Monomers
[98, 122]	Seed kernel of *Tamarindus indica*	Xylose-glucose (3:1)
[105]	Chia seed (*Salvia hispanica L.*)	Xylosa-glucose (2:1)
[118, 119]	Chia seed (*Salvia hispanica L.*)	Xylose, glucose and glucuronic acids (2:1:1)
[120]	Aspen wood (*Populus tremula*)	Xylose: glucuronic acids
[66]	Pine wood	Arabinose, galactose, glucose, mannose and xylose
	Switchgrass (*Panicum virgatum*)	Arabinose, galactose, glucose, mannose and xylose
	Coastal bermudagrass	Arabinose, galactose, glucose and xylose
[101]	*Hymenaea courbaril* seed	Glucose: xylose: galactose (4:3:2)
[100]	Quinoa (*Chenopodium quinoa W.*)	Glucose: galacturonic acid: arabinose: galactose: mannose: xylose
	Amaranth (*Amaranthus caudatus L.*)	Glucose: galacturonic acid: arabinose: galactose: mannose: xylose
[123, 124]	Almond Gum	Arabinose: galactotose: xylose: glucose: rhamnose: glucuronic acid
[125]	*Neolamarckia cadamba* (Rubiaceae)	Xylose: galactose: rhamnose: glucuronic acid: mannose: fucose

Table 3. Monomers and hemicellulose extraction sources.

the main monomer on the three fractions. The mannose glucuronic acid, rhamnose, galactose and fucose content is in the range 22–28%.

2.2. Extraction methodologies

Recent research has shown interest in the xyloglucans extraction from several vegetal sources. The methodologies involve the hydration of matter in water, the methods major differences are based on the raw material/ water (w/v) proportion, temperature (°C), drying methods (vacuum drying or lyophilization) and the process of seeds separation (filtration or sieving).

Specifically, for the mucilage extraction from chia seed, the treatments involve hydration with distilled water (1:40, w/v) at 80°C, for 2 h. Finally, the gum drying process is carried out at 50°C and the seed detachment is performed by sieving [119]. Another method involves soak the seed into water (1:30 w/v, ratio) at 25°C for 2 h to moisturize the chia seed, afterwards the mucilage is separated by centrifugation and vacuum filtration to remove the solid waste, subsequently the gum is stored as lyophilized material [106].

Capitani *et al*. [118] discuss two methods for chia seed mucilage extraction, in the first, it is proposed to submerge the seeds in distilled water (1:10, w/v) for 4 h, followed by a lyophilization process, the mucilage separation occurs by friction in a sieve. The second method involves the same water repose and vacuum filtration separation, followed by a pre-concentration on a rotary evaporator and complemented by lyophilization. After evaluating its rheological properties, it was concluded that the second extraction method provides a greater consistency to the mucilage.

Simi *et al*. [97] studied the xyloglucan physicochemical properties extracted from tamarind seeds. The procedure involves a pulverized seed deproteinization step with protease (at 30 ± 2°C, pH 6). For grease extraction, they used hexane as solvent, subsequently a 95% (v/v)

ethanol solution was used to precipitate the XG, once extracted it is lyophilized and sprayed before use.

Alternative methods of hemicellulose extraction involve alkaline treatments with NaOH combined with an ultrafiltration process. The previous has been applied to aspen wood (*Populus tremula*), the final product is obtained by spray drying [120].

Xyloglucan extraction from *Hymenaea courbaril* L. is carried out with 80% ethanol (80°C, for 10 min). Water is then added and maintained at 80°C for 3 h. The insoluble material is extracted with 4 M KOH. The polymer extracted with alkali is neutralized with acetic acid, followed by a dialysis process and lyophilization [113].

Researchers performed a hemicellulose alkaline extraction from sugarcane bagasse with NaOH (1:25, w/v), to precipitate the hemicellulose, four different ethanol solutions were tested and the pellet was dried at 40°C for 24 h [126] the extract was used to prepare biodegradable films.

Arruda *et al.* [101] studied the biological activities of xyloglucan extracted from courbaril seeds (*Hymenaea courbaril*). The extraction procedure included enzymes inactivation, as well as treatments with NaCl and ethanol at 46% (1:3, v/v) to precipitate the gum.

In another trial, alkaline extraction and delignification with toluene-ethanol (2:1, v/v) is proposed, for obtaining *Neolamarckia cadamba* (Rubiaceae) hemicellulose monomers. The ethanol precipitate was finally lyophilized [125].

2.3. Properties

Xyloglucans possess important applications, especially in pharmaceutical formulations for the gel production [122]. Furthermore, they participate in the control of cellular expansion, own an effect on cell growth, and act as a seeds carbon reserve of many dicotyledons. XG are neutral, non-mutagenic, non-irritating, non-toxic and blood compatible [127, 128]. Additionally, increase the viscosity, have wide pH tolerance, high temperature regimes resistance and salt, also possess adhesiveness, non-carcinogenicity and biocompatibility properties [101, 102]. Considering its attributes, xyloglucans have promising biotechnological purposes [129].

This polysaccharide is considered a hydrocolloid, as a result of its viscosity and ability to retain water, particularly when highly viscous solutions are formed. Currently they are used in a wide range of industries for different applications [118]. Other investigators have attributed properties such as thickener, binder, as a controlled release, texture modifiers, gelling agent, emulsion stabilizers and syneresis control [106, 119]. In Japan xyloglucan is widely used as a food additive, provide texture and can be used in combination or replacement of starch [108]. The previous attributes are due to the xyloglucan possess a high viscosity degree and stability in acid pH and resistance to high temperatures [130].

Researchers evaluated the solubility and water vapor transmission rate (WVTR) effect in xyloglucan films from sugarcane bagasse [126] concluded that the purification process intervenes directly in the micro-structural properties of biodegradable films. In agreement with

other authors, xyloglucan films can be industrially used as coatings in ready-to-eat foods and with health benefits because of their high content of soluble fiber [106].

Biosorption is the property possessed by some biomolecules to bind and concentrate selected ions and other molecules in aqueous solutions [131]. Hemicelluloses mainly conformed by xyloses have application field in the ecology. Thus their combinations with biopolymers such as chitosan have been investigated to produce biosorbent materials in the desalination and heavy metals (Ni, Cu & Pb) removal from water [66]. Other research suggests its application for the development of flocculant-adsorbents for remove several types of dyes from textile wastewater [132].

Bioadhesion can be defined as the state in which two materials, being at least one of them from biological nature, are maintained together by interfacial forces for long periods of time [133]. Natural polymers have been widely used as bioadhesives because of their biocompatibility, specifically the xyloglucan extracted from tamarind seeds has been studied as a mucoadhesive polysaccharide for the transport of medicament administered through the oral route [109].

3. Chitosan-xyloglucan hydrogels

3.1. Preparation

The use of natural polymers with different mechanical, physical and biological properties is frequent in the design and development of biomedical matrices [134]. Biopolymers, which include polysaccharides such as cellulose, chitosan, wool, silk, gelatin and collagen, have been found promising for multitudinal applications in different forms [21].

Chitosan is compatible with a wide variety of biologically active components [18]. The inclusion of carbohydrates such as glucose, cellulose and hemicellulose in chitosan particles generate changes in their structure and by consequence in the biomaterials properties [135]. However, the addition of biopolymers such as xyloglucan (hemicellulose) for the formulation of hydrogels confers resistance properties that increase the value and suitability of the polymer. The main component of chitosan is glucosamine, being a natural substance produced by the body from glucose and it is related to the production of glycosaminoglycans (GAG) that form cartilage tissue in the body and that is also present in ligaments and tendons. It is a biocompatible material that slowly decomposes into harmless products that are completely absorbed in the body [21].

The antimicrobial effect of chitosan has been shown to be beneficial for its application as implants and drug liberators [23]. Hydrogels have attracted attention in various investigations because of their great ability to absorb liquids and their swelling-deswelling capacities sensitive to stimuli without disintegration, what makes it of interest in biomedical and pharmaceutical applications [36, 67]. The swelling is associated by itself with the bioadhesiveness, this depends on the concentration of the polymer, ionic strength, as well as the presence of water, during the dynamic process of bioadhesion, the maximum bioadhesion in vitro occurs with a

optimal water content [41]. The addition of xyloglucan for the formulation of chitosan-xyloglucan hydrogels increases the swelling capacity, because it increases the amount of hydrogen bonds and facilitates the absorption of water [135]. Investigations on the formulation of films from chitosan-hemicellulose demonstrated the capacity and biocompatibility for the application of coatings for wounds, because both polymers are of natural origin and the native properties of chitosan and xyloglucan are beneficial for cell growth [2].

During the healing process, it is indispensable to count on wound coatings for regenerate and repair dermal and epidermal tissue. For a passive coating is essential to stimulate wound healing and to preserve a humidifying environment [70, 136]. In addition, it should prevent the loss of body fluid, prevents accumulation of exudate and protects the lesions from external contamination [21].

Hydrogels have attracted attention because their three-dimensional polymer networks [74] have high capacity to absorb and retain large amounts of water, saline or physiological solutions [137] and present stimulus of response to the swelling-deswelling without disintegration [121]. They owe their mechanical stability to the cross-links introduced between the macromolecular chains that allow flexibility and sufficient resistance [66]. From the science of materials point of view, biological tissues, the essentially moist and soft materials have an elastic modulus of 10^4–10^7 Pa and a water content of 50–85% [138].

In **Table 4**, some biocomposites formulated with mixtures of chitosan, xyloglucan, crosslinkers and other materials are described. Some hydrogels experience continuous and discontinuous changes in swelling, these are regulated by external stimuli such as changes in pH, temperature, ionic strength, solvent type, electric and magnetic field, light and the presence of chelating agents [129].

Reference	Components	Application
[139]	Chitosan: cellulose	Films
[110]	Plasticized xyloglucan	Films
[122]	Bacterial cellulose/xyloglucan (10, 20, 30 wt%)	Films
[106]	Xyloglucan (1%) - glycerol as plasticizer (25, 50 and 75% w/w), based on XG weight.	Films
[2]	Chitosan (0.1%): Hemicellulose, (1:1)	Films
[121]	Chitosan 1%: hemicellulose 1% (70:30, 30:70)	Hydrogels
[128]	Oxidized xyloglucan-chitosan (1:0.5, 1:1, 1:2, 1:3, 1:4) in acetic acid solution	Transparent hydrogels
[140]	Chitosan: xylan hemicellulose, (3:1)	Hydrogels for bone tissue regeneration
[141]	Pharmaceuticals coated with xyloglucan (0.5% or 3%)	Nanocomposites for drug delivery
[96]	Hemicellulose citrate: chitosan, (1:1 w/w)	Aerogel foams
[142]	Chitosan: cellulose	Sponges

Table 4. Biocomposites for medical applications.

The films formation by mixtures of cellulose and chitosan in the presence of ionic liquid has shown a successful miscibility in the solid state [139].

The characterization of bacterial cellulose compounds added with xyloglucan (10, 20 and 30 wt %) by means of drying techniques, show that the inclusion of XG promotes better fiber adhesion and orientation [122].

The addition of plasticizers such as sorbitol, glycerol, urea and polyethylene glycol has been studied in order to facilitate thermal processes and to improve the mechanical properties of xyloglucan films. Thus, the results have shown considerable thermal stability as well as strength and hardness for the xyloglucan and sorbitol (20–30%) combination [110].

Results on the films characterization from xyloglucan extracted from *Salvia Hispanica L.* seeds added with glycerol (25, 50, 75% w/w) indicate that the moisture content, water vapor permeability and solubility of the film in water increased with increasing plasticizer. A high solubility could be advantageous in various applications such as transporter of bioactive compounds [106].

The addition of cross-linking agents such as epichlorohydrin (ECH) for the films preparation of hemicellulose and chitosan (0.1%) involves the reaction of ECH with the chitosan hydroxyl groups. As a result, the smooth, homogeneous and porous surfaces of the obtained films can be beneficial for the breathing of the skin [2].

In the studies on the hydrogels preparation for various applications have proposed the integration of oxidized xyloglucan in combination with chitosan solutions 1% (w/v), the results have evidenced that the strength of the gel depends on the concentration of both polymers [128].

Some researchers have used hemicellulose extracted from woods in combination with chitosan and cross-linked with glutaraldehyde for the hydrogels formation, in consequence the results showed a high response to swelling with increasing hemicellulose content [121].

In the hydrogels preparation studies for the regeneration of osseous tissue, xylan hemicellulose has been incorporated to improve the chitosan properties and was found improvement in the healing of tibia fractures caused by blows, the films preparation was achieved with chitosan: xylan (3:1) mixtures [140].

Researchers have proposed the use of xyloglucan as a protective agent for controlled release of drugs that are limited by the pH of the stomach (pH~1.2), for this, mixtures of xyloglucan (0.5% and 3%) and Enalaprilat (Enal) as pharmaceutical component have been implemented. The results suggest that formulations with 3% xyloglucan can be used as slow drug releaser, especially when needed in other parts of the intestinal tract [141].

The incorporation of carboxylic acid groups by the reaction with citric acid, followed by chitosan addition, has shown an improvement in the properties of sponge-like products, with regard to its elasticity, softness, durability and high porosity [96].

The use of chitosan microparticles to reinforce cellulose biocomposites prepared by lyophilization has been studied, researchers found a more uniform pore size distribution, additionally increasing the chitosan concentration from 0.0 to 1.0% improves the sponge's resistance to breakage, also an antibacterial behavior against *S. aureus* and *E. coli* is exhibited [142].

Xyloglucans show no activity as a bacterial growth inhibitor. In contrast, chitosan exhibits activity against a broad spectrum of microorganisms with absence and absence of hemolytic activity, so it can be potentially applied in the health industry [101].

Assessments of antimicrobial activity of xyloglucan hydrogels from *Tamarindus indica* seeds and chitosan did not show growth of microorganisms on nutrient agar plates exposed to air pollution [128].

Studies on the biological properties of hemicellulose obtained *Prunus amygdalus* showed that it could be a promising component to replace synthetic antioxidants [123].

Some alternatives for the conjugates preparation involve the xyloglucan dissolution in chitosan solutions in oil bath for the heat reaction [60]. Other published methods involve the dissolution of hemicellulose xylan, chitooligomers and glucosamine hydrochloride in distilled water, after adjusting the pH with NaOH (1 M) in an oil bath at 100°C for 4 h [143]. The previous oxidation of xyloglucan with sodium periodate has been proposed for the preparation of complex with chitosan to form hydrogels [128]. In other investigations, the addition of hemicellulose to chitosan solutions is carried out in a water bath at 60°C during the preparation of conjugates [2].

Although it is known that cellulose by itself does not possess antimicrobial activity to prevent infection in wounds [81]. Cellulose and its derivatives have been used extensively in combination with chitosan for the preparation of new materials with antimicrobial activity. Some studies report that cellulose and chitosan can be bound by intermolecular inclusion interaction and also based on their antimicrobial capacity against *Escherichia coli* (Gram−) and *Staphylococcus aureus* (Gram+), such materials can be used as wound coverings, due to their potential to prevent excessive dehydration and wound infection [95]. Similar studies on the antimicrobial activity of xyloglucan-chitosan hydrogels exposed to atmospheric contamination showed no growth of microorganisms on nutritive agar [128]. Studies on the characterization of chitosan microparticles to reinforce cellulose biocomposites showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* with an average zone of inhibition>2 mm and an inhibition rate greater than 80% [142].

3.2. Interaction between functional groups

In hydrogels based on polysaccharides the term “cruising zone” is used to describe crosslinking, because each aggregate involves molecular chains in the form of helices. Generally, the helices are united by non-covalent bonds such as hydrogen bonds, hydrophobic interactions, ionic bonds, etc. [128].

The molecular interaction of both polymers, chitosan and xyloglucan, results in the improvement of hydrogel properties. It is known that cellulose derivatives can act as reinforcement to improve the mechanical and thermal barrier properties. Chitosan by itself has no mechanical and barrier properties [95]. Some factors that influence the combination of both polymers for the formulation of hydrogels is the order of the addition, the concentration and molecular weight of both polymers, temperature, pH and ionic strength of the medium in which they are immersed.

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The process for the formation of hydrogels from chitosan and xyloglucan involves the previous dissolution of chitosan in acid solutions, and xyloglucan in distilled water at elevated temperatures and under continuous agitation to prepare the mixtures of both solutions [121]. Other proposed methods involve the oxidation of xyloglucan with periodate prior to the mixing of both polymers [128].

The molecular structure from chitosan and the main chain of xyloglucans are very similar, the difference is the functional group bonded to carbon two in both carbohydrate. Recent research has reported that the addition of hemicelluloses in chitosan polymer biomaterials increases crystallinity and water retention capacity, especially at low pH [135].

Crystallinity occurs because the hemicellulose is capable of interacting with the bonds formed by the chitosan molecules. The ability to retain water is improved by increasing the concentration of hemicellulose increases the amount of hydrogen bonds, which favors the absorption of water. A graphical representation of the physical interaction between chitosan and xyloglucan is shown in **Figure 3**.

Studies on the characterization of hemicellulose and chitosan hydrogels report that the FTIR analyzes showed deformation in the amide II bands and stretching between the OH and NH groups. This is due to the intermolecular interactions between hemicellulose and chitosan, this could be attributed to events such as hydrogen bonds and hydrophobic attractions. Some ionic

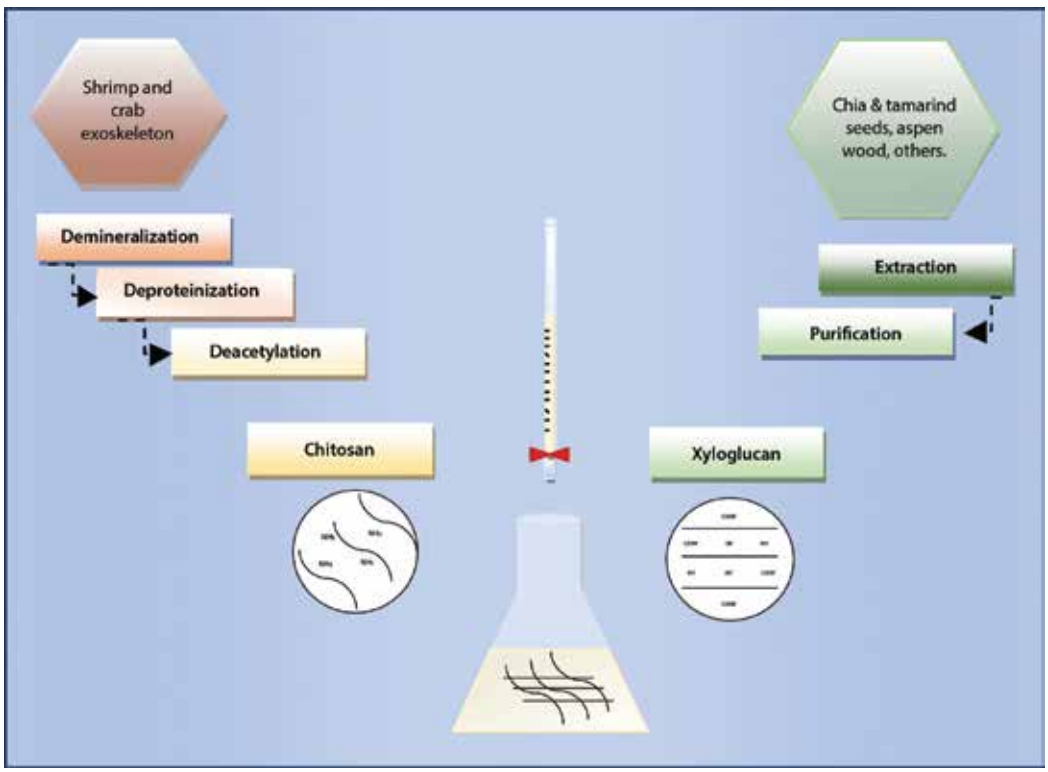

Figure 3. Graphical representation of physicochemical treatments involved in chitosan and xyloglucan extractions.

interactions could take place between carboxyl groups in hemicelluloses and free amino groups in chitosan, although the presence of carboxyl groups in hemicellulose is relatively low [121, 139, 144].

In research on the properties of hemicellulose hydrogels from aspen wood and their interactions with chitosan solutions, it is concluded that the stability of films and hydrogels formation is attributed to the crystalline arrangements and electrostatic interactions of the acidic groups in the hemicellulose and the amino groups in the chitosan [120].

Hydrogels are capable of transforming into a variety of physical forms including slabs, membranes, waits, microspheres, microgels, nanoparticles and porous materials once they have been lyophilized [81]. The biological properties of chitosan and xyloglucan allow the formation of conjugates that transformed into sponges by the process of lyophilization allow obtaining flexible, porous materials and with ability to absorb large amounts of physiological fluids. Properties that make them suitable for biomedical applications in the preparation of coatings [150].

3.3. Mechanical stability

A biocomposite for the restoration of biological tissue based on repair and/or regeneration strategies must meet criteria such as: (a) force to resist application manipulation, (b) biocompatibility with natural polymers, (c) defined structure at the micro-molecular and macromolecular levels and (d) deformation recovery capacity without fracture [74].

Researchers have proposed numerous strategies to promote the chitosan stability and the materials based on this compound. The stability of the chitosan mixtures depends on specific interactions such as hydrogen bonds, ionic bonds, dipole interference; finally, the final properties depend on the miscibility of its components [95]. The addition of plasticizers is necessary to improve the mechanical and permeability properties of some polymer matrices. Such properties could be attributed to the lubricating action to reduce the frictional forces between the chains of the polymers [135].

Plasticizers are usually small molecules that have been employed to increase flexibility and improve the handling of polymeric films. Among plasticizers, glycerol is one of the most widely applied in the films elaboration. It has been successfully introduced in the production of films based on polysaccharides. Features such as water solubility, polarity, non-volatility, and low molecular weight have converted glycerol into a plasticizer compatible with water-soluble polymers (Dick *et al*. 2015).

The addition of 20% of glycerol to the chitosan films causes the reduction in the tension modulus and the increase in the values of elongation at break [19]. The addition of sorbitol (20–30%) in combination with XG results in a considerable thermal stability superior than that of other plastics, which have disadvantages by evaporation or decomposition at high temperatures [110].

Researchers implemented a methodology for the hydrogel elaboration with a high degree of resistance, through the combination of extracts of bamboo hemicellulose (*Phyllostachys pubescens*), polyvinyl alcohol (PVA), and chitin nano-cylinders, this methodology allows to associate their

molecular chains through physical cross-linking. The polymer segments guarantee the connectivity around the porous membrane, while the water fills the pores and acts as a swelling agent forming hydrogen bonds between the hydroxyl groups of the three polymers [145].

Investigations suggest that the mechanical properties of microporous membranes based on chitosan and hemicellulose are related to the concentration of the cross-linking agent and the reaction temperature, because the increase in the concentration of epichlorohydrin (ECH) during the preparation of membranes results in an increase in tensile strength [2].

3.4. *In vivo* and *in vitro* assays

Skin lesions are a common problem affecting the world's population. Traditionally, the normal healing process is divided into several systematic, and coordinated [2] but overlapping phases: hemostasis/coagulation, inflammation, proliferation (granulation tissue formation), re-epithelialization and remodeling [146, 147]. Usually wounds are classified as wounds from trauma, abrasion or secondary events, wounds without tissue loss, and wounds with tissue loss such as burns [148].

Burn wounds are one of the most complex and painful conditions to treat and handle [56]. These lesions are highly susceptible to infection mainly due to the deterioration of the skin, which acts as a protective barrier against microorganisms [54].

The safety and effectiveness of medical devices made of resorbable biomaterials depends largely on its complete biocompatibility [149]. That is, the tissues and the human body accept the implantable material completely and do not provoke a massive immune response as a result of a threatening material.

3.4.1. Mixture of both polymers

Guan *et al*. [2] carried out tests on cell viability with films made of hemicellulose—chitosan, and found to exhibit drug-loading capability, non-toxicity, and good compatibility for wound healing application. In addition, the films showed good mechanical properties, uniform porous structure and adequate optical transparency. The trial demonstrates the potential use of natural polymers for the production of films with future perspectives in the biomedical area.

Bush *et al*. [140] proposed the addition of xylan to chitosan hydrogels to improve restoration of bone fractures in mice. The hydrogels were injected into the site of the lesion. The differences between treatments of chitosan and chitosan with xylan were presented between the 3rd and 4th week. Chitosan hydrogels added with xylan revealed improvement in healing and reduction in fracture size. The untreated fracture was maintained without union after 6 weeks. The above demonstrates that the composite is capable of accelerating the normal healing process in severe wounds, without the need to add growth factors.

3.4.2. Without xyloglucan

One of the essential features that is considered for medical devices is the biocompatibility with the host cells, the anterior is to reduce collateral damage. Biomaterials must accomplish specific requirements in relation to their interactions with the blood elements, thus the materials should

not induce coagulation or thrombus formation. Studies on hemocompatibility have established that the lower the value of the hemolysis ratio, the better the biomaterial compatibility with blood. Some authors have reported that a value of up to 5% hemolysis is permissible for biomaterials. Investigations on the in vitro evaluation of chitosan-based nano-materials have shown hemolysis values at 1.14% after 60 min of the material contact with the blood [58].

Investigations in relation to the homeostatic mechanism of biomaterials, through the reaction with erythrocytes, have compared and tested the conventional dressing gauze, polyurethane sponges, chitosan absorbents and aqueous chitosan solution [150]. Experimental studies showed a change in the shape of erythrocytes in contact with conventional gauze and polyurethane sponges, this can be attributed to the morphological changes of the red blood cells by external stimuli; however, no aggregation was observed. Nevertheless, when the erythrocytes were in contact with the porous chitosan sponges showed deformation and its incorporation on the surface of the chitosan sponges, as a result of a hemostatic interaction. The coagulation of blood cells by the chitosan action could be the result of the interaction of the polymer positive charges with the receptors on the cell surface of the erythrocytes.

Lysozyme is present in the human body as body fluid and in tissues, and hydrolyzes the glycosidic bonds (1–4) of chitin and some peptidoglycans. Studies on the *in vitro* biodegradation of chitosan: gelatin dressings with lysozyme, suggested a total incubation time of 8 days at 37°C [151]. Researchers observed biodegradation of the prototype by 28% after day 8, through the evident destruction of the structure. Studies on the enzymatic activity in lyophilized chitosan-collagen hydrogels exhibited a dynamic degradation from day 14 [152], the anterior could be attributed to the hydrophilicity in sponges that consequently causes the biodegradation and cleavage of the bonds after the swelling of the matrix.

Burns can be classified according to their degree of severity as wounds with loss of tissue and wounds without loss of tissue. To evaluate the curative activity in second degree burns, chitosan coatings were used in rat assays. The coating was replaced every 2 days in the inflammatory phase, 3–4 days in the proliferative phase and every 4–7 days during the maturation phase [153]. It was observed that 90% of the burn healing was reached between the 9th and 12th day. Similar results were obtained in a study where chitosan dressings were loaded with antibiotics. A reduction in wound size was observed between 80 and 90%, with no significant difference on the 15th day after wound induction [154].

To evaluate the efficiency in the quality of cicatrization, female pigs were used for the application of chitosan hydrogel as a coating for third degree burns. Dermal-epidermal reconstruction and re-epithelialization of the affected area were observed without irritation or noxious effects. After 10 months of the cutaneous condition [155], it was observed that the quality of healing, especially in thickness, was better with chitosan hydrogels than with commercial gauze.

Histological observations on the effect of chitosan, heparin and mixtures of both on partial depth burns in adult rats, indicate that burns with local application of chitosan powder are much less severe than control wounds [156]. It also was observed that the mixture of chitosan and heparin inhibited the inflammatory reaction.

Studies on the speed and effectiveness of second degree burns healing in rabbits using chitosan gel, microscopically confirmed the acceleration of wound healing on the 20th day,

as a consequence of the epidermal cells proliferation with complete re-epithelialization of the affected area [157].

Clinical studies reported on the healing of human wounds with chitosan-based materials are the result of previous observations in animals. The use of chitosan membranes to cover fresh wounds as a result of a skin graft donor site [53], reported that the wound adherence was uniform, which is a requirement for a successful biomaterial. It was concluded that chitosan membranes promote hemostasis, healing and rapid re-epithelialization of the affected area, transforming it into new, healthy and esthetically acceptable tissue.

Studies on the development and characterization of chitosan-based microparticles added with *Aloe Vera* and vitamin E, incorporated into a gel for the burns treatment showed mucoadhesive properties influenced by the presence of chitosan. A high degree of re-epithelialization was found after 14 days of treatment [56].

Researchers proposed combining sodium alginate, compatible biopolymer, with chitosan for the films production, this with the aim of improving the skin burn healing in rats as a research model. They concluded that the combination of low level laser therapy with a film based on chitosan and alginate improves the healing process, specifically with respect to re-epithelialization and supply of blood vessels [134].

Chitosan hydrogels with gelatin and honey have been used as coatings for wounds caused by second degree burns in rabbits as an experimental unit. A positive synergistic effect was found with the chitosan and honey mixture in terms of antibacterial activity. Thus, the primary objective in the treatment of burns is achieved, which involves preventing infection and acting as an effective promoter of wound healing caused by burns [45].

Magnesium meets various characteristics to be used as a candidate for applications in biodegradable implants. However, some studies have shown that the rate of magnesium corrosion is too rapid in body fluid to meet medical application characteristics. To counteract the effect, researchers have applied chitosan as an effective corrosion resistant coating, reducing the hydrogen released by the corrosion of the magnesium matrix [158].

Researchers developed nanoparticles of encapsulated insulin as an oral delivery system, which were administered in diabetic rats. The study concluded that oral administration of nanoparticles could be a promising tool to counteract adverse reactions, which are associated with subcutaneous insulin application [159].

3.4.3. Without chitosan

The effectiveness of a polymeric biomaterial for cell restoration depends on the binding of the cells to their surface. In evaluations of in vitro cell adhesion of animal fibroblasts on films with xyloglucan, values of 83% in cell adhesion to membranes have been reported [160]. Due to the anterior, the cell adhesion of the coatings determines its potential biotechnological application in biocompatible wounds.

Recent investigations have focused on the incorporation of growth factors (FGF-18) into injectable xyloglucan gels to promote cartilage reconstruction [161]. These authors report that the porosity and mechanical properties of the gels are highly dependent on the concentration of

the polymer, in addition, they exhibit a weight loss between 15 and 20%, followed by a slow disintegration, with increase in rheological properties and porosity. Also, they demonstrated that growth factors are not released by the gel, so that the uncontrolled growth of cartilage in healthy areas is avoided. The cell viability of chondrocytes in xyloglucans with growth promoters involved a suitable environment to grow and proliferate.

In vitro studies with 3% (w/v) xyloglucan covered nanocomposites for the transport of drugs (Enalaprilate) through the gastrointestinal tract, report that these formulations could be used for slow release of drugs when needed in other regions of the gastrointestinal tract [141].

Researchers developed and evaluated xyloglucan-based ocular films as a possible antibiotic-releaser agent such as ciprofloxacin. They performed an eye irritation test on rabbits, in order to determine their ability to cause damage to the cornea, and an acceptable tolerance was reported, with no redness or inflammation. Therefore, ocular administration of xyloglucan is suggested because of the potential absence of irritation [162].

In studies on the biological characterization of xyloglucan extracted from seeds of *Hymenaea courbaril var.*, an evaluation was made on its hemolytic activity. In the essay, the red blood cells were diluted with saline solution to a 1% (v/v) suspension, then was mixed with a xyloglucan solution. The researchers concluded that the polysaccharide has no hemolytic activity, verifying its potential application in the health industry [101].

Tests on the application of cellulose membranes in patients with burn injuries showed that cellulose offers advantages over conventional treatments such as the use of gauze with Vaseline. Total re-epithelialization occurred in about 7 days [163]. The rate of re-epithelialization is closely related to the age of the patient. The healing process slows down with aging.

Some other important properties of xyloglucan include non-carcinogenicity, mucoadhesivity, biocompatibility and high thermal stability. Therefore, researchers have used it for the preparation of microspheres and the encapsulation of anti-asthmatic agents. *In vitro* pulmonary pharmacokinetic evaluation indicated the potential use of xyloglucan as a release of anti-asthmatic agents through the pulmonary route [164].

4. Conclusion

The review presented in this chapter shows studies on the effectiveness of biomaterials for medical application based on chitosan and xyloglucan, promising results for application in tissue restoration are reported because of their multiple biological properties. Xyloglucans of various sources are defined as a promising biopolymer for biomedical purposes because of their ability to form gels, biocompatibility, adhesiveness, non-carcinogenicity and compatibility with blood. On the other hand, chitosan has remarkable characteristics such as biocompatibility, biodegradability, non-toxicity, bioadhesivity, antigenic capacity and hemostasis. According to the published *in vivo* and *in vitro* tests, both polymers could be used for the hydrogels preparation and their application for the wounds with a high level of dehydration to improve their ability to re-epithelialize. In addition, the interaction of xyloglucan with chitosan confers on these biomaterials the antimicrobial capacity over a wide range of pathogenic microorganisms that cause

infection. Additionally, the introduction of xyloglucan favors the characteristics of fluid absorption and mechanical resistance in chitosan hydrogels.

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Chapter 11

An Overview of Chitosan-Xanthan Gum Matrices as Controlled Release Drug Carriers

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Additional information is available at the end of the chapter

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Abstract

Naturally occurring polysaccharides and/or their chemically modified derivatives have been widely investigated in relation to their use as components of controlled release systems for drug delivery. The aforementioned is due, in part, to their distinct properties such as abundant availability and biocompatibility as well as environmental and economic advantages. Chitosan (CS) and xanthan gum (XG) based matrices have received growing scientific/pharmaceutical interest as oral controlled release drug carriers. Herein, recent advances spanning the last two decades in CS-XG based drug delivery systems are reviewed with the emphasis being on oral tablet formulations, due to their versatility as pharmaceutical dosage forms. The mechanism of interaction between CS and XG, by means of computational and experimental approaches, is scrutinized. Results obtained from the literature establish the possibility of fabricating a controlled release drug delivery system based on CS and XG matrices. This can be achieved by monitoring and manipulating the physiochemical properties of the two polymers as well as the experimental variables affecting their drug retardation efficiency, without the need to employ special equipment or sophisticated experimental techniques/methodologies.

Keywords: drug delivery, controlled release, polymeric matrices, natural polysaccharides, xanthan gum, chitosan, polyelectrolyte complexes, molecular dynamics simulation

1. Introduction

The ultimate goal in drug design and development is to optimize a carrier that ensures the delivery of the active pharmaceutical ingredient(s) (APIs) to the systemic circulation in a safe

and stable manner [1]. Patient compliance is a key aspect to consider when designing a new pharmaceutical dosage form [2]. Therefore, the way the drug will be introduced to the body should be optimized to ensure the availability of the drug at its site of action, at levels within the range of its therapeutic window (**Figure 1**).

Despite emerging advances in drug delivery, the oral route remains the predominant route of drug administration. It is the simplest route, non-invasive and provides ~200 m^2 of readily available surface area for drug absorption [3]. Conventional oral dosage forms usually release drugs immediately in the body, via first order release kinetics for both absorption and elimination processes [4]. Since the efficacy of the administered drug is limited to its residence time in plasma, frequent administration is required for APIs which exhibit a short biological half-life. As a result, low patient compliance and high fluctuation of drug levels in plasma is expected [5, 6]. In order to counter the foregoing drawbacks of conventional dosage forms, a new term in drug delivery was introduced; modified release dosage forms [7].

The United States Pharmacopeia defines modified release tablets as "coated or uncoated tablets that contain special excipients or are prepared by special procedures, or both, designed to modify the rate, the place or the time at which the active substance(s) are released". Modified release delivery systems can be divided into delayed release systems and prolonged/extended release systems. Extended release delivery systems can further be subdivided into sustained and controlled release delivery systems, which differ in the rate at which they deliver APIs to the human blood circulation. Sustained release formulations function by continuously releasing APIs for a prolonged period of time. On the other hand, controlled release (CR) delivery systems do not only retard the release of the drug, but they deliver the drug to the body at a predetermined release rate or location [8]. Consequently, constant drug levels can be achieved (**Figure 2**).

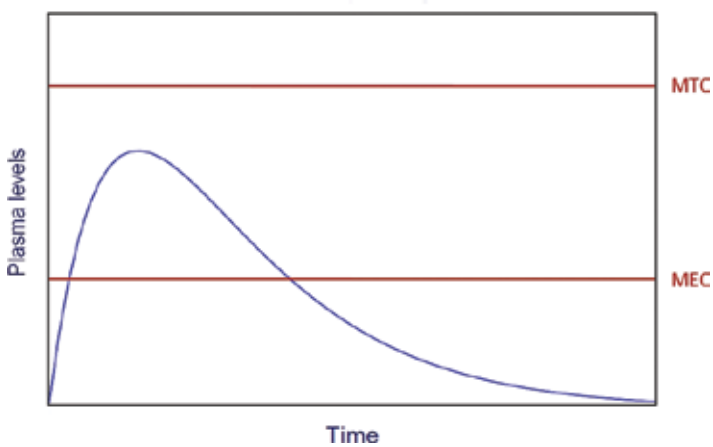

Figure 1. Plasma levels and therapeutic range of a drug following an oral administration of a single dose. MTC: minimum toxic concentration, and MEC: minimum effective concentration.

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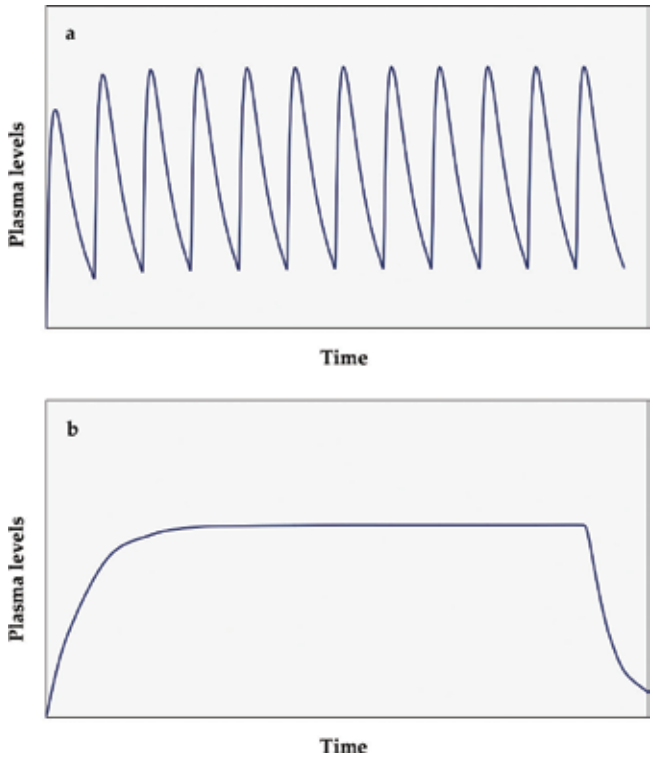

Figure 2. Comparison of plasma concentration-time profiles of drug release following multiple dosing from: (a) conventional, and (b) controlled-release dosage forms.

2. Controlled drug delivery

CR systems are composed of inactive pharmaceutical ingredient(s) that entrap the API(s) and release it/them at a time different from the immediate release form [4]. Researchers in the field of drug delivery have, and are currently still trying to acquire a better understanding of CR by attempting to integrate pharmaceutical technology with the relevant pharmacokinetic parameters associated with different drugs [9]. The rational underpinning controlled drug release includes, but are not limited to: masking the undesired side effects of drugs, attaining a constant drug release profile with minimal drug level fluctuations, and enhancing patient convenience by reducing administration frequency [10]. CR dosage forms are not only capable of extending the time over which drugs are released and providing constant drug levels but also with the potential of protecting therapeutic biomolecules such as peptides and proteins from enzymatic degradation in the gastrointestinal tract (GIT) [11]. CR systems can also be formulated to target the delivery of APIs to the desired site of action [12, 13].

Aside from the substantial need for CR formulations in drug delivery and the potential advantages they offer, the reproducibility and cost of equipment and techniques needed for the preparation of CR dosage forms on a large scale present a major obstacle towards the

widespread production of CR delivery systems in pharmaceutical manufacturing. **Figure 3** summarizes the key factors which require to be taken into account when optimizing a new CR dosage form.

2.1. Design of CR systems

2.1.1. APIs

There are several criteria and properties that should be taken into consideration in the proposed use of an API when designing a controlled release formulation [14, 15].

- The elimination half-life of the drug should be short. Drugs with long half-lives, greater than 8 h, provide a sustained release profile without the need to be formulated in a controlled release system.
- Drugs with a wide therapeutic window are better candidates since higher doses need to be incorporated in CR formulations and dose dumping could occur.
- The absorption rate of a candidate drug should be high to make sure that the release of drug from the CR delivery system is the rate determining step, not the absorption rate.
- Drugs which exhibit high protein binding are retained in the plasma for a long time; thus, they do not require a CR delivery system.
- Drugs that undergo extensive first pass metabolism are poor candidates for CR, since releasing the drug at lower rates will decrease its bioavailability. APIs with a bioavailability index higher than 75% are preferable.

Figure 3. Key factors to be considered when developing a new dosage form in the pharmaceutical industry.

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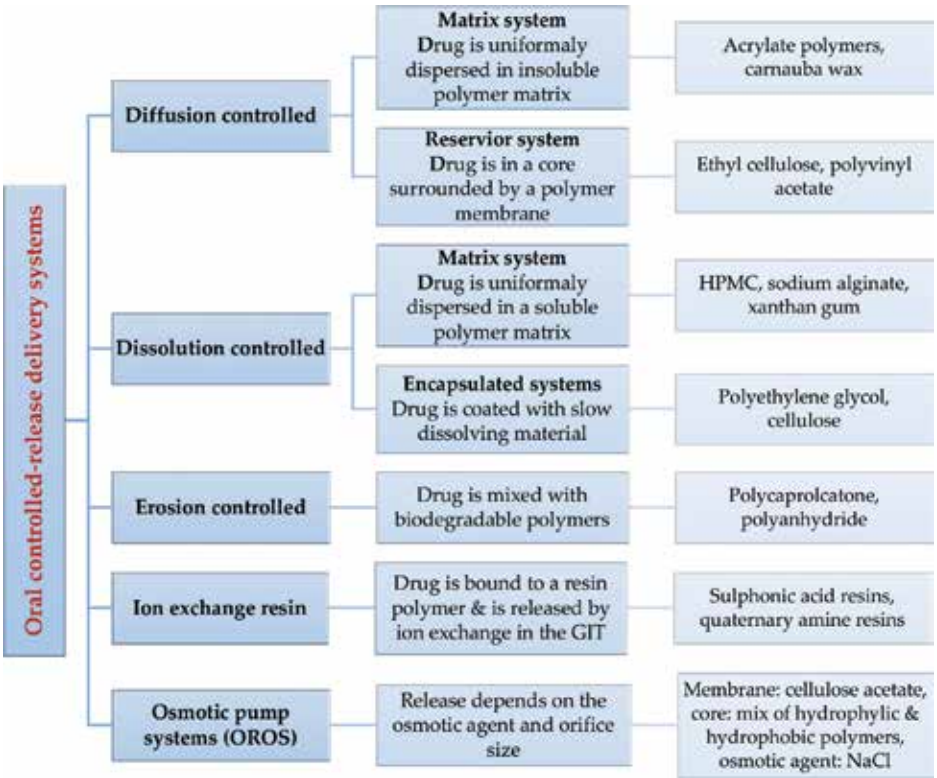

Figure 4. Classification of controlled release drug delivery systems combined with the main mechanisms involved in drug release and examples of inactive ingredients used to achieve CR.

Model	Equation	Mechanism of release
Zero order	$Q_t = Q_0 + K_0 t$	Release is independent of drug concentration within the matrix or device
First order	$\text{Ln}\, Q_t = \text{Ln}\, Q_0 + Kt$	Release is dependent on drug concentration within the matrix or device
Higuchi	$Q_t = K_H t^{1/2}$	Drug released via diffusion through an insoluble polymeric matrix
Hixon-Crowel	$Q_0^{1/3} - Q_t^{1/3} = K_{HC} t$	Drug release is dependent on drug dissolution rate in the media
Korsmeyer-Peppas	$Q_t/Q = K t^n$	This model is used when several mechanisms are involved in drug release from the system

*Where Q_0 is the initial amount of drug in the dissolution media, Q_t is the fraction released at time t, K is the rate release constant, and n is the release exponent.

Table 1. Mathematical models of drug release kinetics from CR formulations*.

2.1.2. Carriers and mechanism of drug release

Controlled drug release can be achieved by utilizing special techniques and devices. As the release of a drug from the delivery system is the rate limiting step in controlled release formulations, CR systems are classified according to the mechanism involved in drug release [3, 16].

In some preparations, more than one mechanism can be involved in the release of the API(s) from the CR systems (**Figure 4**).

2.1.3. In-vitro drug release kinetics

Since the objective of utilizing CR systems is to deliver a drug, or drugs, over a known time interval, several mathematical models (**Table 1**) have been suggested to describe drug release from the systems as a function of time.

3. Natural polysaccharides

Polymers are the most used materials to control the release of APIs. They can be classified as synthetic (silicons, polyesters and cellulose derivatives) and natural polymers (proteins and polysaccharides). Many naturally occurring polymers are inert, biodegradable, and cost-effective in relation to their industrial use [17]. In addition, their chemical structure can usually be easily modified to achieve the desired properties for a specific purpose [18]. Hence, the utilization of natural polymers as components of drug vehicles is gaining extensive attention [3]. The most used polymers are saccharides (carrageenan, cellulose) or proteins (collagen, gelatin) [19].

Natural polysaccharides are hydrophilic polymers consisting of repeating monosaccharide units linked via glycosidic bonds [20]. They are obtained from various sources, mainly vegetal (cellulose, starch), microbes (xanthan gum, dextran), crustaceans (chitin) and algae (alginate, carrageenan) [21, 22]. Depending on the identity of the constituent monomer(s), polysaccharides can be divided into homo-polysaccharides which are composed of the same repeating unit, such as cellulose, or hetero-polysaccharides which are built up from different saccharide units e.g., CS and XG [23, 24]. They can also be classified according to their ionic charge: non-polyelectrolyte (starch, cellulose), and polyelectrolyte polysaccharides. Polyelectrolytes are further sub-divided into negatively charged polymers; such as alginate and XG, or positively charged polymers, which are few in number, such as CS [25, 26].

The unique physicochemical characteristics of each polysaccharide are related to the type of monosaccharide building unit, position of the glycosidic bond, chain substitution and the overall molecular weight [25, 27]. Due to the presence of various functional groups attached to the polymer backbone (carboxyl ─COOH, amine ─NH2 and hydroxyl groups ─OH), polysaccharides have the ability to form non-covalent bonds with a wide range of synthetic and biological molecules [28, 29]. Moreover, they can attach to body tissues and mucus layers and sustain the release of encapsulated active ingredients [13]. The aforementioned properties have attracted attention towards the usage of polysaccharides in major industries including food, agronomy, cosmetics, biochemical engineering and pharmaceutical manufacturing [30, 31].

3.1. Chitosan (CS)

CS is a linear polysaccharide produced by the *N*-deacetylation of chitin [32]. Chitin is found mainly in the exoskeleton of marine crustaceans as well as insects and fungi [33]. Glucosamine and *N*-acetyl glucosamine are the building units of CS. They are linked via β(1-4)glycosidic bonds (**Figure 5a**). The degree of acetylation and distribution of acetyl groups along the polymer

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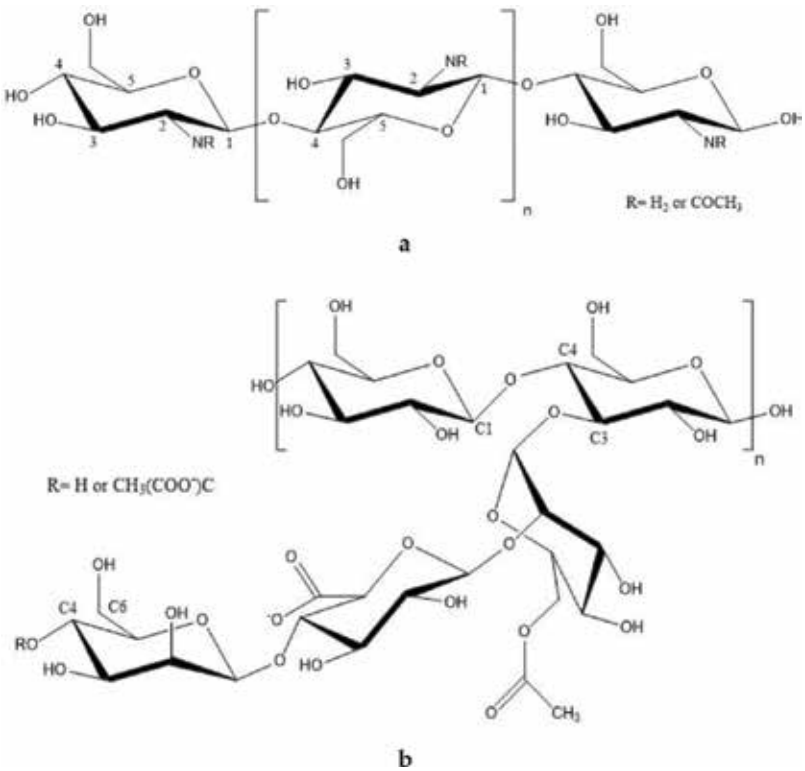

Figure 5. Schematic chemical structures of the building units of: (a) CS and (b) XG.

chain (either block or random distribution) are dependent on the duration of the deacetylation process and preparation method for CS [34, 35]. Following deacetylation of chitin, CS is (unlike chitin) soluble in acidic media. Moreover, the presence of primary amine groups leads to the unique properties of CS over all other natural polysaccharides [36]. It is the only saccharide possessing a high density positive net charge, which allows it to interact with a wide range of anionic polymers and biological molecules [32]. In addition, CS shows high mucoadhesion in the GIT which increases the residence time and enhances the permeation of active molecules [30]. Hence, CS is used commonly in the food industry, for pharmaceutical drug delivery and tissue engineering [37, 38].

3.2. Xanthan gum (XG)

XG is a branched, hetero-polysaccharide produced via microbial fermentation of the microorganism *Xanthomonas campestris* [39]. The primary unit of XG (**Figure 5b**) consists of a cellulosic backbone composed of two D-glucose units (1-4) β-linked to a side-chain of D-mannose and D-glucuronic acid units at a ratio of 2:1, respectively [40]. D-Mannose, which is connected to the main backbone, is attached to an acetyl group at O6, while approximately half of the terminal D-mannose forms a pyruvic acid group between carbons C4 and C6. This side-chain is found at the O3 atom of each alternate glucose unit on the backbone. Due to the presence of carboxylic groups in its structure, XG exhibits a net negative charge and can form complexes

with cationic polymers [41]. In the last decade, the demand for XG, in industry, has been increasing at about 5–10% per annum [42]. It is used in a broad variety of industries, including cosmetics, agriculture, food, textiles and oil [43, 44]. This is due to its safety (non-toxic), desirable rheological properties, high stability over a wide range of pH and temperature, together with its high resistance against enzymatic degradation [45, 46].

4. CS and XG matrices as controlled release drug delivery systems

4.1. Advances and applications

Matrix systems, based on polyelectrolyte polysaccharides, used to retard the release of APIs have been reported in the literature and some of them have been commercialized [29, 47]. The long term instability of their corresponding preparations due to the existence of charged groups limits their application in pharmaceutical manufacturing [48]. Introducing a cross-linker, such as tripolyphosphate or glutaraldehyde, to neutralize the polymeric matrix is a necessary approach to confront such a shortcoming. Though, substituting the cross-linker with an oppositely charged copolymer aids and abbets the synergistic effect of drug release retardation. XG proved to be a potential CR drug carrier. In aqueous solutions, XG shows high viscosity and water uptake capacity encapsulating the drug inside a thick gel-like layer which hinders the release of the incorporated drug. XG has been used alone and with other polymers such as HPMC, karaya gum, guar gum, and polyvinylpyrrolidone (PVP), or ethyl cellulose [49–53]. Formulated matrices were able to sustain the release of caffeine, azithromycin, ibuprofen and propranolol HCl. XG demonstrates a high capability of generating a near zero drug release profile.

Being the only known positively charged natural polymer in aqueous solutions, CS has been extensively investigated as a potential drug vehicle. CS has the ability to preserve the stability of active biomolecules, namely insulin, and enhance their absorption from the GIT [54–56]. CS was mixed with various polymers with the aim of modifying the release of active ingredients, protect genes and therapeutic peptides in the GIT and improve their permeation across the intestinal epithelium and to immobilize antibodies [57–59]. Alginate, carrageenan, pectin, hyaluronic acid and XG are amongst the many natural polymers to be used with CS [47, 60, 61].

The combination of CS and XG was first used in the form of a polyelectrolyte complex (PEC) hydrogel [62]. The hydrogels formed displayed pH dependent swelling behavior and addressed the possibility of developing a gastrointestinal drug delivery system. PECs are formed due to the attractive ionic forces between the positively charged amino groups in CS and the negatively charged carboxyl groups in XG [63]. Therefore, features of the PECs produced can be controlled by manipulating the physicochemical properties of each polymer [64]. Molecular weight, degree of acetylation (DA) of CS, and pyruvic acid content in XG are amongst the most crucial factors to be addressed [47, 65, 66]. Complexation conditions (including concentration of each polymer, mixing ratios, and pH) have a significant influence on the behavior and stability of the resulting PEC [67]. The combination of CS and XG has been extensively studied as a platform for CR drug delivery, resulting in many patents and the publication of research articles.

4.1.1. Patents on CS-XG based controlled release drug delivery system

CS-XG hydrogels have been studied in order to immobilize biological materials. This is due to their insolubility and high stability in acidic medium allowing the system to preserve biological activity and release the materials at neutral pH. CS-XG hydrogels served as a promising candidate for sustained release dosage forms [68]. CS-XG hydrogels were capable of stabilizing and controlling the release of highly sensitive active ingredients such as vitamins, amino acids, nucleic acids and polypeptides when applied topically or orally as dietary supplements [69]. Moreover, the prepared hydrogels were shown to play a role in regulating the dissolution rate of poorly water-soluble drugs as disclosed by the patent WO 2002003962 where fenofibrate, ursodeoxycholic acid, nifedipine and indomethacin were used as models of poorly water-soluble APIs [70].

Tablets comprising CS and XG as a hydrophilic matrix for oral controlled release were first presented by Badwan et al. [71]. A wide range of basic drugs were tested (e.g. ambroxol, salbutamol, metoclopramide, anti-infective, non-steroidal and anti-inflammatory agents (NSAIDs)). Tablets formulated using CS and XG have been used to deliver basic APIs in a controlled release pattern. The drug to polymer ratio used was 1:3, respectively and the preferred XG to CS ratio was 1:1. When the system was studied in-vivo on human volunteers, it produced constant serum levels of ambroxol over a period of 24 h. This study paved the way for further research on approaches and mechanisms involving tablet formulations based on the foregoing combination. A tablet dosage form based on CS-XG for the treatment of hypercholesterolemia was prepared [72]. A combination of lycopene and *Monascus purpureus* were used as active ingredients. When testing the preparation on human volunteers, a significant decrease in the plasma levels of cholesterol, LDL and triglycerides was reported. Moreover, HDL values were reported to be increased.

4.1.2. Research articles on CS-XG based controlled release drug delivery system

The applicability of CS-XG combinations to a wide range of dosage forms with different routes of administration has been investigated. Examples of the preparations reported in the literature together with a brief description of preparation methods, application and examples of incorporated APIs are summarized in **Table 2**.

4.2. Ionic interaction between XG and CS

In spite of the significant amount of research work conducted on XG and CS based matrices, a lack of understanding of the nature of the interaction between the two polymers and their behavior at the molecular level still exists. It was suggested that physico-chemical conditions in the stomach are an ideal environment for the formation of insoluble gels between the two polymers, which retards the release of APIs resulting in a sustained drug release profile [87]. Moreover, in vitro residence time evaluation on porcine mucin and in vivo studies using sheep models have addressed the bioadhesive nature of CS-XG matrices [88].

In order to acquire an understanding of the interaction between XG and CS and factors governing it, a molecular dynamics simulation (MDs) study was conducted by Dadou et al. [89]. The contribution of the DA of CS and protonation was evaluated. The resulting trajectories

Dosage form	Preparation method	Application	Incorporated ingredient
Hydrogels	Solution mixing under heat	Drug delivery, tissue engineering, immobilization of biological active materials	Probiotics [73], enzymes [64]
Films	Solution casting	Drug delivery, tissue engineering, food industry	Wound healing [74], amoxicillin [75], scaffolds [76]
Capsules	Complex coacervation, encapsulation of physically mixed powder	Drug delivery	Theophylline [77], ciprofloxacin HCl [78]
Beads	Extrusion-dripping technique, complex coacervation mechanism	Drug delivery, immobilization of biological active materials	Probiotics [79], glipizide [80], antibodies [59]
Microspheres	Spray drying, ionotropic gelation method	Drug delivery	Meclizine HCl [81]
Micro-emulsions	Homogenization with oil phase	Drug delivery	Progesterone [82]
Liposomes (chitosomes)	Thin film hydration method, spray drying	Drug delivery	Rifampicin [83]
Cryogel	Freeze-drying	Immobilization of biological active materials	Enzymes [84]
Tablets	Direct compression, granulation, hot melt extrusion	Drug delivery	Metformin HCl [85], terbutaline sulfate [48], propranolol [86]

Table 2. Main applications of CS-XG based matrices.

and binding free energy calculations revealed that electrostatic forces (polar interactions, ΔE_{ele}) are the driving force for the interaction, and that the interaction occurs regardless of the DA and state of protonation of CS (free energy values are negative for all complexes). Protonation of CS molecules increases their penetration between the branched chains of XG and produces more stable complexes with lower free binding energy (**Table 3**). Intermolecular interactions (Van der Waals) showed a positive contribution to the formation of CS-XG PECs. This can be explained by the presence of a large number of hydroxyl groups along the chains of the polymers which can induce an instantaneous dipole attraction with the surrounding atoms. High positive solvation free energy (ΔG_{solv}) values justify the resultant insoluble PECs upon complexation between CS and XG in the laboratory. ΔG_{solv} increases with protonation, reaching a maximum value when CS is fully protonated, indicating a higher extent of interaction with XG.

4.2.1. Mixing ratio

Since the interaction between CS and XG is electrostatically driven, the properties of the resultant PECs can be modified by controlling the net charge density. This can be achieved either by altering the mixing ratios or the initial concentrations of the polymeric solutions. Films of CS and XG were prepared and examined by scanning electron microscope (SEM) for additional information relating to the behavior and the interaction between the two polymers in

CS	ΔE_{ele}	ΔE_{vdW}	ΔG_{sol}	ΔG
0% P*, 0% DA	−21.290	−14.03	21.890	−13.43
50% P, 0% DA	−227.53	−24.47	222.77	−29.22
100% P, 0% DA	−419.95	−23.27	412.57	−30.65
0% P, 50% DA	−25.080	−21.12	28.460	−17.74
50% P, 50% DA	−232.68	−23.96	227.36	−29.28
0% P, 100% DA	−25.070	−25.79	30.150	−20.71

Values presented are in kcal/mol.*P represents state of protonation.

Table 3. Binding free energy calculations for XG-CS complexes.

aqueous solutions at different mixing ratios [90]. SEM images (**Figure 6**) show the rough surface of CS, whilst XG films produce a smooth surface. Combining the two polymers resulted in a pronounced alteration in the surface morphology of the films. The resulting PECs form irregular and fibrous surfaces with a porous structure. PECs at a mixing ratio of 1:1 (w/v %) showed a dramatic change in the surface structure and it is suggested that they represent the maximum interaction between the two polymers.

4.2.2. Initial concentration of XG

Argin-Soysal et al., studied the effect of polymer solution concentration on the formation of stable capsules and their subsequent swelling behavior [67]. The initial concentration of the XG solution was found to be the determining factor in relation to complexation density, more than CS. This is due its high molecular weight and the highly viscous hydrogels it forms when in contact with water [91]. The physical cross-linking between XG and CS was complete when the concentration of XG was 1.5%, regardless of other experimental conditions. Consequently, the degree of swelling was shown to be dictated by the initial aqueous concentration of XG.

4.2.3. pH and initial concentration of CS solutions

Dumitri et al., found that the pH of CS solutions has a moderate effect on the extent of interaction between XG and CS [65]. PECs where readily obtained within a wide range of pH (3.6–8.0). At lower pH values, the carboxyl groups of XG become protonated (uncharged) while the amine groups in CS are fully charged; hence, the interaction between CS and XG is

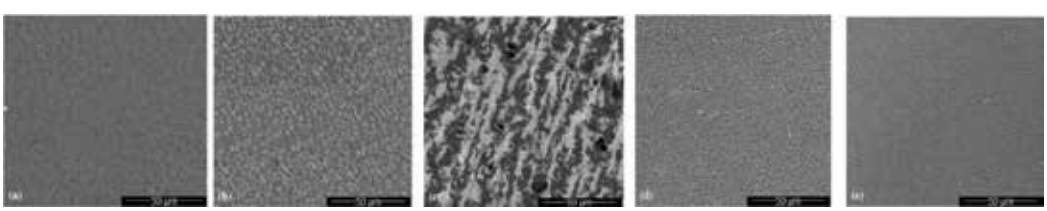

Figure 6. SEM images at magnification power of x2000 of: (a) CS, (b) CS-XG (2:1), (c) CS-XG (1:1), (d) CS-XG (1:2) and (e) XG films, from Eftaiha et al. [90].

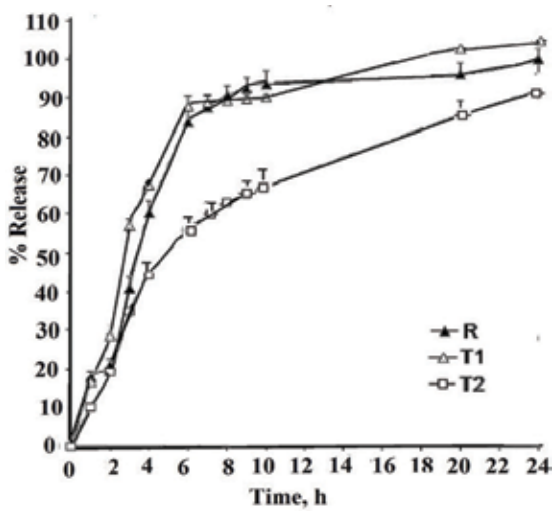

Figure 7. In-vitro release of ambroxol HCl from: (R) reference product, prepared tablets at a P:D of (T1) 1:1, and (T2) 3:1, as reported by Al Remawi et al. [94].

impeded and reduced drug retardation occurs. The effect of pH was more pronounced when preparing low concentration solutions of CS. A considerable increase in the degree of swelling with the pH of solutions at CS concentrations of 0.65–0.7% (w/v) occurs.

4.2.4. Molecular weight (Mw) of CS

The swelling capacity of CS-XG based PECs were found to be influenced by the Mw of CS [68]. Lower water retention capacity was achieved by using a higher Mw of CS. The absorption of water increased noticeably with around 1000% weight gain at lower Mw of CS. The increase in water absorption causes the formation of more PEC layers which results in potentially more drug retardation. The aforementioned claim was supported by the slow release of diclofenac sodium from low molecular weight CS tablets (13 and 30 kDa) [92]. AlAkayleh et al., found that the release rate of terbutaline sulfate from XG-low molecular weight CS tablets (viscosity 38 mPa s) was slower than XG-high molecular weight CS (70 mPa s) [48].

4.2.5. DA of CS

The PEC between XG and CS is formed due to the electrostatic attraction between oppositely charged groups. Increasing the DA content decreases the number of available free amine groups that are readily protonated. In addition, the rigidity of CS chains increased with DA owing to strong intramolecular hydrogen bonds dictated by amide groups [93]. As a consequence, the extent of interaction between the polymers is reduced. Release of propranolol HCl from an CS-XG matrix was studied as a function of the degree of deacetylation (DDA) of CS [86]. Release of drug from the matrix was faster from the acetylated form of CS. This result is in accord with the outcomes of the molecular dynamics simulation study (**Table 3**) [89].

4.2.6. Ionic strength of solution

Adding ionic species to the solution resulted in a large decrease in water uptake of CS-XG PECs. Competition takes place between free ions and water molecules for the hydroxyl groups of the polymers and reduces the hydration of CS and XG chains. Thus, the degree of swelling is lower which, in turn, will have an effect on the drug retardation capability of the system [63].

4.2.7. Concentration of incorporated API

Hydrophilic matrices need to be used at high polymer to drug ratios in order to exert their effect in sustaining the release of APIs [27]. Thus, their application is restricted to low strength drugs, as addressed by Badwan et al. [71]. Al Remawi et al. studied the effect of polymer to drug ratio (P:D) on the release of ambroxol HCl from CS-XG based tablets [94]. The release rate of ambroxol was highly dependent on the P:D ratio. Greater retardation of drug release was attained at higher polymer ratios (**Figure 7**).

5. Tablets comprising CS-XG

Oral solid dosage forms remain the most favorable choice to deliver APIs. The main reason is that they preserve the physicochemical stability of chemical entities more than liquid forms [95]. Additionally, tablets offer advantages for both manufacturers and patients which include ease of handling, low production cost, dose precision and self-administration capability [96].

Utilization of CS as an efficient excipient in tablet formulation is gradually increasing. CS powder exhibits a high surface area and porosity [93]. It produces tablets with high tensile strength that form a network-like structure when examined by microscopy [97]. The aim of using a combination of polymers, as tablet excipients, is to enhance compressibility and flowability properties. Furthermore, a polymeric mixture can increase the overall retardation performance of the system. CS-XG based tablets were formulated by compression of one layer and multi-layers; they were used solely or with other polymers such as galactomannan, seed gum or β-cyclodextrin [48, 85, 86, 98]. Moreover, they were used in immediate release, floating mucoadhesive and buccal tablets [99, 100]. According to Badwan et al., combining XG with CS has the advantage of improving the mechanical properties of both polymers [93].

5.1. Tablet preparation methods

5.1.1. Direct compression

Direct compression is a technique for formulating tablets which limits the use of solvents, temperature and equipment. It is the first choice whenever the API and inactive materials are suitable for direct compression and are stable at high pressure [101]. Powders of both active and inactive ingredients are mixed homogeneously, then sieved to the desired particle size. Finally, the prepared blend is compressed using a tablet press machine at a predetermined

pressure [102]. CS-XG based tablets prepared via direct compression, showed a high potential towards sustaining the release of terbutaline sulfate and ambroxol [48, 103].

5.1.2. Dry granulation

Dry granulation is utilized to improve compaction properties of ingredients. It can influence flowability, stability, content uniformity of the powders and enhance the bioavailability of the API. This is attained by increasing the particle size of powder materials via aggregation of particles by either roller compaction or slugging and then milling to produce granules with the desired size [104].

5.1.3. Wet granulation

Wet granulation of tablet components is usually achieved using water, ethanol or a mixture of both. Following drying at an appropriate temperature, granules are mixed with other excipients if needed, passed through a sieve and finally compressed using a press machine at a predefined pressure [105]. Wet granulation is used to produce dust free granules, enhance flowability and cohesion. Eftaiha et al., investigated the ability of CS-XG tablets prepared by wet granulation using an aqueous solution of XG 1% (w/v) to modify the release of metronidazole [87]. The preparation was able to sustain the release of metronidazole, both in-vitro and in-vivo. A mucoadhesive behavior was observed when applying the tablets on sheep duodenum.

5.1.4. Hot melt extrusion (HME)

In HME the powdered API, functional polymers and any other excipients are blended in a mortar and pestle then fed into the hopper of a single or double screw extruder. Fukuda et al., prepared CS-XG tablets using HME to study the release of chlorpheniramine maleate [106]. The processing temperature was 90°C (zone 1), 95°C (zone 2), 105°C (zone 3) and 110°C (die) with a screw speed of 15 rpm. The processing time needed for powders inside the barrel of the extruder is usually ~3–4 min. The extruded materials were then manually cut into tablets of the desired weights. Chlorpheniramine release from the prepared CS-XG tablets occurred in a sustained manner and was independent of the pH and ionic strength of the dissolution media. HME offers the advantage of continuous processing and process analytical technology (PAT) which enables quality control testing throughout the process [107].

5.2. Mechanism of drug release

Drug release from CS-XG matrices is suggested to be governed by the dissolution rate of the drug and the polymers in the media as well as the diffusion of the drug from the matrices and erosion of the polymers. The data in **Figure 8** illustrates the processes of drug release from a tablet composed of CS and XG. When the tablet is first exposed to aqueous media, an insoluble gel layer forms on the top surface of the tablet, as a result of polyelectrolyte complexation between the two charged polymers [108]. Water molecules start to penetrate this layer towards the matrix owing to the high water uptake capability of XG. [91]. Accordingly, both polymers and drug are dissolved and a rubbery hydrated region is formed (white area) [27].

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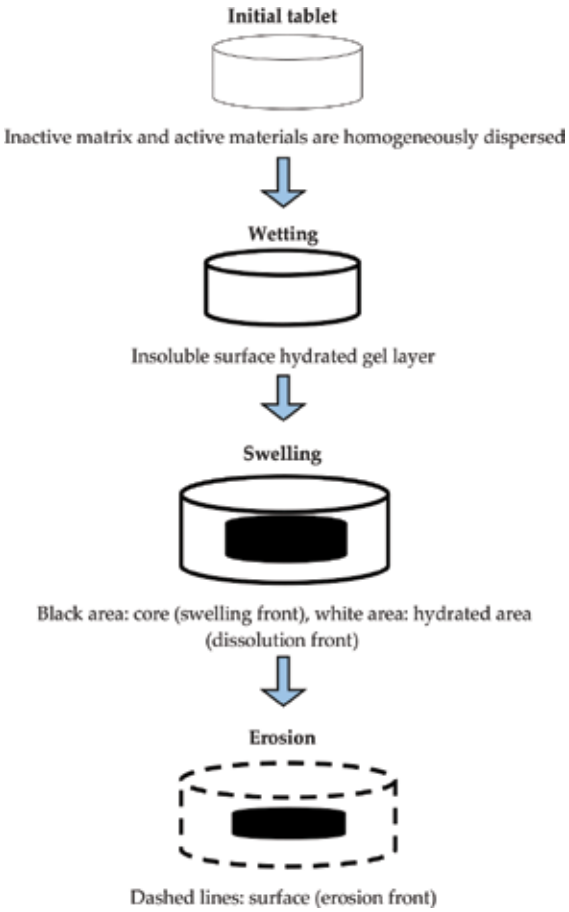

Figure 8. Schematic representation of the behavior of CS-XG tablets in an aqueous medium.

On the other hand, a non-hydrated glassy area is formed at the core of the tablet, where no water molecules reach the system (black area) [109]. As time lapses, further penetration of water molecules into the tablet occurs resulting in the polymers chains being solvated. Consequently, swelling of the matrix occurs [110]. At this stage, water molecules enter between the polymer chains, the radius of gyration of the polymers increases and the end-to-end distance of the polymer backbones also increases [111]. This phenomenon of polymer relaxation is referred to as "swelling of the matrix" [91]. As more water molecules pass into the matrix, the polymer concentration on the outer surface of the tablet decreases, losing its integrity, and starts to dissolve in the medium. This phenomenon is termed "polymer erosion" [112].

The rate of drug release from such a matrix could occur as a function of diffusion of the water molecules into the matrix, dissolution of both polymers and drug, polymer relaxation and erosion [91, 113]; this depends on the previously mentioned factors (Section 4.2) [111, 114].

6. Conclusions

Controlling the release of active ingredients is one of the fastest growing applications of CS-XG based matrices. Various drug delivery systems and newly emerging technologies have been developed in order to optimize the foregoing mixture. XG-CS matrices show a high potential towards controlling the release of a wide range of active biomolecules. The efficiency of CS-XG matrices to control the release of drugs can be reinforced by manipulating the physicochemical properties of CS and XG and the experimental conditions used. Thus, incorporation/use of expensive devices and the method of preparation can be kept to a minimum. With further optimization and the utilization of newly emerging computational and quality by design tools, relatively simple and straightforward CS-XG based matrices can be formulated as potentially universal carriers to control the release of APIs.

Conflict of interest

The authors declare no competing financial interests.

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Chapter 12

Ampicillin-Loaded Chitosan Nanoparticles for In Vitro Antimicrobial Screening on *Escherichia coli*

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Abstract

Purpose: To develop ampicillin-loaded chitosan nanoparticles by modified ionic gelation method for evaluating their antimicrobial activity onto *Escherichia coli*.

Methods: Ampicillin-loaded chitosan nanoparticles (CHT-NPs) prepared by ionic gelation method with sodium tripolyphosphate as cross-linking agent. Drug release parameters (zeta potential, particle size, entrapment efficiency, and *in vitro* drug release) were assessed in relation to CHT-NP antimicrobial profile on *E. coli*. Antibacterial properties of CHT-NP formulation with ampicillin were found better than mere ampicillin without CHT-NPs.

Results: SEM, AFM images revealed dimensions of CHT-NPs with irregularity in shape/size. Optimized concentrations of chitosan 0.5% w/v with three different ratios (0.05% to 0.3% w/v) of TPP proved optimal for the evaluation of antibacterial profile of CHT-NPs. *In vitro* ampicillin-loaded CHT-NP delivery studies revealed an initial burst followed by slow sustained drug release, demonstrating superior antimicrobial activity than plain ampicillin, due to the synergistic effect of chitosan and ampicillin.

Conclusion: Chitosan content and cross-linking agent concentrations are control factors in synthesis of the optimized CHT-NP formulation. CHT-NPs with ampicillin cargo capably sustained ampicillin delivery due to NP size and increased surface charge, resulting in efficient growth inhibition in assays with *E. coli*.

Keywords: ampicillin, chitosan nanoparticles, drug delivery, *Escherichia coli*, ionic gelation

1. Introduction

Drug delivery systems are effective for implementing sustained release of many kinds of drugs. Chitosan (CHT), in particular chitosan nanoparticles (CHT-NPs), have been frequently used in drug delivery applications [1]. CHT is the generic name for a family of strongly polycationic derivatives of poly-N-acetyl-D-glucosamine (chitin) found in the exoskeletons of crustaceans such as crabs and shrimps. It can also be found in the cell wall of fungi and bacteria. Structurally, it is a linear polymer of cationic character formed by units of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose linked by 1–4 bonds [2]. Having a positive charge, CHT is ideal for many drug delivery applications [3, 4]. CHT is biodegradable, non-toxic, non-immunogenic and biocompatible as well as the only naturally occurring polycationic polymer. Along with its derivatives, CHT has received a great deal of attention from the pharmaceutical industry as antimicrobial and antifungal agent [3, 4]. An extensive review of biocompatibility, hydrophilicity, biodegradability and broad spectrum gram-negative/positive antibacterial and anti-fungal effects of chitosan can be found [2].

CHT-NPs exhibit great drug encapsulation efficiency (*EE*) and diverse release profiles, adequate for transporting different types of drugs in many environments [5]. By controlling synthesis parameters conveniently (i.e. reagent concentrations, stirring speed), their diameter, surface charge and other properties can be easily modified thereby leading to a versatile delivery vehicle. CHT-NPs can be synthesized using the ion gelation method with tripolyphosphate (TPP) as cross-linking agent [6, 7]. Ionic cross-linking of CHT is a typical non-covalent interaction, which can be realized by association with negatively charged multivalent ions of TPP. There is a considerable body of literature on the production of CHT-TPP using this method, with many variations concerning the concentrations and ratios of the initial components, of which [1, 7] are seminal works in these matter. Ion gelation allows encapsulation of various compounds, including β-lactams.

Ampicillin is a beta-lactam antibiotic with amino-penicillin skeleton that easily penetrates outer membrane gram-positive/negative bacteria and irreversibly inhibits the transpeptidase enzyme—needed for synthesis of the bacterial cell wall [8]—in the third and final stage of synthesis in binary fission leading cell lysis (bacteriolytic) [9]. Bacterial resistance to antibiotics, including ampicillin, has been observed and investigated for three decades as a serious threat and health crisis [10]. Several mechanisms have been found liking transpeptidase synthesis and activation pathways to bacterial resistance to ampicillin in *E. coli*. Given that ampicillin drug acts as a broad-spectrum penicillin/antibiotic in treatment of infections caused by gram-positive/negative bacteria, and the broad-spectrum β-lactamases among *Enterobacteriaceae* [11] that is evolutionary conserved, finding novel synergistic ways to curb such general and versatile resistance mechanisms is essential for ensuring continued effectiveness of antimicrobial agents [12].

In this work, ampicillin-loaded CHT-NPs were synthesized and the effectiveness of their antimicrobial activity was evaluated against ampicillin and CHT-NPs on *E. coli*. A set of synthesis conditions was investigated in terms of their effect upon particle size, morphology and

surface charge. After selection of the most viable delivery system from the later analysis, the β-lactamic antibiotic (ampicillin) was encapsulated in order to evaluate the encapsulation efficiency of the CHT-NPs. A release profile was obtained in order to assess their applicability as antimicrobial agents against gram-negative bacteria. The aim of the present study is the development of a simple method of synthesis of nano-carriers capable of transporting and releasing a drug such as ampicillin, ultimately inhibiting growth on *E. coli*.

2. Materials and methods

2.1. Reagents

CHT with molecular weight between 100,000 and 300,000 (ACROS Organics™), sodium tripolyphosphate and glacial acetic acid were used. Ultra-pure water was obtained using the Milli-Q A10 system (Millipore). Ampicillin sodium salt (Fisher BioReagents) was used as the antimicrobial agent.

2.2. Bacterial strain

E. coli ATCC 25922 was used for this study.

2.3. Synthesis of chitosan nanoparticles

CHT-NPs were produced using a modified ion gelation method. Briefly, CHT was dissolved at 0.5% w/v in 2% v/v acid acetic solution. Sodium tripolyphosphate was dissolved in ultra-pure water to obtain a 0.1% w/v concentration. An ampicillin volume of 1 ml was then added and later 1 ml of the TPP solution was added drop-wise to 1.5 ml of the CHT solution and magnetically stirred for 1 h. The final suspension was centrifuged at 11,000 rpm for 20 min [6, 13]. Three concentrations of CHT (0.1, 0.3 and 0.5% w/v) and five concentrations of TPP (0.005, 0.01, 0.05, 0.1 and 0.3% w/v) were employed in order to determine their optimal ratio in terms of particle size and surface charge.

2.4. Statistical analysis

In order to evaluate the role of synthesis parameters on particle size and surface charge, including random variations in CHT and then TPP concentrations, selection of linear mixed effects models was performed using standard the Akaike information criterion (AIC), the Bayes information criterion (BIC) and the negative 2 log likelihood criterion (−2LL) [14]. The generalized form (Ω_0^2) of the usual coefficient of determination (R_2) was used [15]. A total of five models (including interactions) were evaluated for each response variable, that is, particle size and ζ potential. Calculations were performed using the *R* Statistical Computing package [16] with the lmer4 package for linear mixed effects models [17]. The Staterthwaite approximation of *p* values was computed for the corresponding observable (**Table 1**) [18].

Model	Response variable	Fixed effects
1	Size	1
2	Size	CHT concentration
3	Size	TPP concentration
4	Size	CHT + TPP concentrations
5	Size	CHT + TPP + CHT*TPP concentrations
6	ζ potential	1
7	ζ potential	CHT concentration
8	ζ potential	TPP concentration
9	ζ potential	CHT + TPP concentrations
10	ζ potential	CHT + TPP + CHT*TPP concentrations

The value of 1 in effects represents the case of a purely random model used as the baseline.

Table 1. Statistical models for evaluating formulations.

2.5. Determination of particle size and ζ potential

The determination of particle size (apparent hydrodynamic diameter) was performed by dynamic light scattering (DLS) and surface electric charge using a Zetasizer Malvern Nano SZ-90 particle analyser, reported as either ζ potential or electrophoretic mobility [7].

2.6. Morphology analysis of CHT-NPs

In order to study the morphology of nanoparticles, topographic images of CHT-NPs were taken on a multi- mode atomic force microscope (AFM) Asylum Research MFP-3D. The AFM probes used for this study were rectangular silicon probes with a nominal spring constant of 40 nN/nm. Similarly, image visualization was carried out in a scanning electron microscope (SEM) Hitachi S-3700 with a 15 nm gold coating on the diluted samples (1/10) using an aluminum base at an acceleration voltage of 15 kV [7].

2.7. Determination of encapsulation efficiency of loaded CHT-NPs

The encapsulation efficiency (*EE*) of the CHT-NPs was determined according to the method described in the previous studies [19]. In brief, the nanoparticle suspensions were separated by centrifuging at 11,000 rpm for 20 min and the contents of ampicillin in the supernatants were measured by HPLC-DAD Perkim Elmer. A blank sample of CHT-NPs without ampicillin was obtained but treated similarly as the drug-loaded CHT-NPs. All samples were measured in triplicate. The *EE* were calculated using.

$$EE = \frac{F}{T} \times 100\% \quad (1)$$

where F is the free amount of ampicillin in the supernatant, T is total amount of ampicillin.

2.8. Release profile of loaded CHT-NP

Release studies were carried out in PBS (pH 7.4) as follows: 1.5 ml ampicillin-loaded CHT-NPs and 1.5 ml PBS were incubated at 37°C and shaken at 200 rpm. Triplicate samples were analyzed at each time step, between 0 and 24 h. The samples were centrifuged and the concentrations of ampicillin released in the supernatant were determined by HPLC-DAD.

2.9. Determination of antimicrobial activity of loaded CHT-NPs

The spectrophotometrically adjusted inoculum (100 μl) of 10^4 bacterial cells was added to each well in the sterile flat-bottomed microtiter plate containing the test CHT-NPs. The design of experiments includes duplicated wells of ampicillin-loaded nanoparticles with three different concentrations of ampicillin (5, 10, 20 mg/ml), two wells with ampicillin as growth inhibition control, two wells containing bacterial suspension with CHT-NPs (growth control) and two wells containing only media (background control) were included in this plate. For the case of wells with ampicillin and CHT-NPs, dilutions were halved at each consecutive level in the gradient. Optical densities were measured for 24 h at 37°C using a multi-detection microplate reader Biotek Synergy HT at 600 nm and automatically recorded for each well every 30 min. Turbidimetric growth curves were obtained depending on the changes in the optical density of bacterial growth for each CHT NP sample and the drug-free growth control.

3. Results and discussion

3.1. Particle size and surface charge of loaded CHT-NPs

Figure 1 corresponds to measures of nanoparticle size (a) and ζ potential (b) according to the concentrations indicated above for both CHT and TPP. As TPP concentration increases, diameter decreases at 0.3 and 0.5% w/v CHT concentrations (a). The opposite occurs at a CHT concentration of 0.1% w/v. In the case of ζ potential (b), all CHT concentrations yield decreasing values as TPP concentration increases.

Statistical models revealed that the best alternative for explaining nanoparticle size in terms of CHT and TPP concentrations is model 5, which includes both factors as well as their interaction (**Table 2**). Both AIC and −2LL have a larger distance from the baseline than in model 3 (size only depending on TPP concentration), being also more significant and with a better fit than it. **Figure 1(a)** shows that nanoparticle size decreases as TPP concentration increases and, simultaneously, CHT concentration decreases. In that sense, model 5 also indicates that while TPP is the largest driver of nanoparticles diameter ($\chi^2 = 3.536$, $df = 4$, $p(\chi^2) < 0.001$), the interaction between CHT and TPP is responsible for the observed change in slope (dotted lines for each line). CHT concentration seems not to have a large role by itself.

A similar analysis was carried out for ζ potential (**Figure 1(b)**). By the same criteria, model 10 was the best criteria for all AIC, BIC and −2LL simultaneously. While all factors are statistically significant, TPP concentration.

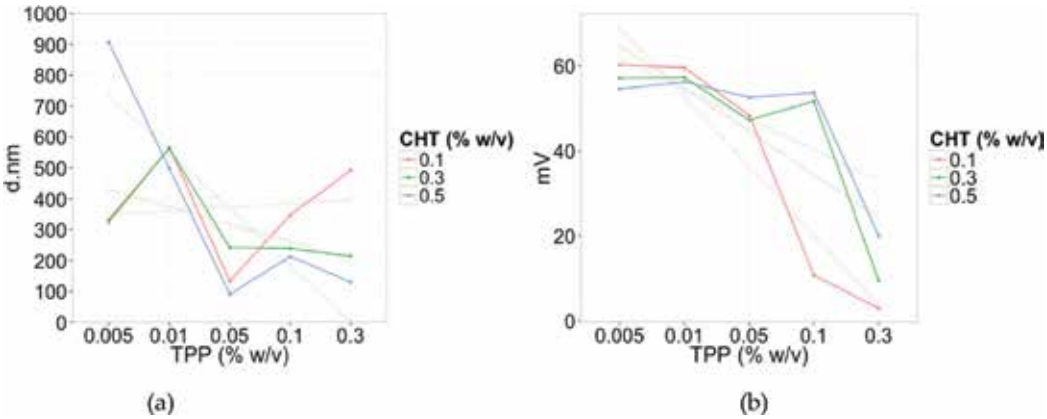

Figure 1. Nanoparticle diameter (nm) and ζ potential (mV) at different concentrations of CHT and TPP. CHT concentration has a marked inverse effect to that of TPP on nanoparticle size, evidence of interference between both factors. A similar trend is identifiable for ζ potential between the lowest and highest TPP concentrations. (**a**) Nanoparticle diameter. (**b**) ζ potential.

Model	*df*	AIC	BIC	−2LL	χ^2	*df* (χ^2)	$p(\chi^2)$	Ω_0^2
2	6	597.35	608.19	−292.67	0.206	2	0.902	0.853
3	8	590.54	604.99	−287.27	10.805	2	0.005*	0.844
4	10	594.11	612.17	−287.05	0.433	2	0.805	0.843
5	18	566.67	599.19	−265.33	43.440	8	<0.001*	0.852

Model 1 is not included (comparison baseline). *Significant *p* values.

Table 2. Results for selection of linear mixed effects models using maximum likelihood for nanoparticles size as the response variable.

central ($\chi^2 = 6604.98$, $df = 4$, $p(\chi^2) < 0.001$): as it increases, ζ potential decreases thanks to a lower amount of available binding sites in the CHT matrix (**Table 3**).

Finally, the most significant levels for both size (diameter in nm) and ζ potential that include CHT and TPP concentrations were identified ($p < 0.001$) as candidates (**Table 4**). In agreement with existing literature, nanoparticles with a large ζ potential ($\zeta \geq 40$ mV) are desirable due to their good stability [20, 21] having sizes in between 200 and 580 nm [5, 22] for the particular case of CHT-NPs. From **Figure 2**, it is clear that levels 1 and 2 reported in **Table 4** (matching Samples M and N in **Table 5**) comply with these requirements. The choice of sample N maximizes the value for the ζ potential while preserving a small nanoparticle size within the range mentioned above.

3.2. Morphology analysis of loaded CHT-NPs

A batch of CHT-NPs without cargo was synthesized and visualized using AFM imaging according to sample preparation N (**Figure 3**). Scan areas are (A) 5 μm × 5 μm and (B) 1 μm × 1 μm respectively for 3A and 3B. The distribution of nanoparticle diameters is reported in **Figure 4** after post-processing of the AFM image.

Model	*df*	AIC	BIC	−2LL	χ^2	*df* (χ^2)	*p*(χ^2)	Ω_0^2
7	6	278.95	289.79	−133.477	0.862	2	0.650	0.995
8	8	261.73	276.18	−122.866	21.222	2	<0.001*	0.995
9	10	261.64	279.71	−120.822	4.087	2	0.13	0.995
10	18	196.41	228.93	−80.204	81.2370	8	<0.001*	0.995

Model 6 is not included (comparison baseline). *Significant *p* values.

Table 3. Results for selection of linear mixed effects models using maximum likelihood for ζ potential as the response variable.

Level	Concentrations
1	CHT 0.5%w/v, TPP 0.05%w/v
2	CHT 0.5%w/v, TPP 0.1%w/v
3	CHT 0.5%w/v, TPP 0.3%w/v

Only levels with $p < 0.001$ are shown here.

Table 4. Significant levels for CHT and TPP concentrations simultaneously reported in model 5 for nanoparticle size and model 10 for ζ potential.

Additionally, SEM images were performed upon samples with and without ampicillin cargo (**Figure 5**). Images 5A, 5B and 5C are from a CHT-NPs sample. It verifies that CHT-NPs have a dispersed, corrugated and spherical morphology with a diameter between 100 and 200 nm. Complementary, images 5D, 5E and 5F belong to 10 mg/ml ampicillin-loaded CHT-NPs sample with an *EE* more than 40% (**Figure 6**) these samples have a smooth spherical morphology with a diameter between 500 and 1000 nm, showing an aggregation effect between nanoparticles.

3.3. Encapsulation efficiency of loaded CHT-NPs

For encapsulation efficiency (**Figure 6**) the initial ampicillin concentration was compared against final encapsulated concentration (**Figure 6(a)**) and later transformed into *EE* (**Figure 6(b)**). *EE* lies within a range of 15–41% with a peak value at a concentration of ampicillin of 4 mg/ml in the final CHT-NPs solution volume.

3.4. Release profile of loaded CHT-NP

A release profile was obtained for the ampicillin-loaded CHT-NPs (**Figure 7**). Released percentage was calculated in relation to the encapsulated ampicillin concentration. Release percentage oscillates between 5 and 20% across 24 h. The burst effect is clearly observable (between 0 and 2 h), to be later succeeded by a more stable behavior (from 2 to 18 h) and rising finally in the last stage (from 18 to 24 h). The observed pattern suggests that swelling of the first layer in the polymeric matrix releases a large amount of ampicillin in the medium (0–1 h), and becomes more stable until the innermost layers are reached, where the remaining

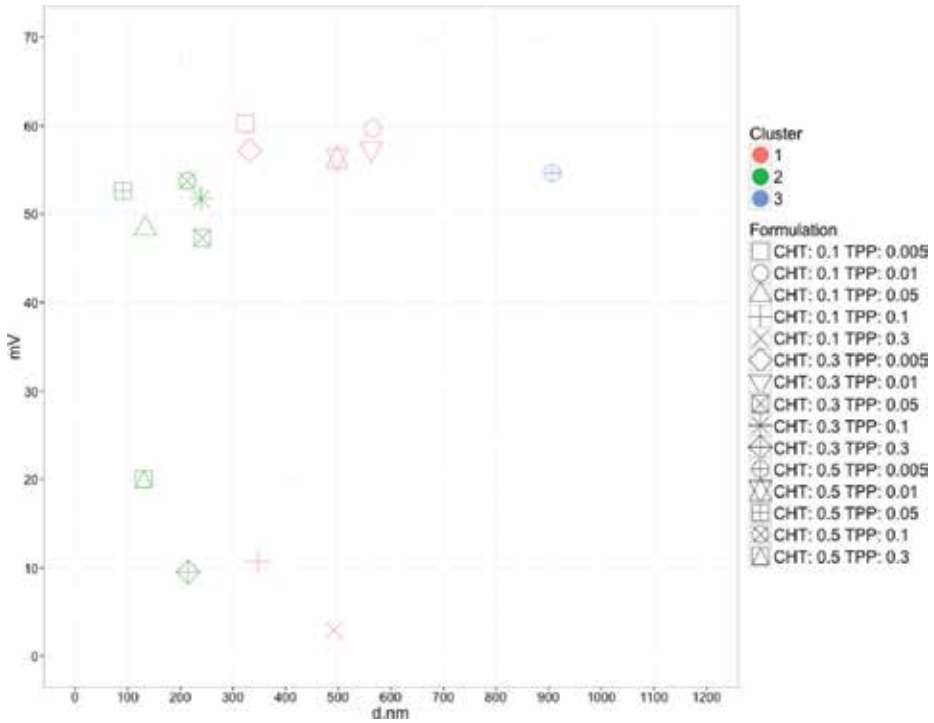

Figure 2. Scatter plot for diameter and surface charge of different CHT-NPs. The classification (clusters) was computed using the Hartigan-Wong *k*-means clustering algorithm [23]. Formulations indicate both CHT and TPP concentrations used for each nanoparticle type. The top left points in the green cluster (above 45 mV and below 300 nm) are the best delivery targets with respect to size and ζ potential, which appear at medium to low concentrations of TPP (0.05–0.1% w/v) and mostly at medium and high concentrations of CHT (except for C with 0.1% w/v). Most experimentally found diameters are below 600 nm. In the case of surface charge, data are partitioned in two groups: above 40 mV and below 20 mV with no nanoparticle in the middle.

Sample	CHT	TPP	*d*	ζ
A	0.1	0.005	323.57 ± 186.84	60.23 ± 2.85
B	0.1	0.01	565.60 ± 97.49	59.67 ± 0.92
C	0.1	0.05	133.60 ± 18.34	48.37 ± 0.31
D	0.1	0.1	347.43 ± 17.60	10.73 ± 0.59
E	0.1	0.3	492.63 ± 16.35	2.92 ± 0.16
F	0.3	0.005	331.73 ± 168.06	57.17 ± 2.28
G	0.3	0.01	562.73 ± 155.08	57.33 ± 2.06
H	0.3	0.05	241.90 ± 34.51	47.30 ± 0.53
I	0.3	0.1	239.30 ± 27.14	51.73 ± 1.25
J	0.3	0.3	214.93 ± 11.40	9.53 ± 0.40
K	0.5	0.005	906.97 ± 264.60	54.63 ± 2.26
L	0.5	0.01	498.30 ± 49.73	56.27 ± 0.95

Sample	CHT	TPP	d	ζ
M	0.5	0.05	90.88 ± 30.90	52.63 ± 2.53
N	0.5	0.1	213.50 ± 29.76	53.73 ± 3.33
O	0.5	0.3	130.56 ± 15.65	20.03 ± 1.46

CHT and TPP concentrations are given in terms of w/v percentage. Diameter d is given in nm. ζ potential is given in mV. Standard deviation is included in both cases.

Table 5. CHT and TPP concentrations for synthesis of nanoparticles in **Figure 2**.

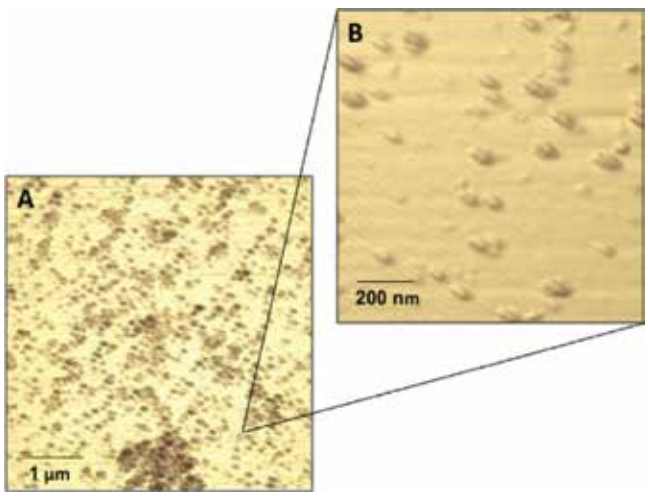

Figure 3. AFM image of CHT-NPs without ampicillin cargo. AFM images were performed as a first assessment of CHT-NP synthesis process. **Figure 5** provides the contrast between these nanoparticles and ampicillin loaded CHT-NPs. **A** corresponds to a scan area of 5 µm × 5 µm and **B** to 1 µm × 1 µm.

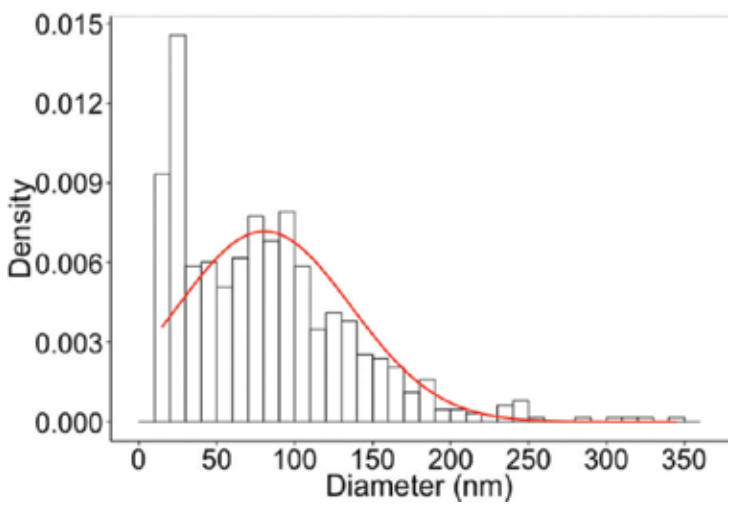

Figure 4. Histogram of CHT-NPs diameters for the AFM image in **Figure 3**. $N = 632$, $\bar{d} = 80.5$ nm, $\sigma_d = 55.6$ nm.

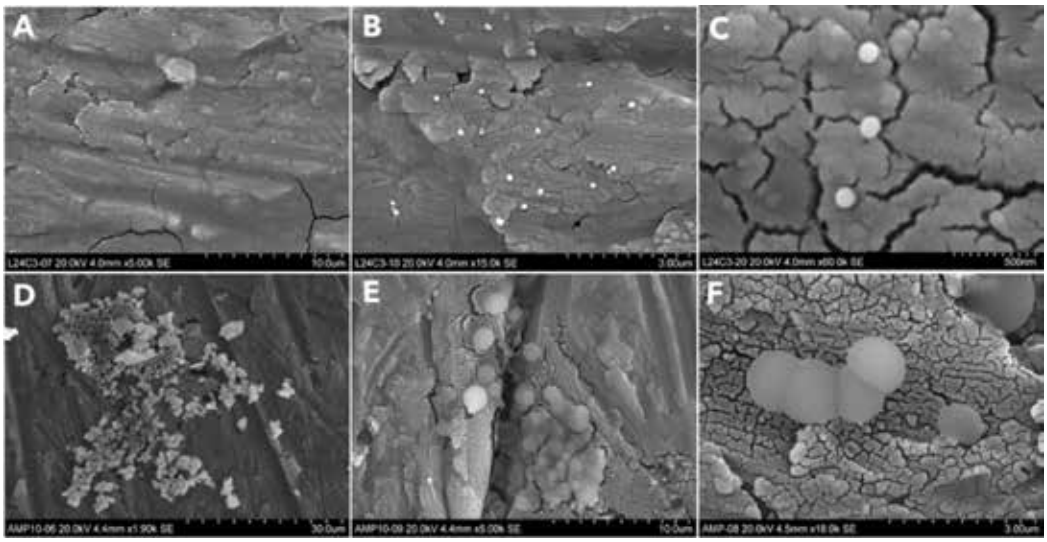

Figure 5. SEM image of CHT-NPs, without and with antibiotic cargo (ampicillin). **A**, **B** and **C** are images of nanoparticles without cargo and **D**, **E** and **F** encapsulate ampicillin. Nanoparticle diameter and size appears to increase in order to gain stability in ampicillin-loaded nanoparticles, which also has an additional aggregation effect.

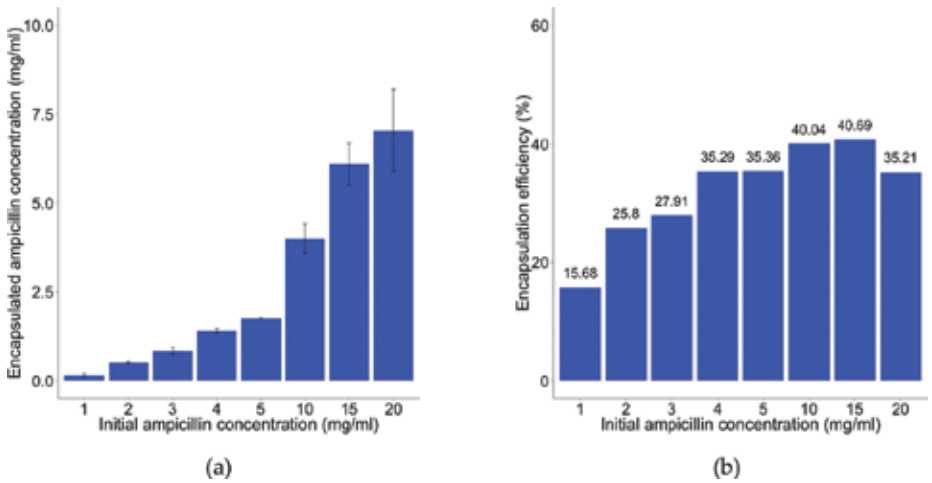

Figure 6. Cargo efficiency of the CHT-NPs from initial concentrations, obtained as described in [19]. (**a**) Measured encapsulated ampicillin concentration through HPLC. (**b**) Corresponding encapsulation efficiency, calculated as *EE* in Eq. (1).

contents are finally released. The latter is consistent with what is described by Carbinato et al. [24] for cross-linked pectin/high-amylose starch matrices.

3.5. Growth inhibition assays for *E. coli* ATCC 25922

Figure 8 shows the growth inhibition assays for *E. coli* ATCC 25922, an ampicillin–susceptible reference strain. Under the conditions of this assay, this strain shows a minimal inhibitory

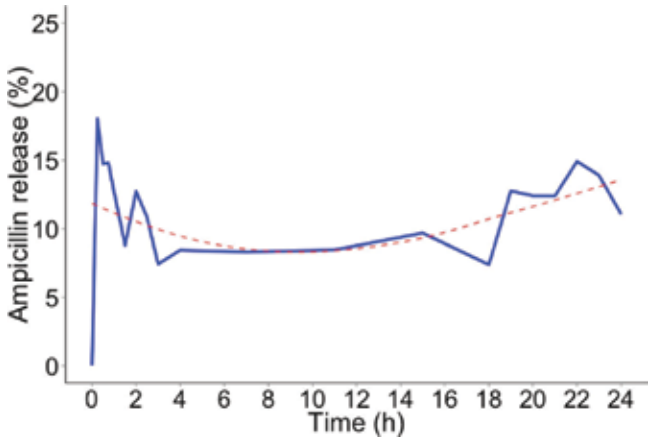

Figure 7. Release profile of ampicillin-loaded CHT-NPs. A burst effect was observed for the first hour, followed by a stabilization phase (1–4 h), a steady release phase (4–15 h) and posterior relative increase (15–24 h).

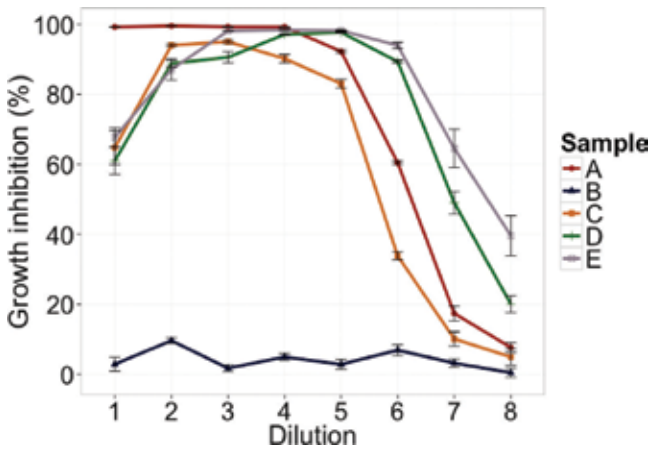

Figure 8. Growth inhibition assays for *E. coli*. Samples are as follows: (**A**) ampicillin 20 mg/ml, (**B**) CHT-NPs, ampicillin-loaded NPs at (**C**) 5 mg/ml, (**D**) 10 mg/ml and (**E**) 20 mg/ml. As expected, sample **B** produces no inhibition, while **A**–**C** remain effective until the fifth dilution and **D**–**E** to the sixth dilution.

concentration (MIC) of 2 μg/ml, which is in agreement with the range values (2–8 μg/ml) previously reported [25]. A clinical *E. coli* isolate is considered as non-susceptible when its MIC value is >8 μg/ml. The highest ampicillin concentration used as control was 500 μg/ml (dilution 1, **Figure 8**), generating a concentration gradient by double dilutions through 3.91 μg/ml (dilution 8, **Figure 8**). The three ampicillin-loaded CHT-NPs systems (C, D and E) show a similar growth inhibition pattern with respect to the positive control (ampicillin), indicating that under these conditions ampicillin is released from CHT − NPs to concentrations high enough to inhibit bacterial growth. Moreover, both CHT − NPs systems D and E, loaded with 10 mg/ml and 20 mg/ml of ampicillin respectively, maintain growth inhibition with more dilutions with respect to ampicillin. It is worth noting that inhibition in samples A and C lower their effectiveness rapidly after the fifth dilution, but samples D and E have a much wider range of inhibition at lower concentrations for all remaining dilutions.

4. Conclusions

CHT-NPs synthesis was successfully achieved and the manipulation of CHT and TPP concentrations towards optimization for drug delivery resulted in smaller particles and increased surface charge. Besides, a detailed physicochemical characterization of CHT-NPs was obtained. CHT-NPs were able to encapsulate and release ampicillin. The encapsulation efficiency of ampicillin CHT-NPs exceeds 30% in most cases while antibiotic release maintains a relatively stable profile, ranging between 5 and 20% in a 24-hour period. A microbiological assay was used as proof of principle in order to verify the release of the antibiotic ampicillin from the CHT-NPs systems. Growth inhibition of the ampicillin-susceptible *E. coli* ATCC 25922 reference strain was achieved in a similar pattern compared with ampicillin alone.

In terms of the obtained release profile in a period of 24 h, several comparisons can be drawn in terms of other alternatives. The cumulative release profile, computed from data shown in **Figure 7**, indicates that less than 40% of the cargo was released after 24 h. Ampicillin-loaded electrospun poly(ε-caprolactone) nanofiber yarns have a much faster release profile, releasing more than 90% of its cargo in the same period [26]. By contrast, our formulation has a release profile faster than that reported for ampicillin-conjugated gum Arabic microspheres [27]. In the later cases, no additional antibiotic or bacteriostatic effect is present in the polymeric matrix. CHT-NPs loaded with ampicillin reported here are comparable to chitosan microgranules loaded with diclofenac sodium in contrast to chitosan beads loaded with diclofenac sodium in terms of the order of magnitude of released antibiotic [28]. Compared against ampicillin loaded methylpyrrolidinone chitosan and chitosan microspheres, our nanoparticles have a significantly lower encapsulation efficiency [29], yet the former are not suitable for sustained release. CHT-NPs are similar in size compared to existing literature on ampicillin-loaded nanoparticles and liposomes [30], with a release profile closer to that of liposomes. In the later study, liposomes had a larger inhibition halo at lower dilutions than nanoparticles of higher density and longer release time.

Finally, the synthesis protocol of CHT-NPs elaborated in this study constitutes a platform for the analysis of the encapsulation of other antibiotics with different structures as well as for assays on other bacteria.

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Conflict of interest

The authors have no conflict of interest to declare.

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Chapter 13

Chitoneous Materials for Control of Foodborne Pathogens and Mycotoxins in Poultry

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Billy M. Hargis and Guillermo Tellez

Additional information is available at the end of the chapter

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Abstract

Public concern with the incidence of antibiotic-resistant bacteria, particularly among foodborne pathogens has been challenging the poultry industry to find alternative means of control. Chitosan is a modified, natural biopolymer derived by deacetylation of chitin. The antimicrobial activity and film-forming property of chitosan makes it a potential source of food preservative or coating material of natural origin for improvement of quality and shelf life of various foods of agriculture, poultry, beef and seafood origin. In addition to its use as an antimicrobial, it has been shown that it has good properties as a mycotoxin adsorbent. The purposes of the present chapter is to summarize our experience using chitin-chitosan from Deacetylated 95% food grade chitosan (Paragon Specialty Products LLC Rainsville, AL) or *Aspergillus oryzae* meal (Fermacto®, PetAg Inc., Hampshire IL) to control foodborne pathogens, improve performance, biological sanitizer and mycotoxin binder in commercial poultry.

Keywords: chitosan, Fermacto®, *Salmonella*, mycotoxins, gut health

1. Introduction

Chitin ($C_8H_{13}O_5N$)n) is a long-chain polymer of a N-acetylglucosamine (**Figure 1(a)**), a derivative of glucose, and is found in many places globally. It is the main component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans (e.g., crabs, lobsters and shrimps) and insects, the radula of mollusks, and the beaks of cephalopods, including squid and octopi [1]. In terms of structure, chitin may be compared to the polysaccharide cellulose and, in terms of function, to the protein keratin [2]. Depending on its source, two types of chitin allomorphs

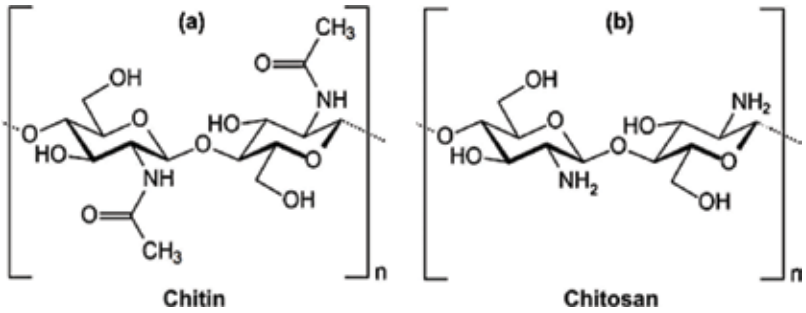

Figure 1. Chemical structure (a) of chitin poly(N-acetyl-β-d-glucosamine) and (b) of chitosan (poly(d-glucosamine) repeat units.

can occur, the α and β forms, which can be differentiated by infrared and solid-state NMR spectroscopy together with X-ray diffraction [3]. Chitosan is a high molecular weight polysaccharide linked by a β-1,4 glycoside and is composed of N-acetyl-glucosamine and glucosamine (**Figure 1(b)**). It is a natural biopolymer derived by deacetylation of chitin and the most widespread polycationic biopolymer [3]. However, although chitosan is obtained from chitin, the applications of the latter compared to chitosan are limited because it is chemically inert [4] and because of its poor solubility [5]. Unlike chitin, chitosan is soluble but in an acidic media since at neutral or alkaline pH it is insoluble. The properties of chitosan can be modified by changing the degree of deacetylation, pH and ionic strength. At neutral pH, most chitosan molecules will lose their charge and precipitate when it is in solution [6].

The application of chitin is focused on obtaining soluble derivatives in aqueous media such as chitosan [3]. Chitosan has several applications in fields such as waste and water treatment, agriculture, fabric and textiles, cosmetics, nutritional enhancement, and food processing. Given its low toxicity and allergenicity, and its biocompatibility, biodegradability and bioactivity, it is a very attractive substance for diverse applications in the pharmaceutical and medical fields, since it has been used for systemic and local delivery of drugs and vaccines [7]. However, one of the most important application is its antimicrobial activity against bacteria, filamentous fungi and yeasts. Chitosan has wide spectrum of activity against Gram-positive and Gram-negative bacteria but it is more effective against Gram-negative bacteria [8, 9]. Furthermore, it has been reported that the antimicrobial activity and film-forming property of chitosan makes it a potential source of food preservative or coating material of natural origin for improvement of quality and shelf life of various foods of agriculture, poultry, beef and seafood origin [3, 10]. The mechanism of the antimicrobial activity of chitosan has not yet been fully elucidated, but several hypotheses have been proposed. The most feasible hypothesis is a change in cell permeability due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes [11, 12]. Other mechanisms include the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis and chelation of metals, spore elements, and essential nutrients [5, 13].

The antimicrobial activity of chitosan depends on both intrinsic and extrinsic factors. Among the intrinsic factors are the molecular weight and degree of deacetylation of chitosan. While the extrinsic factors include pH, temperature and reactive time [14]. Moreover, it has been observed that when the chitosan is in nanoparticle form, it has better antimicrobial

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properties since its small particle size gives it a greater surface area and high reactivity which could enhance the charge interaction with the bacterial surface and of this way to produce a superior antimicrobial effect [15].

2. Antimicrobial effect of chitosan on *Salmonella* in broiler chickens

Salmonella enterica serovars continue to be among the most important foodborne pathogens worldwide due to the considerable human rates of illness reported, the wide hosts species that are colonized by members of this remarkable pathogen genus, which serve as vectors and reservoirs for spreading these agents to animal and human populations. **Figure 2** shows the distribution of the major serotypes of Salmonella with importance in public health. Furthermore, the public concern for the appearance of resistant strains to many antibiotics, particularly among zoonotic pathogens such as common *Salmonella* isolates, is also challenging the poultry industry to find alternative means of control [16]. For these reasons, continued research on sustainable alternatives to antibiotic growth promoters for animal production is needed.

Interest in chitosan, a biodegradable, nontoxic, non-sensitizing, and biocompatible polymer isolated from shellfish, arises from the fact that chitosan is reported to exhibit numerous beneficial effects, including strong antimicrobial and antioxidant activities in foods [18]. Its application in agriculture, horticulture, environmental science, industry, microbiology, and medicine are well reported. A significant interest in the antimicrobial activities of chitosan either as solution, or as powders, edible films and coating against foodborne pathogens, spoilage bacteria, and pathogenic viruses and fungi in several food categories has been extensively investigated [19]. We have evaluated the effect *in vitro* and *in vivo* of chitosan on *Salmonella typhimurium* in broiler chickens [20]. In our *in vitro* crop assay experiments (**Table 1**), 0.2% chitosan significantly

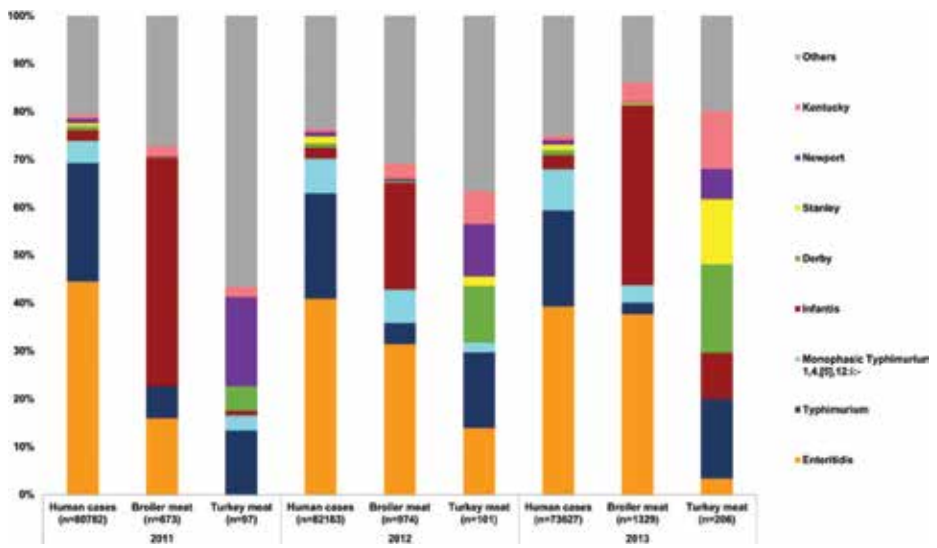

Figure 2. Distribution of the major serotypes of non-typhoidal *Salmonella* associated with human cases (salmonellosis) and poultry meat in EU, 2011 to 2013 [17].

Treatment	Trial 1		Trial 2		Trial 3	
	30 min	6 h	30 min	6 h	30 min	6 h
Control	5.22 ± 0.15[a]	7.62 ± 0.01[a]	5.19 ± 0.11[a]	6.99 ± 0.03[a]	6.05 ± 0.18[a]	7.95 ± 0.31[a]
Chitosan (0.2%)	3.94 ± 0.20[b]	3.04 ± 0.20[b]	3.49 ± 0.24[b]	4.40 ± 0.19[b]	5.05 ± 0.19[b]	5.31 ± 0.26[b]

[a-b]Values within columns with different lowercase superscripts differ significantly (P < 0.05).

Table 1. Antimicrobial activity of chitosan on *Salmonella typhimurium* in an in vitro crop assay.

Treatment	Trial 1		Trial 2	
	Cecal tonsils (CT)	Log_{10} ST/g of CT	Cecal tonsils (CT)	Log_{10} ST/g of CT
Control	15/20 (75%)	4.20 ± 0.82[a]	15/20 (75%)	5.00 ± 0.62[a]
Chitosan (0.2%)	9/20 (45%)	2.28 ± 0.75[b]	12/20 (60%)	3.34 ± 0.72[b]

[a-b]Values within columns with different lowercase superscripts differ significantly (P < 0.05).

Table 2. Effect of chitosan on *Salmonella enteritidis* cecal tonsils colonization in 7-days-old broiler chickens.

reduced the cfu of ST at 30 min or 6 h compared with control (P < 0.05). In the *in vivo* experiments with 40 day-of-hatch broiler chicks and challenged with 2×10^5 cfu ST, dietary 0.2% chitosan significantly reduce the cfu/g of ST in the ceca in both experiments (**Table 2**). However, no significant reduction in the incidence of ST in cecal tonsils colonization was observed, suggesting that 0.2% chitosan significantly reduced the cfu of ST/gram *in vitro* and *in vivo*.

3. Effect of chitosan as a biological sanitizer on chicken skin

Chickens contain large numbers of microorganisms in their gastrointestinal tract and on their feathers and feet; therefore, storage quality of fresh chicken is partially dependent on the bacteria present on the integument prior to slaughter. Pathogenic microorganisms present in chicken carcasses after processing and throughout scalding and picking can contaminate equipment and other carcasses [21]. Pathogenic bacteria such as *Salmonella spp.* and *Campylobacter* spp. are able to attach to skin and penetrate in skin layers or feather follicles, facilitating their presence on chicken skin and carcass during poultry processing [22]. Critical control point determination at broiler processing has become very important, especially because of the recent attention on

Treatment	Trial 1		Trial 2	
	1 h	24 h	1 h	24 h
Control	6.57 ± 0.11[a]	6.03 ± 0.02[a]	6.78 ± 0.06[a]	7.36 ± 0.06[a]
Chitosan (0.5%)	6.23 ± 0.03[a]	5.81 ± 0.06[b]	7.06 ± 0.08[a]	6.60 ± 0.17[b]

[a-b]Values within columns with different lowercase superscripts differ significantly (P < 0.05).

Table 3. *Salmonella typhimurium* (log10 cfu ± standard error)/square cm of chicken skin treated with 0.5% chitosan solution.

Hazard Analysis and Critical Control Points (HACCP) for reduction of microbial contamination of meat and poultry [23]. For all these reasons, strategies to reduce bacterial contamination on poultry carcasses are important. However, most of the bacterial reduction strategies for poultry comprise the use of antimicrobial chemicals in rinses or washes and their efficacy is reduced by the presence of organic matter. Therefore, it grows the need of biological sanitizers in the processing plant to prevent carcass to carcass cross-contamination by pathogenic bacteria and to lower the potential of foodborne diseases. Interest in chitosan, a biocompatible polymer derived from shellfish, as a biological sanitizer arises from reports showing several beneficial effects such as antimicrobial and antioxidative activities in foods [2]. The use of chitosan in industry, agriculture, and medicine is well described [13]. The antimicrobial activities of chitosan against foodborne pathogens has been broadly investigated in the food industry [24]. Research conducted in our laboratory on the effect of chitosan as a biological sanitizer in chicken skin contaminated with *Salmonella* Typhimurium and aerobic Gram-negative spoilage bacteria present on chicken skin, have revealed that 0.5% chitosan for 30 s dipping ST contaminated skin samples in a solution of 0.5% chitosan reduced ($P < 0.05$) the recovery of ST by 24 h as well as the presence of spoilage-causing psychrotrophic bacteria below detectable levels [19], (**Table 3**). The antimicrobial activity and film-forming characteristic of chitosan makes it a potential source of food preservative, increasing quality and shelf life of different types of foods [10]. The mechanism of the antimicrobial activity of chitosan has not yet been fully elucidated; nevertheless, different hypotheses have been proposed. The most realistic hypothesis is that chitosan is able to change cell permeability due to interactions between the positive charges of its molecules and the negative charges of the bacterial cell membranes [1]. Other hypotheses include the chelation of metals and essential nutrients, inhibiting bacterial growth had also suggested that high molecular weight chitosan could be able to form a polymer membrane around the bacterial cell, preventing it from receiving nutrients [25].

4. Prebiotic properties of *Aspergillus oryzae* to control foodborne pathogens improve performance and bone mineralization in poultry

Prebiotics are non-digestible food ingredients that are selectively fermented by gut bacteria and are known to have positive effects on gastrointestinal (GI) physiology. Some prebiotics have been shown to selectively stimulate the growth of endogenous lactic acid bacteria in the gut thereby improving the health of the host [26]. Prebiotics selectively modify the colonic microflora and can potentially influence gut metabolism [27]. The commercially available mycelium product of *Aspergillus oryzae*, Fermacto® (PetAg Inc. Hampshire, IL 60140 USA), referred to as *Aspergillus* meal (AM), has no live cells or spores and is proven to enhance the digestive efficiency of the GI tract [28]. *Aspergillus* fiber contains beta-glucans [29], fructooligosaccharides (FOS) [30], chitosan [31], and mannanoligosaccharides (MOS) [32]. Beta-glucan is considered as a powerful immune-enhancing nutritional supplement that affects the intestinal villi and primes the innate immune system to help the body defend itself against viral and bacterial invaders [33]. MOS protect the GI tract from invading toxins and pathogens by binding toxin active sites [34]. FOS and chitosan refer to a class of non-digestible carbohydrates that are readily fermented by beneficial bacteria in the intestine [30]. A healthy population of these beneficial bacteria in the digestive tract enhances the digestion and absorption of nutrients, detoxification and elimination processes,

and helps boost the immune system [35]. With an increase in the dependence on livestock as an important food source, it becomes crucial to achieve good health in order to make rearing of animal food sources safe and beneficial to both animals and humans.

Several studies have demonstrated that prevention of *Salmonella* colonization in chickens can be achieved by feeding prebiotics [36]. According to Lowry et al. [37], dietary beta-glucan reduces SE colonization significantly in chickens. In their experiment, SE from L/S was recovered from 76% of non-treated birds, while only 7% of the birds were positive for SE in the treated group, (**Figure 3**). Moreover, in the same study, heterophils isolated from birds treated with dietary beta-glucan contained 40% ($p < 0.05$) more SE than heterophils isolated from untreated birds, (**Table 4**). Heterophils form the first line of defense and killing of *Salmonella* by heterophils is well-described. This corroborates the immunostimulatory effect of beta-glucans and FOS are widely used as prebiotics in a broad range of animal species, and these carbohydrates have been tested with success for protection against *Salmonella* infections in chickens and other avian pathogens [35, 38, 39]. Kim et al. conducted a study where dietary MOS (0.05%) and FOS (0.25%) had an effect on intestinal microflora of broiler chickens, suggesting the use of these prebiotics as an alternative to the use of growth-promoting antibiotics [40]. Finally, chitosan is a modified, natural biopolymer derived by deacetylation of chitin, the main component of the cell walls of fungi and exoskeletons of arthropods. As mention before, chitosan exhibits numerous beneficial effects, including strong antimicrobial and antioxidative activities. Its application in agriculture, horticulture, environmental science, industry, microbiology, and medicine are well reported [10]. According to Huang et al., the use of 0.01 or 0.015% of oligo-chitosan in the diet increased serum levels of immunoglobulins in broiler chickens, suggesting a potential immunomodulatory effect [41]. There have been numerous studies that report the use of chitosan as a mucosal adjuvant, by enhancing IgA levels. It is well known that IgA is active across mucosal surfaces and is the predominant class of antibody against enteric pathogens [42, 43]. The commercial prebiotic supplement derived from *Aspergillus* sp. mycelium is

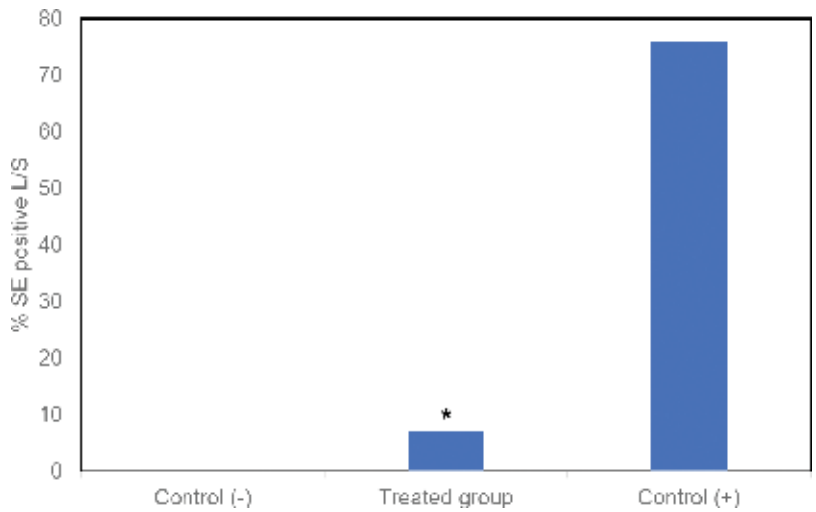

Figure 3. Effects of dietary β-glucan on SE organ invasion in immature chickens. β-glucan fed as a nutritional supplement to neonatal chickens 3 days prior to SE challenge. (*indicates statistically significant differences, $P < 0.05$).

Treatments	Percent heterophils + SE	Mean #SE/heterophil	Phagocytic index (PI)
Control feed	38.54 ± 0.05[b]	4.38 ± 1.08[b]	175.54 ± 44.92[b]
β-glucan feed	78.84 ± 0.03[a]	8.20 ± 0.76[a]	644.10 ± 57.07[a]

[a-b]Values within columns with different lowercase superscripts differ significantly ($P < 0.05$).

Table 4. Effects of feeding β-glucan on chicken heterophil phagocytosis.

unique because it contains all of the above mentioned prebiotic ingredients. Additionally, AM contains 16% protein and 45% fiber and may be used with low levels of protein and amino acid diets to improve performance in commercial poultry [28, 44]. Even though the exact mechanisms of action for prebiotics have not been defined, it may be speculated that the effect is due to changing intestinal flora that promotes the growth of beneficial bacteria. This product has also been shown to benefit poultry through stimulation of growth, most probably by increasing absorption of feed ingredients and improving digestibility [45, 46].

In a recent study conducted in our laboratory we evaluated the effect of 0.2% dietary *Aspergillus* meal (AM) against horizontal transmission of *Salmonella* spp. in turkeys and chickens [36]. The results of this study showed that dietary supplementation with 0.2% *Aspergillus* Meal was able to reduce *Salmonella enteritidis* horizontal transmission in turkeys, (**Table 5**) and *Salmonella* Typhimurium horizontal transmission in broiler chickens, by reducing the overall colonization levels in birds, (**Table 6**). Although the mechanism of action is not totally understood, the reduction in *Salmonella* colonization may be related to a synergistic effect between beta-glucan, MOS, chitosan, and FOS present in the *Aspergillus oryzae* mycelium. In a previous work, we showed that dietary AM induces important changes on intestinal morphometry in turkey poults such as increased number of acid mucin cells in the duodenum, neutral mucin cells in the ileum, and

Groups	Day 10 cecal tonsils	Day 20 cecal tonsils	Day 30 cecal tonsils
Control—No AM	20/20 (100%)	18/20 (90%)	15/20 (75%)
AM	15/20 (75%)*	12/20 (60%)*	8/20 (40%)*

*(a) in the Control-No AM group and (b) in the AM group in both tables.

[a-b]Values within columns with different lowercase superscripts differ significantly ($P < 0.05$).

Table 5. Effect of dietary *Aspergillus* meal against horizontal transmission of *Salmonella enteritidis* at 10, 20 and 30 days of age in turkeys.

Groups	Trial 1		Trial 2	
	Liver/spleen	Cecal tonsils	Liver/spleen	Cecal tonsils
Control—No AM	18/20 (90%)	20/20 (100%)	19/20 (95%)	18/20 (90%)
AM	6/20 (30%)*	5/20 (25%)*	8/20 (40%)*	6/20 (30%)*

*(a) in the Control-No AM group and (b) in the AM group in both tables.

[a-b]Values within columns with different lowercase superscripts differ significantly ($P < 0.05$).

Table 6. Effect of dietary *Aspergillus* meal against horizontal transmission of ***S**almonella typhimurium* at 10 days of age in chickens.

	Control	Aspergillus meal
Body weight (Kg)	600.32 ± 52.26[b]	720.87 ± 63.82[a]
FC (feed: gain)	1.34 ± 0.03[a]	1.23 ± 0.02[b]
Mortality (%)	2.00%[a]	2.50%[a]

[a-b]Values within columns with different lowercase superscripts differ significantly ($P < 0.05$).

Table 7. Effect of *Aspergillus* meal on productive parameters in turkey poults at 30 days of ages.

sulphomucin cells in the duodenum and ileum, as well as increased villi height and villi surface area of both duodenum and ileum when compared to control, suggesting that AM prebiotic has an impact on the mucosal architecture and goblet cells proliferation in the duodenum and ileum of neonate poults [45]. Our extended studies using dietary AM prebiotic supplemented for 30 days, have shown significantly increased the body weight of neonate poults and improved feed conversion when compared with poults that received the control basal diet, and interestingly, energy and protein content in the ileum was significantly lower in poults that received dietary AM prebiotic compared with control poults suggesting better digestibility, absorption of those nutrients and bone mineralization [46]. FOS have been shown to stimulate calcium (Ca) and magnesium (Mg) absorption in the intestine and increase bone mineral concentrations in humans and rats as well as stimulate net Ca transport from the epithelium of the small and large intestine [30, 38].

The gastrointestinal tract serves as the interface between diet and the metabolic events that sustain life. Intestinal villi, which play a crucial role in digestion and absorption of nutrients, are underdeveloped at hatch and maximum absorption capacity is attained by 10 days of age [47]. Understanding and optimizing the maturation and development of the intestine in poultry will improve feed efficiency, growth and overall health of the bird. Studies on nutrition and metabolism during the early phase of growth in poults may, therefore, help in optimizing nutritional management for maximum growth. By dietary means it is possible to affect the development of the gut and the competitiveness of both beneficial and harmful bacteria, which can alter not only gut dynamics, but also many physiologic processes due to the end products metabolized by symbiotic gut microflora [48]. Additives such as enzymes, probiotics and prebiotics are now extensively used throughout the world [49–51]. Our studies suggest that the increase in performance and bone parameters in neonatal poults fed with 0.2%AM (**Table 7**), may be related to a synergistic effect between beta-glucan, MOS, chitosan and FOS from *Aspergillus niger* mycelium [45, 46].

5. Evaluation of chitosan as binding adsorbent material to prevent mycotoxicosis poultry

Mycotoxins are secondary toxic metabolites produced by filamentous fungi which, even at low concentrations, represent an important danger for both animal and human health [52, 53]. Currently, over 300 mycotoxins have been identified worldwide, being aflatoxins, ochratoxins, zearalenone, trichothecenes, and fumonisins, the most frequently found with synergistic toxic effects reported when more than one of these mycotoxins are present in the feed [54, 55]. Mycotoxins are chemically and structurally different, representing serious public health risk

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factors since mycotoxins have been shown to have carcinogenic, teratogenic, nephrotoxic, and hepatotoxic effects after the consumption of contaminated grains or animal food products [56]. On the other hand, mycotoxins are equally important in the animal food industry, causing significant economic losses due to diminished performance and productivity, decreased reproductive parameters, and an increased mortality rate associated with the toxicological effects in liver, kidneys, and immune system [52, 57, 58]. Researchers have developed some methods in order to reduce the harmful effects of grains contaminated with mycotoxins. These include physical (thermal and irradiation inactivation); chemical (ozonation and ammoniation); and, biological (bacterial degradation or adsorption [57, 59, 60]. Nevertheless, toxin sequestering agents are the most common and reliable products used for the feed industry due to its economic practicality and aptness for nutritional insight [61, 62]. Several studies have demonstrated that cellulosic materials have adsorption capacities for heavy metal ions and other pollutants [63, 64]. Similarly, some researchers have evaluated the binding activity of chitosan (CS) against several mycotoxins [2, 65]. As a biological polymer, chitosan has been shown to have promising uses as an adsorbent for the removal of various mycotoxins, heavy metal ions, and dyes [65]. Furthermore, it has been tested in the removal of OTA from contaminated drinks, demonstrating that chitosan can reduce the levels of this mycotoxin [1, 66]. On the other hand, some in vitro methods have been developed to evaluate the adsorbent capacity of mycotoxin sequestering products [67, 68]. However, these methods may not be directly applicable to poultry diets because they do not use the successive incubation at different pH and enzyme activity conditions similar to the different gastrointestinal compartments of poultry. Recently, we evaluated the adsorption capacity of CS on Aflatoxin B1 (AFB_1); Fumonisin B1 (FUB_1); Ochratoxin (OTA); Trichothecene (T-2); Deoxynivalenol (DON); and, Zearalenone (ZEA), using an *in vitro* digestive model that simulates three gastrointestinal compartment of poultry [69]. In that study, deacetylated 95%, high molecular weight (350 kDa) Chitosan (CS, Paragon Specialty Products, LLC, Rainsville, AL, USA) was tested and acetylated with an aqueous solution of acetic acid 1% (v/v). Then, this solution was dropped into NaOH

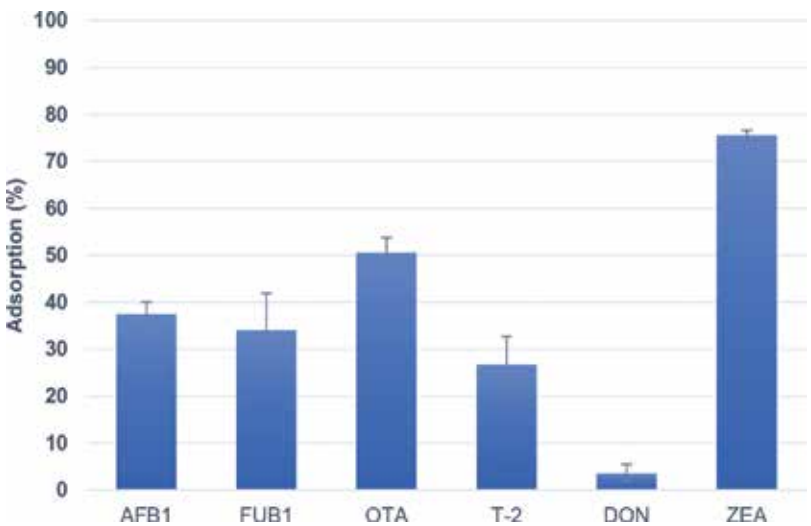

Figure 4. Percentage of adsorption of different mycotoxins on chitosan. Bars are the mean values. Error bars displays an interval around each mean, which are based on Fisher's least significant difference (LSD) procedure.

0.5 M solution and the formed chitosan particles were rinsed three times with pure water and dried [69, 70]. The results showed a moderate adsorbent capacity of CS against five of the six mycotoxins evaluated, except for DON since only 3.5% was adsorbed, (**Figure 4**). Similar results were obtained in another study using non-crosslinked chitosan against different mycotoxins but it is a fact that cross-linking is related to a higher adsorption capacity and pH can affect it [70]. The mycotoxins adsorption capacity of CS is due to the electrostatic interactions. At alkaline pH, the CS is positively charged, while mycotoxins such as AFB1, FUB1, OTA and ZEA are negatively charged [70–72]. In the case of DON and T-2, the interactions appeared to be minor, causing poor adsorption. These results are very similar to those obtained in other studies [70]. Therefore, it could be said that ionic interactions are the main mechanism of mycotoxin adsorption of chitosan.

6. Chitosan nanocarriers: a strategy to improve solubility, permeability and stability of drugs

Another application of chitosan (CS) is its use in nanotechnology for the development of drug delivery systems such as nanoparticles and nanocapsules. These systems emerge as a strategy to improve the dissolution of drugs with low solubility and increase its permeability, which translates into an increase in bioavailability, a greater specificity and also an increase in the stability of drugs against physiological and environmental conditions [73]. In our laboratory, we have developed two nanocapsular systems capable of loading a phytopharmaceutical named Curcumin. This molecule has also been the subject of study in the poultry industry, given its properties, including its antioxidant action, the immunomodulatory, anticoccidial, anti-inflammatory, antimicrobial and growth promotion effects, the latter as an alternative to antibiotic growth promoters in order to maintain the performance and health of the birds [74–76]. In our laboratory, we have already evaluated the antimicrobial activity of curcumin against Salmonella *enteritidis* in an in vitro model that simulates the three compartments of the chicken gastrointestinal tract. The results obtained show that at a dose of 1%, the concentration of Salmonella *enteritidis* decreases slightly but not significantly with respect to the control [77]. However, one of the problems of curcumin, even when it is administered at high doses (12 g/day) is its low bioavailability due to its poor solubility and therefore poor absorption, as well as its rapid metabolism and systemic elimination [76]. In this sense, the development of nanocapsular systems aimed to increase the solubility, permeability and stability of curcumin. Such systems were named chitosan nanocapsules (NC-CS) and Alginate nanocapsules (NC-ALG) and were composed of an oily core of vitamin E surrounded by a biodegradable polymeric shell of either chitosan or alginate respectively.

Both systems were obtained by the a slightly modification of the solvent displacement technique [78]. However, the formation of NC-CS is based on the electrostatic and hydrophobic interactions as well as the hydrogen bonding and van der Waals forces that take place between the chitosan dissolved in an acidic aqueous phase and the lipid cores of Vitamin E formed in the organic phase, causing the polymer to be adsorbed on the surface of the lipid cores [4, 79–81], (**Figure 5(a)**). While NC-ALG were prepared using the "Single-stage procedure" based on the dipolar ionic interactions between the polymer (ALG), which is dissolved in the aqueous phase and the cationic surfactant (CTAB) present in the organic phase which also contains the oil [82], (**Figure 5(b)**).

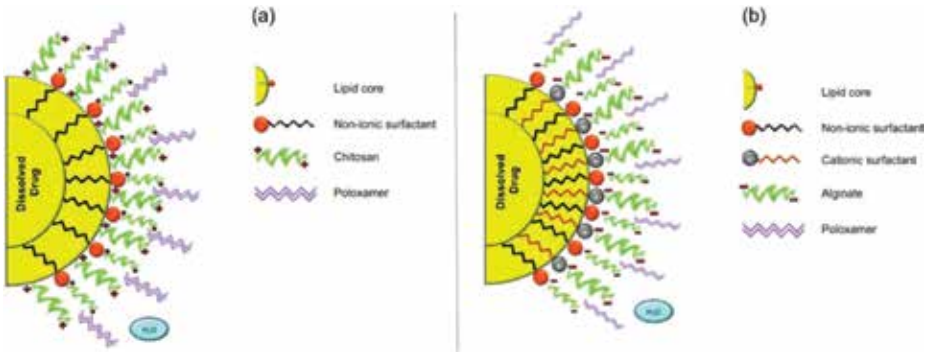

Figure 5. Structural illustration of NC-CS (a), NC-ALG (b) and its components.

Formulation	Size (nm)	PDI	ζ potential (mV)	%E. E.	pH
NC-NC	116.7 ± 3.2	0.107	24.4 ± 2.1	>98	4.67 ± 0.08
NC-ALG	178 ± 7.9	0.149	−49.0 ± 2.3		6.08 ± 0.06

PDI: polydispersity index; EE: encapsulation efficiency. Values are given as mean ± SD; n = 3.

Table 8. Physicochemical characteristics of the nanocapsules obtained.

The systems were characterized physicochemically in terms of particle size, surface charge, polydispersity index (PDI) and curcumin encapsulation efficiency, (**Table 8**). Particle size and (PDI) were determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nanoseries 3600 (Worcestershire, UK). The zeta potential values were calculated from the mean electrophoretic mobility values, as determined by Laser Doppler Velocimetry (LDV) using a Malvern Zetasizer Nanoseries 3600 (Worcestershire, UK). The particle size of NC-CS was round 116.7 nm with a PDI of 0.107 and presented positive surface charge (24.4 mV) while NC-ALG was round 178 nm with a PDI of 0.149 and a negative surface charge (−49.0 mV). Curcumin encapsulation efficiency was determined indirectly by Centrifugation-Filtration. Quantification of curcumin was performed by high performance liquid chromatography (HPLC, Merck-Hitachi, Japan) at 425 nm, using a reverse phase Hypersil® Division C8 column (150 × 3 mm, 5 μm; ThermoQuest, Hemel Hempstead, England). Curcumin encapsulation efficiency of both formulations, was >90%, with a final concentration of curcumin around 750 μg/ml [Unpublished work from our laboratory].

The stability to storage conditions is a parameter that must be evaluated in nanoparticulate systems (**Table 9**). In that study, the storage stability of NC-CS and NC-ALG was around 3 and 2 months respectively. In the case of NC-Cs, after 3 months of storage, the decrease in particle size and the precipitation of CUR were presented with greater magnitude since the chitosan begins to hydrolyze gradually and the viscosity of the formulation based on nanocapsules decreased during the storage period [83]. On the other hand, the results obtained for NC-ALG suggest that the stability of this type of formulation is around 2 months [Unpublished work from our laboratory]. These results are very similar to those reported in other studies, in which they report that the particle size of NC-ALG decreases between month 1 and 5 of storage [84].

Formulation	NC-CS			NC-ALG		
Time (months)	**1**	**2**	**3**	**1**	**2**	**3**
Z-average (nm)	115.2 ± 2.9	101.3 ± 4.1	95.45 ± 3.0	161.9 ± 2.1	157.7 ± 4.77	149.3 ± 2.9
PDI	0.115	0.145	0.196	0.178	0.213	0.245
ζ potential (mV)	23.8 ± 2.1	23.9 ± 3.2	24.5 ± 2.8	−48.7 ± 1.9	−46.8 ± 2.5	−44.3 ± 2.7

Values are given as the mean ± SD; n = 3.

Table 9. Physicochemical characteristics of the nanocapsules during stability studies under storage conditions (4°C).

An important parameter that was take into account in these nanosystems was the cellular toxicity on caco-2 cells. For this, the conditions for the maintenance of the cell cultures were made according to Déat-Lainé et al. [85] with slight modifications. Before starting the study, the formulations were diluted in cell culture medium (DMEM: Dulbecco's Modified Eagle Medium) in order to obtain the treatments with different polymer concentrations. Cell viability was determined by MTT assay [86]. In **Figure 6**, the results showed that even at high polymer concentrations (500 μg/mL) the cell viability is above 80%. However, it is a fact that the toxicity increases as so does the polymer concentration. Other studies in Caco-2 cells have shown similar results to those obtained in our laboratory and agree that the toxicity of chitosan nanoparticles is due to their physicochemical properties such as size and surface charge and also to the molecular weight of the chitosan and the concentration at which the cells are exposed [87, 88]. In the case of NC-ALG, the toxicity was lower since the interactions between the carboxyl groups of alginate and cell membranes are weaker because they are of the electrostatic type. The toxicity in these systems is more related to the particle size [89]. From the toxicity study, the polymer concentration to carry out the permeability studies was selected. It should be mentioned that this concentration did not compromise cellular viability.

Permeability studies were carried out on a monolayer of caco-2 cells and the quantification of curcumin was performed by UPLC-TQ-ESI-MS/MS (Waters ACQUITY UPLC system, Milford, MA, USA). Chromatographic analysis was performed on a Waters ACQUITY BEH Shield RP 18 column (2.1 × 100 mm, 1.7 μm). The polymer concentration used was 500 μg/mL of each polymer. Results show that the permeability of curcumin increased 28.6 and 14.6 times when it was in NC-CS and NC-ALG respectively, compared to the dispersion of curcumin in cell culture medium (DMEM: Dulbecco's Modified Eagle Medium) [Unpublished work from our laboratory]. The increase in the permeability of curcumin in NC-CS is due to the ability of chitosan to temporarily open the tight junctions, which are related to a decrease in the value of transepithelial electrical resistance (TEER, MERSSTX01 electrode, Millicell ERS-2, Millipore, Billerica, MA, USA) (**Table 10**). The mechanism by which chitosan has this capacity is based on the interaction of its protonated amino groups with cell membranes, followed by a reversible structural reorganization of the binding proteins and a specific redistribution of the actin F cytoskeleton and the ZO-1 protein [90, 91]. Furthermore, it has been reported that particles positively charged, with spherical shape and with a monodisperse population have improved cellular uptake through the caveolae-mediated endocytosis and macropinocytosis pathway

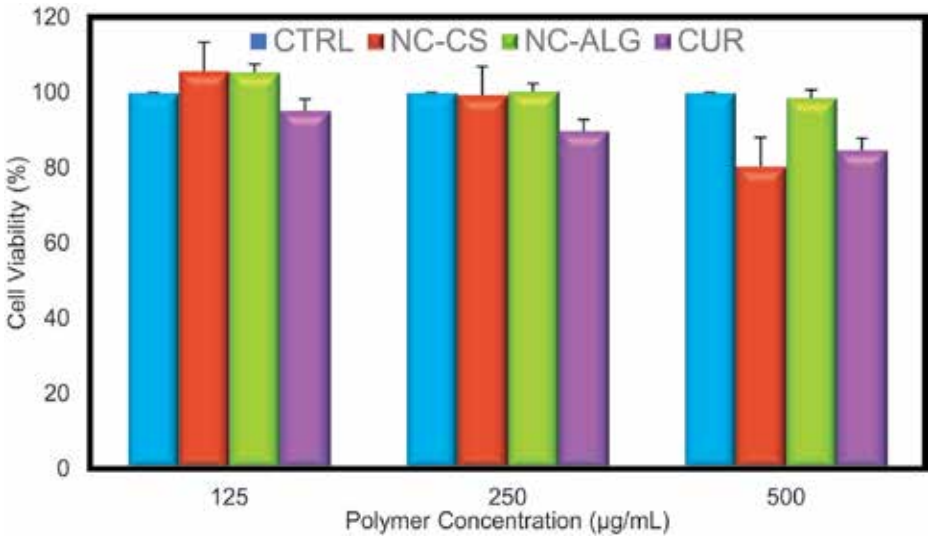

Figure 6. Cell viability by the MTT assay on Caco-2 cells 2 h after the addition of NC-CS, NC-ALG and CUR at different concentrations. Values are given as mean ± SD; n = 3.

Formulation	$P_{APP} \times 10^{-6}$ (cm/s)	R	TEER (%)			
			0 (h)	1 (h)	2(h)	12 (h)
CUR	4.96 ± 0.36	—	100	92 ± 1	93 ± 2	99 ± 4
NC-CS	141.60 ± 37.62	28.6*	100	81 ± 3	82 ± 4	97 ± 6
NC-ALG	72.38 ± 19.33	14.6*	100	90 ± 2	88 ± 6	100 ± 2

Values are given as the mean ± SD;
n = 3.*p < 0.05 significantly different from CUR.

Table 10. Mean apparent permeability (Papp) and the absorption enhancement ratio (R) of NC-CS, NC-ALG and CUR across Caco-2 cells monolayers after 2 h incubation, as well as the values of transepithelial electrical resistance (TEER) determined at different times.

[65, 92]. Meanwhile, the mechanism of passage of NC-ALG through the monolayer of caco-2 cells depends largely on the particle size mainly. So, the main mechanisms are endocytic such as clathrin-mediated endocytosis, caveolae-mediated endocytosis and micropinocytosis [92, 93]. The results suggest that the use of NC-CS and NC-ALG to improve the bioavailability of curcumin is an interesting strategy to enhance the antimicrobial effect. Previous studies using an *in vitro* digestive model that simulates three gastrointestinal compartments of poultry have demonstrated that raw curcumin does not have good antimicrobial activity against *Salmonella enteritidis* [77]. However, when a solid dispersion of curcumin/PVP K30 was used, it decreased the concentration of *Salmonella enteritidis* more than 3 log in the compartment that simulates the intestine [Unpublished work from our laboratory]. Additional *in vivo* studies in 1-day-old chickens challenged with 10^4 CFU of *Salmonella enteritidis*/bird has shown that the solid dispersion of curcumin/PVP K30 administered in the feed at a concentration of 0.1% decreased more than 2 log the concentration of *Salmonella enteritidis* in ceca-cecal tonsil isolates [Unpublished

work from our laboratory]. Since nanocapsules increased the solubility and permeability of curcumin, the antimicrobial activity of nanocapsules loaded with curcumin developed in our laboratory is being carried out both *in vitro* and *in vivo* against *Salmonella enteritidis*.

7. Conclusion

As seen in this chapter, chitin and its derivatives, such as chitosan, are biopolymers with a wide variety of applications in different areas. Chitosan as a functional biopolymer has different properties. Some of these properties are its intrinsic nutritional value, such as antioxidant properties and health-promoting bioactivities against many chronic diseases, including hypercholesterolemia, hypertension, inflammation and immune diseases. In the case of chitin, its application is more limited given its poor solubility in aqueous medium, however, it has been reported that it has practically the same properties as its derivatives.

Every year millions of people are affected and thousands of them die due to infections and intoxication as a result of foodborne outbreaks, which also cause billions of dollars' worth of damage, public health problems and agricultural product loss. A considerable portion of these outbreaks is related to the consumption of contaminated products with foodborne pathogens and mycotoxins. In this sense, one of the main applications of chitosan is its antimicrobial effect against Gram-positive bacteria such as Gram-negative bacteria, having better activity with the latter due to the ionic interaction that takes place between the positively charged chitosan molecules and the negatively charged microbial cell membranes. Studies conducted on chickens and turkeys challenged with Salmonella *enteritidis* and typhimurium show the antimicrobial capacity of chitosan when it is administered in the feed. Furthermore, in vitro studies have demonstrated its properties as an adsorbent, since it can interact ionically with mycotoxins such as AFB1, FUB1, OTA and ZEA given that they are negatively charged, nevertheless, it is a fact that cross-linking is related to a higher adsorption capacity.

Finally, another application of chitosan is its use in nanotechnology for the development of nanoparticles and nanocapsules. These systems are an important strategy to improve the solubility, permeability and stability of molecules that are difficult to formulate. In the case of curcumin, a phytopharmaceutical that has become the subject of study in the poultry industry given its properties, including its antioxidant action, the immunomodulatory, anticoccidial, anti-inflammatory, antimicrobial and growth promotion effects, has problems of solubility and permeability, which causes low bioavailability. However, its association or encapsulation in nanoparticulate systems has shown that the solubility and permeability of this are improved. This suggests that the use of curcumin loaded in chitosan nanocapsules could increase its antimicrobial activity derived from the combination of the effects between chitosan and curcumin on different bacteria.

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Chapter 14

Chitosan: A Good Candidate for Sustained Release Ocular Drug Delivery Systems

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Abstract

This chapter focuses on the eye, one of the most important organs of humans. Current data on pathophysiology of the human eye are presented in direct correlation with a range of therapeutic products, with a well-known and widely used material, namely chitosan. Applications of chitosan biopolymer are described in the development of innovative, modern, therapeutic devices and solutions. Thus, chitosan is a good excipient either for classic drop-type ocular systems, as well as for complex drug systems such as nanostructures (nanoparticles, nanomicelles and nanosuspensions), liposomes, microemulsions, microspheres, in situ hydrogels and inserts or implants. A number of disadvantages for ocular administration of the drugs are thus overcome.

Keywords: chitosan, ocular, delivery systems

1. Introduction

As fascinating as its perfect structure, so difficult to approach due to increased sensitivity and many protective barriers, the human eye continues to be a brainstorming of ideas to formulate and characterize pharmaceutical preparations with optimal action at this level.

The eye can be structured into two large segments: anterior and posterior, the latter representing about two-thirds of the total area. The anterior segment includes the cornea, the conjunctiva, the iris, the lens, the ciliary body and the aqueous humor. Sclera, choroid, retina, vitreous humor and optic nerve are parts of the posterior segment [1].

Following eye drops, the bioavailability of the drug is less than 5% [2] due to factors such as nasolacrimal drainage, lacrimation induction, blink reflexion or corneal barrier [3]. Pharmaceutical formulations given intraocular must be sterile, without pyrogens or endotoxins, isotonic, isohydric and stable. The eye tolerates a pH between 7.5 and 9.5. Alkaline solutions are better supported [4].

Due to the occurrence of diseases such as glaucoma [5], age-related macular degeneration [6], diabetic macular edema [7], diabetic retinopathy [8] or dry eye syndrome [9], which require drug delivery for a prolonged period, it has become necessary to create pharmaceutical formulations that provide sustained release, increased bioavailability with decreased frequency of administration. A significant challenge in achieving this goal is to overcome ocular barriers without causing permanent tissue damage [10].

Introduced on market in 1990, chitosan was the source of numerous studies to harness its potential as pharmaceutical excipient [11]. Obtained by deacetylation of chitin, the second most abundant polysaccharide after cellulose, chitosan consists of D-glucosamine and N-acetyl D-glucosamine linked β-(1-4) [12]. Mucoadhesiveness, biodegradable, biocompatible and non-toxic nature make it a suitable candidate for ocular formulations. Chitosan solutions have pseudoplastic and viscoelectric properties that do not disturb the pre-corneal tear film [13].

New formulations and devices have been obtained to ensure an increased retention time and thus a superior drug delivery system using nanomicelles, nanosuspensions, liposomes, in situ gels, inserts and contact lens [14].

2. Chitosan-based drug delivery systems for ocular administration

2.1. Physiopathology of the eye

The eyeball has a spherical shape and an antero-posterior diameter of about 24 mm. It is structured in to two segments: anterior and posterior (**Figure 1**). The anterior segment of the eye comprises the cornea, conjunctiva, iris and ciliary body, crystalline and aqueous humor [15]. Cornea is transparent, avascular, composed of five layers and provides optimal light transmittance [16]. It continues with sclera through the limbus [17] and the conjunctiva. The conjunctiva is a thin, strongly vascularized, porous [18] membrane where mucus-producing goblet cells are located. The mucin layer interacts with the corneal glycocalyx, facilitating the spreading of the tear film [19]. Aqueous humor provides nutrients needed for the cornea and maintains intraocular pressure at the optimum value [20].

To maintain intraocular pressure at normal values between 12 and 20 mmHg, a proper opening of the anterior chamber angle is required to allow an evacuation of excess through the trabecular meshwork [21]. In the posterior segment of the eye are sclera, choroid, retina, vitreous humor and optic nerve. Choroid has the role of reducing the amount of light that reaches the retina, contributes to thermoregulation through the dissipation of heat and influences the intraocular pressure through the vasculature [22].

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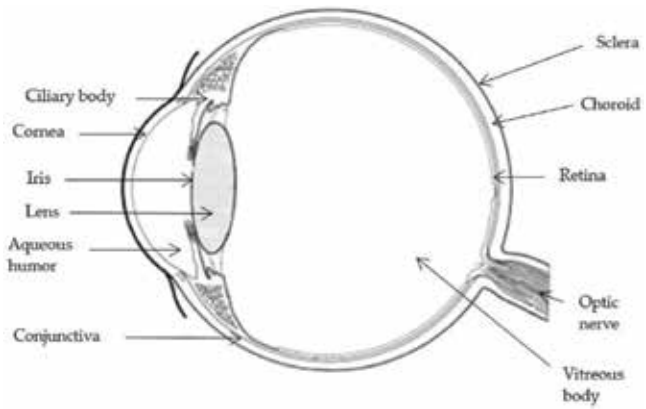

Figure 1. Anatomy of the eye.

The retina is a thin and transparent tissue, made up of 10 layers in which there are two types of receptors: cones and rods. These receptors convert photons into nerve impulse that reaches the brain through the optic nerve [23].

Glaucoma [24–27], conjunctivitis, blepharitis [28], keratitis, dry eye syndrome [29, 30] affect anterior eye segment [31], while posterior segment disorders affecting the vision and even causing complete loss of it: diabetic retinopathy [32], macular degeneration, macular edema and uveitis [33, 34].

Recent studies have made correlations between glaucoma and Alzheimer's disease. Both chronic conditions cause the accumulation of β amyloid associated with inflammatory processes, the appearance of reactive oxygen species and cell apoptosis [35].

The eye is protected by two types of barriers: static and dynamic. Cornea, conjunctiva, ciliary body, aqueous humor and retina are static barriers, while blood flow or lacrimal flow are dynamic barriers. There are situations when their alteration can lead to ocular lesions or hypotonia. The latter consists of penetrating serum proteins into the anterior and posterior rooms with the appearance of edema [36]. Molecules up to 20 kDa can cross the conjunctiva while those up to 5 kDa cornea [37]. In pathological situations, blood retinal barrier alteration causes the permeation of proteins to the retina with the appearance of edema and alteration of vision [38]. In diabetic retinopathy, elevated levels of vascular endothelial growth factor and NO increase the level of reactive oxygen species that generate oxidative stress with neovascularization [39]. The main protector against chemical or microbial aggression is the tear film, a mixture of lacrimal fluid and mucin, an O-glycosylated glycoprotein [40]. It is composed of three different layers [41]. The pH of the tear fluid is about 7.4. It decreases on awakening by the loss of CO_2 resulting from anaerobic metabolism during sleep and increases at contact lens wearers, dry eye syndrome or lacrimal stenosis [42]. Aquaporins play an important role in the transmembranar movements of water through the cornea and conjunctiva in the tear fluid while maintaining the osmolarity of the film [43].

2.2. Chitosan

The benefits of polysaccharides consist of natural abundance, the presence of functional groups available for chemical alterations, and the disadvantages include varied properties depending on the origin, microbial contamination or low microbial resistance [44].

The discovery of chitosan is attributed to Rouget in 1859 when he noticed that he can bring chitin in a soluble form by submitting it to various chemical and thermal treatments [45].

This natural polysaccharide (**Figure 2**) has increased interest because it is non-toxic, biocompatible, biodegradable with various applications in tissue engineering [46–49], food as preservative [50, 51], ruminants' fermentation process [52], in water treatment, medicine and pharmacy as wound dressing [53], implants and medicinal products [54–56]. It is often obtained by deacetylation with an aqueous solution of NaOH from chitin, a polysaccharide from crustaceans' exoskeleton (lobster, crab, squid and shrimp), some fungi and insects [11], insoluble in water but soluble in solutions of dilute acids such as acetic, citric, tartaric and hydrochloric acid at pH < 6.5. It is not soluble in phosphoric or sulfuric acid [57]. This behavior is explained by the protonation of amino groups with the formation of inter-molecular repulsions [11]. It can be dissolved in neutral medium in presence of glycerol-2-phosphate [58].

Biological actions include antimicrobial, antioxidant [59], antiviral [60], antitumoral, antithrombotic and antifungal activity [61]. The positive charge of the molecule binds to the fungal cell membrane, produces an alteration of the K and Ca flux with inhibition of respiration and fermentation [62]. The anti-obesity effect is due to the ability to bind lipids, decreasing their absorption in the digestive tract [63].

Mucoadhesive properties are due to the positive charge that allows interaction with sialic acid from mucin, negatively charged, with the formation of electrostatic bonds [56].

The properties of chitosan are influenced by molecular weight and degree of deacetylation. The biodegradation rate of the polymer is determined by the content in acetyl groups [64]. A degree of deacetylation of 85% or more is preferred due to strong mucoadhesive properties and biocompatibility [65]. In order to obtain oligosaccharides, enzymatic methods are preferred with the use of

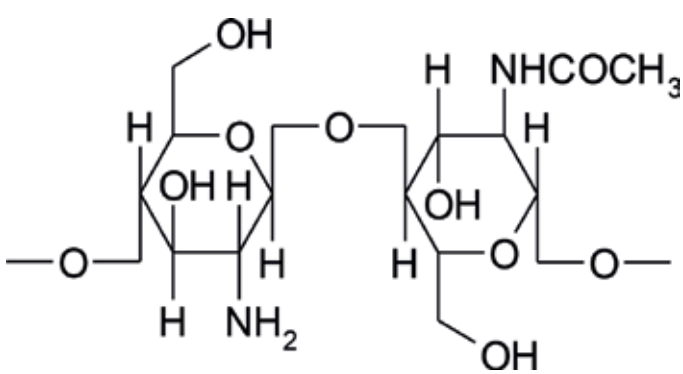

Figure 2. Structure of chitosan.

chitosanases, enzymes with high specificity [66]. Oligosaccharides have anti-inflammatory, antitumoral [67] and antimicrobial action [68].

Low molecular weight chitosan derivatives exhibit water solubility in a wide range of pH, low viscosity and superior biological activities: bactericidal, immunomodulatory, antitumoral, hypolipidemic and hypocholesterolemic [69]. The reactive groups of chitosan are the amino group of C2 and the hydroxyl groups of C3 and C6. Positions C2 and C6 are favorable for substitution. Substitution with carboxymethyl or succinyl groups at this level increases the solubility of the compounds. Due to the presence of a carboxyl group, they can bind calcium, depriving the extracellular matrix of Ca. ions. Thus, they alter tight junctions and its permeability and facilitate paracellular transport through the epithelium. [58]. Chitosan thiolated compounds known as thiomers have strong mucoadhesive properties, increased permeability, antiproteasic activity [70] and inhibit efflux pump [71]. Thiolated derivatives are conjugates with thioglycolic acid or cysteine (**Figure 3**). They exhibit paracellular permeability through the mucosa, forming gels at pH between 5 and 6.8. [72]. Chitosan-N-acetylcysteine has been approved on the market as eye drops under the name Lacrimera, with increased mucoadhesive properties [73].

2.3. Advanced drug delivery technologies

Different strategies have been approached to increase the bioavailability of drug substances at the eye level: increased corneal permeability (prodrugs, permeability enhancers and cyclodextrins), increased viscosity of the vehicle (suspensions, ointments and gels in situ), use of dispersion systems (liposomes, emulsions and nanoparticles), increasing contact time with solid matrix (inserts and contact lenses) [74]. In order to increase eye retention time and reduce the frequency of administration, it is preferred to use natural polymers such as chitosan, gelatin, sodium alginates, sodium hyaluronate, etc. (**Table 1**). At the same time, they are biocompatible, biodegradable and non-toxic [75]. Other advantages of these polysaccharides include natural abundance, nature-friendly materials, relative ease of isolation and low cost [44]. At the same time, they are biocompatible, biodegradable and non-toxic [75]. Other advantages of these polysaccharides include natural abundance, nature-friendly materials, relative ease of isolation and low cost [44].

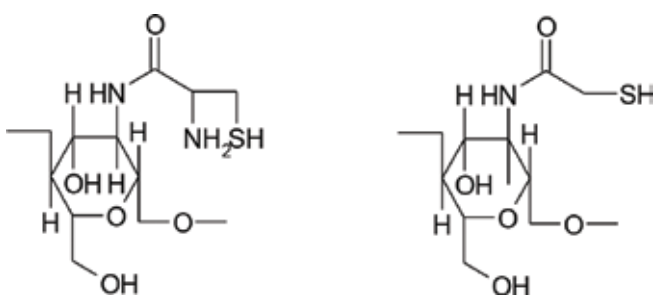

Figure 3. Structures of thiolated chitosans: chitosan-cysteine (left) and chitosan thioglycolic acid.

Polymer	Charge	Solubility	Properties	Ocular dosage forms	References
Chitosan	Positive	Insoluble in water, soluble in solutions of dilute acids such as acetic, citric, tartaric, hydrochloric acid at pH <6.5. It is not soluble in phosphoric or sulfuric acid	Mucoadhesive, biodegradable, biocompatible and non-toxic, pseudoplastic and viscoelastic properties similar to tear film.	In situ gels, nanoparticles, liposomes, micelles microspheres, inserts,	[13, 57, 76]
Sodium hyaluronate	Negative	Soluble in water at room temperature and acidic pH	Biodegradable, viscoelastic properties	In situ gels	[75, 77]
Carrageenan	Negative	Soluble in water, insoluble in organic solvents	Gelling, thickening and stabilizing properties, gelification in presence of Ca^{2+}	In situ gels, microspheres	[58, 75, 78]
Sodium alginate	Negative	Soluble in water, acidic pH. Divalent cations decrease solubility	Gelification in presence of Ca^{2+}, low toxicity, biocompatibility, biodegradability	Ocular mini-tablets, microspheres	[58, 75, 77]
Dextran sulfate	Negative	Soluble in water	Viscosifying, emulsifying, texturizing, stabilizing properties. Excellent biocompatibility and clinical safety	In situ gels	[58, 75, 78]
Colagen	Amphoteric	Soluble in acidic pH	Very compatible with ocular tissues	Ocular films, ocular inserts	[75, 78]
Gelatin	Amphoteric	Soluble in water	Excellent biocompatibility, ease of processing and availability at low cost	Ocular films	[75, 78, 79]
Xanthan gum	Negative	Soluble in water, insoluble in organic solvents	Swelling in basic environment	Viscosity enhancing solutions, gels	[58, 75]

Table 1. Natural polymers used in ocular drug delivery systems to increase eye retention time.

Chitosan increases contact time with cornea, the most commonly used are low molecular weight derivatives [80]. Nanotechnology has been developed to overcome eye barriers and protect active substances [81]. Mucoadhesive nanocarriers increase eye contact time and act as permeability enhancers (**Figure 4**) [82–84].

Thus, innovative formulations have been developed for the anterior segment of the eye, such as preparations based on semifluorinated alkanes applied easy as drops or spray [85], micelles, in situ gels, liposomes, contact lenses [86], inserts [87], dendrimers [88, 89], mini-tablets [90], microspheres [91], nanowafers [92], ocular ring [93] or punctal plug systems [94]. For the posterior segment: micro, nanoparticles, hydrogels, implants and microneedles [95–98].

Characterization of ophthalmic pharmaceutical forms is performed by in vitro and in vivo tests. Determinations include sterility, pH, particle size, viscosity, stability, active substance content and in vitro release. Toxicity studies include the Draize test [99] and the Hen's egg test chorioallantoic membrane (HET-CAM Test) [100]. Particularly, the oxygen permeability is determined for

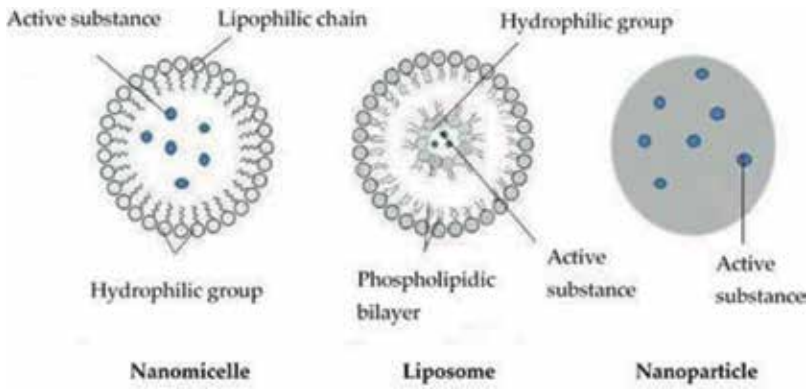

Figure 4. Comparison between different nanostructures.

the lenses, and for the inserts and the contact angle [101]. Measuring the degree of drug release in vitro is vital in the development of a pharmaceutical product, the best known way being with Franz's diffusion cell [102, 103] In a Franz cell, consisting of two compartments separated by an artificial membrane and filled with simulated biological fluid, the formulation to be analyzed is placed. Holding at 37°C, samples are taken at certain time intervals and analyzed to determine the concentration of the substance that crossed the membrane [104].

2.3.1. Nanoparticles

In nanotechnology, the particle size should be between 30 and 200 nm, they should be stable, biocompatible and biodegradable [105]. Chitosan nanoparticles are formed spontaneously by mixing a solution of chitosan with tripolyphosphate (TPP) to form inter and intramolecular bonds. The main mechanism underlying the incorporation of active substances is the occurrence of electrostatic interactions with positively charged chitosan or negative TPP [106].

Basaran et al. have prepared and evaluated chitosan nanoparticles to enhance the ocular permeability of ornidazole for the treatment of bacterial ocular infections. These were prepared by spray-drying method. The nanoparticles were analyzed by morphology, pH, concentration in active substance, in vitro release profile. In 24 h, 98% of the amount of ornidazole was in the simulated biological medium. The authors consider the formulation to be safe and effective for the release of ornidazole at the posterior segment [107].

For the treatment of bacterial endophthalmitis, Silva et al. incorporated daptomycin into chitosan nanoparticles. The preparation was carried out by the ionotropic gelling method, which was subsequently evaluated together with antimicrobial efficiency and stability in the presence of lysozyme and mucin. Using SEM, the particle size was evaluated at about 200 nm. The degree of incorporation varies between 80 and 97%. Total daptomycin release was achieved in 4 h. Incubation with lysozyme did not affect the integrity of nanoparticles [108].

The efficacy of the chitosan-alginate nanoparticles loaded with betamethasone Na phosphate in the treatment of macular edema was studied. With particle size between 16.8 and 692 nm, a rapid initial release was noted, followed by a slow release during 24–72 h [109].

Chitosan nanoparticles were formulated and evaluated by Selvaraj et al. as a potential acyclovir release system at the eye for the treatment of viral diseases. Nanoparticles were prepared by ionic gelling and characterized by SEM, DSC and FTIR. The particle size was between 200 and 495 nm, the encapsulation efficiency was between 56 and 80% and the loading capacity was 10–25%. In vitro release studies demonstrated a sustained release for 24 h, the kinetic release profile following the Higuchi model [110].

The study tracks the potential of montmorillonite in the preparation of prolonged ophthalmic nanoparticles. The nanoparticles were prepared by ionic gelling of chitosan with sodium tripolyphosphate. With a spherical shape between 358 and 585 nm and an incorporation efficiency of between 12.27 and 50.92%, nanoparticles release betaxolol within 10 h, being effective in the treatment of glaucoma [111].

The sustained release of celecoxib from the nanoparticles of chitosan and alginate was proposed by Ibrahim et al. Various blends of polymers were prepared in varying proportions in order to obtain the optimal formulation with the smallest particle size and the highest potential zeta.

Nanoparticles were included in collyria, in situ gels and preformed gel. With TEM, spherical particles with an incorporation efficiency of over 75% have been shown. The release of active substance followed the Higuchi model, and the formulations proved to be non-toxic according to in vivo studies [112].

2.3.2. Nanomicelles

Nanomicelles, amphiphilic molecules that have the ability to form in an aqueous medium organized supramolecular structures, contribute to the solubilization of hydrophobic active substances.

A positive-load nanomicelle increases the retention time and the permeability due to interactions with the negatively charged eye surface. Changing its surface by the addition of a cationic polymer such as chitosan increases contact time to the eye [113].

Another study has proposed the formulation of pluronic/chitosan nanoparticles whose surface has been modified by adding chitosan in order to increase the ocular bioavailability of metipranolol. Nanomicelles were analyzed by diameters, morphology, turbidity, stability and in vitro release. The drug nanoparticle size ranged from 123 to 232 nm with a zeta potential between 6.1 and 9.2 mV. According to the turbidity test, the micelles were stable, preventing the vision from collapsing. The release was 88% in 6 h [114].

A study designed to evaluate rapamycin ocular release from octanoyl-g-chitosan-g-PEG nanomaterials was initiated by Somavarapu et al. Micelle size was determined using dynamic light scattering (DLS), surface morphology with transmission electron microscopy (TEM) and thermal properties with differential scanning calorimetry (DSC). The concentration in the active substance was determined by the HPLC method. Following the study, nanomicelles with a size of 52 nm were obtained and positively charged. The formulation remained stable for 3 days. On visual analysis the preparation is clear with a dispersion index of 0.25. Tissue retention was 24 h [115].

2.3.3. Nanosuspensions

Shi et al. have formulated a chitosan and methoxy polyethylene glycol-poly (β-caprolactone) nanosuspension for the ophthalmic delivery of diclofenac. Nanosuspension was characterized by FTIR, X-ray diffraction and DSC. Nanosuspension was stable at 4 and 25°C for 20 days. Prolonged release of diclofenac was achieved for 8 h without irritation [116].

A nanosuspension of chitosan, sodium alginate and tripolyphosphate was developed as an efficient delivery system of lomefloxacin. Nanosuspension was evaluated for particle size, zeta potential, incorporation efficiency and permeability through the bovine cornea. The incorporation efficiency of the active substance was 70.63%, particle size 176 ± 0.28 nm, zeta potential 13.65 mV. Nanosuspension releases lomefloxacin for more than 8 h and a three-fold increase in bovine corneal permeability to solutions is noted. Also, administration of lomefloxacin in the form of nanosuspension provides the advantage of a prolonged action, protects against enzyme metabolism and increases corneal permeability. Chitosan possesses antimicrobial activity, potentiating the effect of the antibiotic [117].

A chitosan-based nanosuspension with the active substance itraconazole is prepared by co-precipitation. It has been noticed that co-precipitation of itraconazole from the chitosan- lysine system in the presence of poloxamer 100 as a stabilizer causes a nanosuspension with the smallest size, increases drug solubility 12-fold and a very fast in vitro release. Comparative assessment with a commercial suspension determines a significantly increased permeability on the goat's cornea in the first case [118].

2.3.4. Liposomes

Introduced as drug carriers in 1968 [114], liposomes are membrane vesicles composed of one or more phospholipidic or cholesterol layers designed to transport drug substances incorporated either into the core or into one of the layers [36]. They are biodegradable and biocompatible, increasing the permeability of the drug with increasing retention time. These can be administered at both the anterior and posterior segment.

Chitosan-coated liposomes, called chitosomes, increase ocular retention with decreased metabolism of drug substances. Coating liposomes with quaternary ammonium chitosan derivatives such as N-trimethylchitosan reduces particle aggregation due to steric stability and increases mucoadhesiveness [119].

Liposomes with an incorporation efficiency of more than 90% bromfenac were prepared for targeting the retina. Changing liposome surface with chitosan improves mucoadhesive properties. The optimal concentration of chitosan that prevents liposome aggregation was determined at 0.15% [120].

A potential carrier for ocular drug release were low molecular weight chitosan-based liposomes formulated by Li et al. Liposomal morphology was examined with TEM, and cytotoxicity was assessed in rabbit conjunctival cells. By incorporating cyclosporin A, a delayed release profile was revealed as compared to un-coated liposomes. In vivo studies showed that the concentration of cyclosporin in different ocular tissues increased over 24 h [121].

The objective of the study initiated by Ustundag-Okur et al. has been exploiting the potential of nanostructured lipid carriers with chitosan for ocular application of ofloxacin. Particle characterization involved determining the size, potential zeta, viscosity, incorporation efficiency, active substance load or sterility. According to the authors, the system has a 48-h corneal retention time and a substance incorporation efficiency of over 97%. Chitosan improves transcorneal permeability [122].

2.3.5. Microemulsions

The use of microemulsions as drug delivery systems offers advantages such as thermodynamic stability, increased eye retention, improved absorption, incorporation of substances in any of the two phases [123].

Bhosale et al. have formulated several chitosan-based microemulsions as a potential voriconazole release system at the eye level. The formulations were evaluated for thermodynamic stability, physico-chemical parameters, in vitro and in vivo release studies. All the formulations have a particle size of less than 250 nm, potentially zeta positive. In vitro delivery tests have shown that the formulations have a sustained release of over 12 h compared to market formulations. Following in vivo studies in rabbits, it was concluded that the formulations showed an active substance concentration of more than 47% in aqueous humor at 4 h after administration compared to the product Vozole with a voriconazole concentration of approximately 20% [124].

The evaluation of the tear retention of a chitosan-based emulsion containing indomethacin was carried out by Yamaguchi et al. This was compared to a non-chitosan emulsion after instillation in rabbits. The chitosan emulsion has an average concentration of 3.6 and 3.8 higher than that without chitosan at 0.5 and 0.75 h after instillation. The average residence time and half-life for the chitosan emulsion were 1.5 times and 1.8 times higher than the comparative emulsion. It has been appreciated that the chitosan emulsion has a prolonged lacrimal retention time and a wide distribution on the ocular surface due to the mucoadhesive properties of chitosan [125].

2.3.6. Microspheres

Chitosan microspheres determine a controlled release of drug substances and increase the bioavailability of drugs, improving the absorption of hydrophilic substances at epithelial level. They facilitate the transport of substances to the eye or accumulation at the corneal or conjunctival level [126].

Chitosan-based microspheres loaded with ganciclovir were prepared by Kapanigowda et al. Characterization of the formulation was achieved by in vitro release studies, release kinetics and stability of microspheres. The degree of eye irritation, pharmacokinetic parameters and histopathology were evaluated on Wistar rats. In vitro release studies showed an initial burst in the first few minutes, the diffusion following Fick's law. Stability studies were favorable and it was determined that in 75 h, three administrations of this formulation were needed compared to six administrations of ganciclovir as a solution [127].

A study initiated by Rajawat et al. has proposed to develop chitosan and chitosan-N-acetyl cysteine-based microspheres as possible ocular delivery system for acyclovir. The formulations were prepared using emulsification crosslinking process, the microspheres having an active substance incorporation efficiency of 97.86 ± 2.06% for the chitosan microspheres and 76.99 ± 1.14% for the thiolate derivatives. In vitro release studies showed an initial burst followed by a sustained release of acyclovir for 12 h, and in vivo studies did not indicate signs of ocular toxicity [128].

2.3.7. Hydrogels in situ

In situ gels have shown interest since the 1970s.The first gel was synthesized by Kopecek in 1971. It still possesses the "smart" name because they respond to the stimulus by a change in physical or chemical behavior.

Hydrogels are defined as three-dimensional structures that absorb water in large quantities without dissolving into it. Water can not be removed either under pressure [58]. For example, administration of timolol in the form of drops requires two administrations per day, and only one application per day as a gel [129].

Chitosan dissolved in acidic solution and neutralized with β-glycerophosphate undergoes a sol-gel transformation at body temperature, favoring the transfer of protons from chitosan to the weak base.

Because of the amino-positive groups, it is able to interact spontaneously with anionic polymers, forming polyelectrolyte complexes (PECs) with an increased tendency to form hydrogels: chitosan-chondroitin sulfate, chitosan dextran sulfate (**Figure 5**, chitosan alginate [130]. A gel based on chitosan and dextran sulfate was proposed for the ciprofloxacin release study. It has been chemically characterized, morphologically, in terms of stability and concentration in the active substance. Among the analytical techniques used are FTIR, SEM and DSC. Ciprofloxacin release in simulated lacrimal fluid was determined using a UV-Vis spectrometer. The eye tolerance test was evaluated using HET-CAM (Hen's egg test chorioallantoic membrane). The result of the study was a non-irritating product that provides ciprofloxacin release for 21 h in the treatment of susceptible germs infections [131].

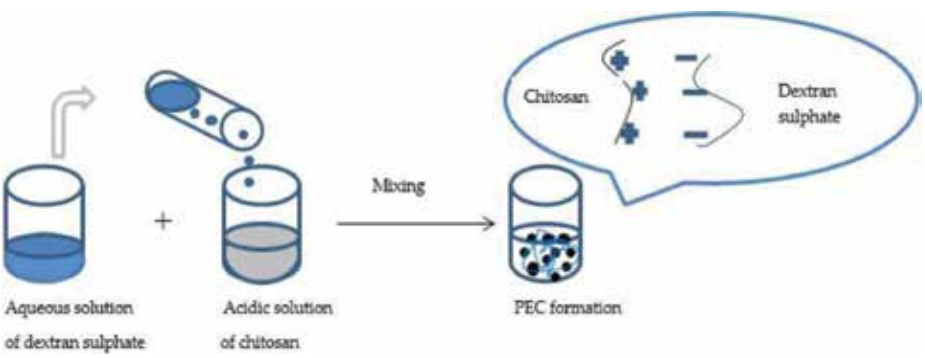

Figure 5. Steps in formation of chitosan-dextran sulfate gel, illustrating the technique described by Jain et al. [131].

The main advantage of this type of gels is the sustained release of the active substance and the absence of blurred vision. Due to the increased contact time with the eye surface, the bioavailability of the active substance is increased, the frequency of administration is reduced [132].

A gel composed of 15% pluronic and 0.1% chitosan with a ciprofloxacin's release efficiency of 46.61 ± 0.41% and a time release of 1.94 ± 0.27 h was developed by Varshosaz et al. Ciprofloxacin release was determined by the dissolution method in artificial tear solution up to 8 h, and the samples were analyzed spectrophotometrically at 272.4 nm. Rheologic behavior and phase transition temperature (PCT) were determined using a Cup and Bob viscometer. The formulation was kept liquid at pH 4 and 25°C and gel transformed to pH 7.4 and 37°C [133].

From several formulations analyzed, Gupta et Vyas proposed a mixture of 0.4% Carbopol and 0.5% chitosan as an optimal ocular drug release system for timolol maleate. It is in a liquid state at room temperature and pH 6 and is a gel under the action of tear fluid at pH 7.4. The formulations were analyzed: pH, viscosity, swelling capacity and concentration in active substance. According to the studies, substance delivery followed Fick's law for 24 h [134].

Zaki et al. attempted to incorporate ketorolac tromethamine into various hydrogels for ophthalmic administration. As polymers, chitosan and Carbopol 940 were used in different concentrations. The visual aspect, pH, viscosity, in vitro delivery behavior and stability were analyzed. The best formulation according to the authors would be the one with 0.5% chitosan in composition [135].

A gel based on chitosan and dextran sulfate was proposed for the ciprofloxacin release study. It has been chemically characterized, morphologically, in terms of stability and concentration in the active substance. Among the analytical techniques used are FTIR, SEM and DSC. Ciprofloxacin release in simulated lacrimal fluid was determined using a UV-Vis spectrometer. The eye tolerance test was evaluated using HET-CAM (Hen's egg test chorioallantoic membrane). The result of the study was a non-irritating product that provides ciprofloxacin release for 21 h in the treatment of susceptible germs infections [136].

The aim of a study initiated by Gilhotra et al. is to evaluate the alginate-chitosan eye film with atenolol in the treatment of glaucoma. The study showed that the addition of Ca gluconate leads to an increased release of atenolol from the chitosan-alginate matrix without the desired sustained effect [130].

Another study proposes a corneal membrane composed of chitosan and collagen. The membrane was prepared by dissolving chitosan in collagen in varying proportions, followed by the addition of 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide as a crosslinker. The membrane was characterized in terms of mechanical properties, contact angle and optical transmittance. In vitro cell culture studies have shown that collagen does not influence cell morphology, viability with good compatibility [137].

Fabiano et al. formulated a chitosan and β-glycerophosphate gel for incorporation of transcorneal 5-fluorouracil nanoparticles. The sol-gel transition takes place in the range of 30–35°C. The concentration in active substance is kept constant for 7 h after administration. The system is a potential candidate for optimal 5-fluorouracil release at eye level [138].

2.3.8. Inserts and implants

Intravitreal injections are the most common method of administering drugs to the posterior segment of the eye. They can be indicated in conditions such as age-related macular degeneration (AMD) with monoclonal antibodies such as bevacizumab (Avastin) or ranibizumab (Lucentis).

An alternative to injections is ophthalmic implants such as Vitrasert (ganciclovir), Retisert (fluocinolone acetonide), Iluvien (fluocinolone acetonide) and Ozurdex (dexamethasone) [139]. Ozurdex is bioerodible [140].

Ophthalmic inserts are solid, semi-solid, sterile, thin, multilayer, impregnated with active substance and placed on the conjunctival sac. Following studies, they have demonstrated increased retention time, sustained release for a longer period of time, dosage accuracy, reduced frequency of administration and lack of preservatives with irritant potential. They can be classified as solubles (with natural or synthetic polymers, insolubles (Ocusert—diffusion mechanism of release; or soft contact lenses—osmosis mechanism) and bioerodibles (Lacrisert) 6 [141].

Chitosan-based ocular inserts have been designed as an alternative to the release of brimonidine tartrate in the treatment of glaucoma. Characterization of inserts was performed from an analytical point of view using FTIR, SEM and DSC. Swelling capacity, active substrate release profile, in vitro bioavailability on Muller cells were also studied. The results of the study were that brimonidine tartrate was physically dispersed between the polymer chains. The inserts release the active substance for 30 days without adverse effects. They also have the advantage of being free of preservatives [142].

Foureaux et al. studied the effects of some antiglaucoma inserts from chitosan. The inserts having diminazene aceturate as active substance were prepared by casting technique and analyzed for swallow capacity, analytically for FTIR, DSC and SEM. Quantification of the active substance from the inserts was performed with the UV-Vis spectrometer and in vitro release studies using a Franz cell. The authors concluded that inserts reduce intraocular pressure by up to 4 weeks [143].

Upadhyaya et al. prepared chitosan-based inserts by casting method for levofloxacin release at the eye level. It has been observed that PVP addition increases levofloxacin release rate. Based on in vitro delivery studies, it was concluded that ocular inserts are suitable for the release of the active substance over 24 h and are useful in the treatment of bacterial infections [144].

The purpose of the study initiated by Franca et al. is to evaluate the effectiveness of some chitosan-based inserts with bimatoprost. The sustained release of the active substance is performed according to in vitro studies at 8 h, which recommends it as a potential alternative in the treatment of glaucoma [145].

2.3.9. Contact lenses

Theoretically, ocular administration of active substances through contact lenses is 35 times more effective than eye drops.

Soft contact lenses are generally made of hydrogels due to their biocompatibility and transparency.

Incorporation of the active substances is accomplished by wetting the lenses with a drug solution, inclusion in a polymeric mixture or in a colloidal structure such as nanoemulsion, nanosuspension, liposomes dispersed in the lens, ligand grafting on the hydrophilic matrix with the formation of inclusion complexes with the drug [146]. If the drug's affinity for the lens is too high, the formulation is stable, but the release is difficult. If the drug is weakly retained by the lens, the release is rapid, followed by a steep decline [147].

Hydration is required when using contact lenses, allowing oxygen to penetrate the cornea. Since the lack of hydration results in dry eye syndrome [148], it is recommended to use contact lenses in association with eye drops [149].

Several advantages are attributed to the use of hydrogel contact lenses: good light transmission, chemical stability and high mechanical properties, increased permeability for oxygen [150].

Behl et al. proposed to increase eye bioavailability of dexamethasone by incorporating it into chitosan nanoparticles which were subsequently imprinted in pHEMA hydrogel contact lenses. Particle size was analyzed by SEM, interactions between dexamethasone and nanoparticles by FTIR. They also studied in vitro release studies. Obtaining an average transmittance of 95–98% demonstrates lens clarity, and dexamethasone release was 55.75% in 22 days. According to the study, the bioavailability of dexamethasone was 72% compared to eye drops within the first 10 days. The conclusions of the study were that the application of contact lenses with chitosan nanoparticles in which dexamethasone was incorporated, leads to therapeutically positive responses [151].

The association of chitosan and gelatin has been shown to be beneficial in the preparation of contact lenses according to Xin-Yuan et al. The film was characterized by permeability, transmittance, water absorption and mechanical properties. The study demonstrated that the film is biocompatible, transparent, permeable and gelatin association has increased water absorption and oxygen permeability [152].

Wearing contact lenses can create certain problems, so Hu et al. have proposed the assembly of a chitosan/hyaluronic acid multilayer on the surface of the lens in order to improve the surface properties such as wettability or deposition of proteins. The chitosan/hyaluronic acid multilayer was loaded with norfloxacin and timolol, respectively. It was observed that the multilayer steadily releases norfloxacin in 1 h, and timolol in 30 min. The purpose of this study is to increase the hydrophilic character of the lenses, increase the water retention and reduce the deposition of the proteins [153].

2.3.10. Mini-tablets

Mini-tablets are devices with a diameter of approximately 2–4 mm inserted into the conjunctival sac. They can gel in the presence of lacrimal fluid or the matrix can dissolve, releasing the active substance [154].

Among the advantages of mini-tablets are easy administration, increased compliance, sustained release, lack of irritation and lack of dilution of drug substance [155].

EL-Gawad et al. prepared ocular mini-tablets based on various polymeric matrices including chitosan for the controlled release of piroxicam. The friability studies showed a 2.36% weight loss in the chitosan mini-tablets, which means they can resist the stresses that occur when administered without producing a foreign body sensation. They also have the ability to quickly disintegrate when administered [156].

Refai and Tag aimed to formulate and evaluate some aciclovir eye mini-tablets to treat keratitis. The spongy nature of the mini-tablets provides fast hydration and gelling at the eye level, reducing foreign body sensation. Several mini-tablets with different polymers including chitosan have been evaluated. Rheological studies have shown pseudoplastic behavior. Optimal release of acyclovir was in the case of chitosan mini-tablets. The chitosan mini-tablets were chosen for the significant sustained release of acyclovir and bioadhesive properties, and the corneal permeability is superior to the Zovirax ointment [157].

Verestiuc et al. were prepared acrylic-functionalized chitosan hydrogels with N-isopropyl acrylamide or 2-hydroxyethyl methacrylate monomers, then pressed to obtain mini-tablets. These have been evaluated for the controlled release capacity of some drugs at the ophthalmic level. By comparison, interpolymeric complexes and pure chitosan were analyzed. The effects of the structure and composition of the network on the properties of swelling, adherence and release of active substances such as chloramphenicol, atropine, pilocarpine or norfloxacin were studied. In vivo studies in rabbits which received pilocarpine indicated that mini-tablets based on chitosan and 2-hydroxyethylmethacrylate are optimal carriers for the delivery of the therapeutic agent [158].

Another study aims to develop and study mini-tablets of sodium alginate, calcium gluconate and chitosan for the purpose of ocular delivery of gatifloxacin. In vivo tests and irritation studies were performed on rabbits. The release was 95–99% on 6–24 h according to the authors. It has been observed that this is enhanced by the increased addition of calcium gluconate. Also, the mini-tablets have been found to be non-irritating and the chitosan and alginate mini-tablets have good antimicrobial properties [159].

3. Conclusions

The human eye is a small, sensitive and complex organ that represents a continuous challenge in pharmaceutical research. The reduced bioavailability (below 5%) of drug substances as eye drops due to factors such as nasolacrimal drainage, blinking reflexes or ocular barriers has made it necessary to develop new ways of administration. Due to its properties, chitosan is considered a good candidate as an excipient in various pharmaceutical formulations for ocular administration. It is biocompatible, biodegradable and non-toxic. It has mucoadhesive properties by interacting with sialic acid residues from the mucin structure and pseudoplastic and viscolectric properties similar to lacrimal fluid. Thiolated derivatives, called thiomers, have enhanced mucoadhesive properties and improve the permeability of active substances through ocular barriers.

The use of chitosan in ophthalmic delivery systems such as nanoparticles, nanomicelles, nanosuspensions, liposomes, microemulsions, microspheres, in situ gels, inserts, contact lenses or mini-tablets increases the retention time of the active substance at the eye level with enhancing its bioavailability. Thus, it will decrease the frequency of administration and will increase patient's compliance with improving his quality of life. These chitosan-based systems do not cause irreversible alterations in ocular barriers, do not damage the tissues, or interfere with tear fluid.

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Chapter 15

Antifungal Activity of Chitosan against Postharvest Fungi of Tropical and Subtropical Fruits

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Additional information is available at the end of the chapter

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Abstract

In the present chapter, results about the efficacy of chitosan (Chi) on sporulation, mycelial growth, germination, as well as quality parameters on fruits are shown. The results demonstrate that chitosan can control various phytopathogen isolates from diverse fruits. The pathogens in the genera *Colletotrichum*, *Fusarium*, and *Rhizopus* are involved in important postharvest disease losses throughout the world. In Nayarit, producers had reported high postharvest losses not only at field but also during the commercial chain with their products, besides the resistance of several pathogens to fungicides, which traditionally are applied for controlling diseases. In this sense, the aim of this research group is focused on the research of alternative and effective methods for controlling postharvest diseases. In vivo results are promising due to a good control in important tropical fruits like banana, avocado, mango, and jackfruit. An enhancement in the chitosan antimicrobial activity is reported with the combination with GRAS substances, as well as the use of nanotechnology. Chitosan can be an environment-friendly alternative to the use of chemical fungicides for controlling postharvest diseases in fruits.

Keywords: chitosan, fruits, fungal growth, vegetables

1. Introduction

Mexico is an important exporter of fruits worldwide [1]. However, important postharvest losses have been reported. In this sense, postharvest diseases represent a major factor of losses during storage and shelf life of produce, due to the deterioration of quality and microbial contamination [2]. In many countries traditionally, postharvest decay control is obtained using chemical fungicides, but nowadays consumers are concerned about food safety and environmental issues [3]. The use of antimicrobial packaging can be effective during the storage period, handling, or transport of fruits [4]. In recent years, various investigations have reported the efficacy of the application of chitosan (Chi) in the control of postharvest pathogenic microorganisms, due to the diverse properties like the ability to form films, biodegradability, antimicrobial properties, and the elicitor function [5]. Chitosan has become a useful compound due to its fungicidal effect and its induction of plant defense mechanisms for controlling postharvest diseases of fruit and vegetables [6]. In a previous study, chitosan was applied successfully on strawberry (*Fragaria x ananassa*); the coating decreased the respiratory rate, reduced water losses, as well as preserved the firmness during storage time on treated fruit [7]. Besides, previous studies reported resistance-inducing properties of chitosan in the form of defense responses (enzymes POD, PPO, and PAL) in fruits [8]. The objective of this chapter article was to summarize information about the application of chitosan with other alternative methods, including GRAS substances and the use of nanotechnology, against important fungi that affect tropical and subtropical fruits.

1.1. Fruits and vegetables

1.1.1. Health issues

Nowadays, an interest in the health benefits of fruit and vegetable consumption is increasing. The easy consumption, the good taste, and the nutritional value of fresh fruits and vegetables are important characteristics that have allowed consumers to be more aware about the benefits of a healthy diet. The consumption of fruits and vegetables contributes to the wellness and nutritional health of consumers, due to their high content of phytochemicals as well as other components that may act synergistically with phytochemicals (ascorbic acid, carotenoids, and phenolic compounds) [9].

1.1.2. Production

Worldwide, the main tropical fruit producers and export countries are the Far East, Latin America, and the Caribbean, most of which are developing countries, while a high percentage of developed countries are importers of these fruits. The main tropical fruits for exportation are mango, pineapple, papaya, and avocado, which represent approximately 75% of the exportation of fresh tropical products [10]. The postharvest losses of fruits and vegetables caused by microorganisms worldwide are of the order of 5–25% in developed countries and 20–50% in developing countries; in developed countries they have technologies that allow to diminish or avoid the attack of microorganisms.

1.1.3. Postharvest losses

The main causes of quantitative postharvest fruit losses are classified as crop and harvest practices, availability and conditions of transport, pests and infections, climatic conditions, consumer preferences and attitudes, infrastructure, as well as financial availability of the markets [11]. Fruits can be infected at field or during the postharvest management [12]. Diseases are the principal cause of postharvest losses in tropical and subtropical fruits; anthracnose is the main postharvest disease in various tropical fruits caused by *Colletotrichum gloeosporioides* [13].

1.2. Chitosan: origin, structure, and antimicrobial properties

Chitosan is a polysaccharide derived from chitin, which is the second most abundant polysaccharide in the world, after cellulose [14]. Chitin is a polysaccharide of animal origin and is the main constituent of the outer skeleton of insects and crustaceans like shrimp, crabs, and lobster [15]. Chitosan is the *N*-deacetylated derivative of chitin [16]. The molecular weight of chitosan ranged between 300 and 1000 kDa depending on the source of chitin. Chitosan is a copolymer of N-acetyl-D-glucose amine and D-glucose amine as shown in **Figure 1**.

Important chemical properties of chitosan are as follows: linear polyamine, reactive amino groups, reactive hydroxyl groups available, and chelates metal ions, specially transition metals. Between the biological properties of the chitosan, the most important one is the biocompatibility (natural, biodegradable, safe, and nontoxic) [17]. Various mechanisms of action have been proposed; however, this process is not fully understood. It is important to mention that the antimicrobial activity of the chitosan on pathogenic microorganisms depends on different factors like the strain, molecular weight, concentration, degree of deacetylation, and type of chitosan, among others [5]. The interaction of chitosan with the microorganism

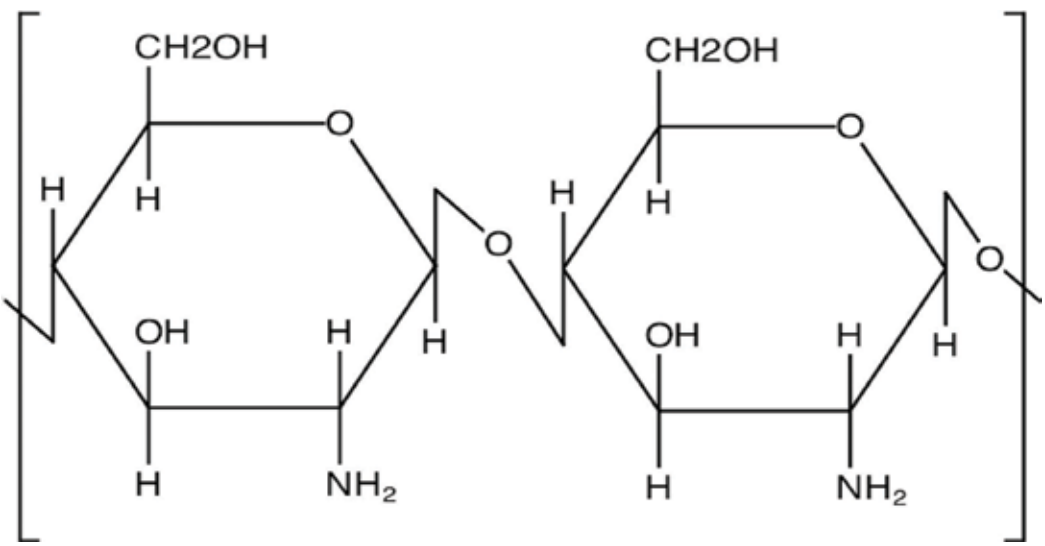

Figure 1. Chemical structure of chitosan.

results in different changes: (a) changes on cell permeability, due to the polycationic nature of the chitosan amino group and the electronegative charges in the outer surface of the fungal or bacteria membrane [18]; (b) affectation on homeostasis (K^+, Ca^{2+}), leading to the efflux of small molecules affecting fungal respiration [19]; (c) microbial starvation, when chitosan acts as chelating agent of metals and essential nutrients affecting microbial development [20]; and (d) inhibition on synthesis of mRNA and proteins, related to their ability to pass through the cell membrane of a microorganism and subsequently bind to DNA [21].

1.2.1. Effects of the antifungal activity of chitosan in the control of postharvest pathogens isolated from various fruits

Chitosan is considered one of the most promising products for the control of several important postharvest fungi in fruits and vegetables [22–25]. In vitro tests used a completely randomized block design. Data were subjected to analysis of variance (ANOVA), and a Tukey test ($p \leq 0.05$) was used for the comparison of means. In our research, the molecular weight of chitosan as well as the concentration used plays an important role in antifungal efficacy against the pathogens tested. Several factors influence the antimicrobial activity of chitosan; among them are the type of chitosan and the concentration [21]. It is reported that the antimicrobial activity of chitosan also depends of the molecular weight, is better when chitosan of low molecular weight and oligochitosan instead of high molecular weight chitosan is applied. In this sense, high molecular weight chitosan cannot pass through the microbial membrane and acts against microbial development [21]. Concerning to the efficacy of chitosan at different concentrations is reported that at lower concentrations, chitosan binds cell surface of microorganisms (negatively charged), disturbing the cell membrane, and causes death of microbial cell by leakage of the intracellular components; however, at higher concentrations, chitosan may coat the microbial surface and prevent the leakage of intracellular components [21].

1.2.1.1. Mycelial growth

The application of chitosan (Chi) at 1.0, 1.5, and 2.0% was effective against the pathogen *Colletotrichum* sp. isolated from banana (*Musa paradisiaca*), 100% of mycelial growth inhibition was observed ($p \leq 0.05$). Conversely, for the pathogen of *Fusarium* sp. isolated from banana, higher concentrations of the chitosan (1.5 and 2%) were applied to obtain a good inhibition (93.2 and 100%, respectively ($p \leq 0.05$)) (**Figure 2**) [26]. In a study, against *Colletotrichum* sp. isolated from banana (*Musa sapientum*), different concentrations where applied (1.0, 1.5 and 2.0%) obtaining a 78.94, 92.1, and 98.68%, respectively ($p \leq 0.05$). Conversely, with the application of chitosan at 0.5%, only 49% of growth inhibition of *Colletotrichum* sp. was obtained ($p \leq 0.05$) [27].

As shown on **Figure 2**, *Colletotrichum gloeosporioides* T147 isolated from avocado (*Persea americana* mill.) c.v. Hass was successfully inhibited as the concentration of chitosan increased, and the percentage of inhibition ranged from 88.85 to 92.97% ($p \leq 0.05$) [28]. In a recent study, the ability of chitosan-pepper tree (*Schinus molle*) essential oil biocomposites against *Colletotrichum gloeosporioides* was evaluated (data not shown) [29]. In this study, an important

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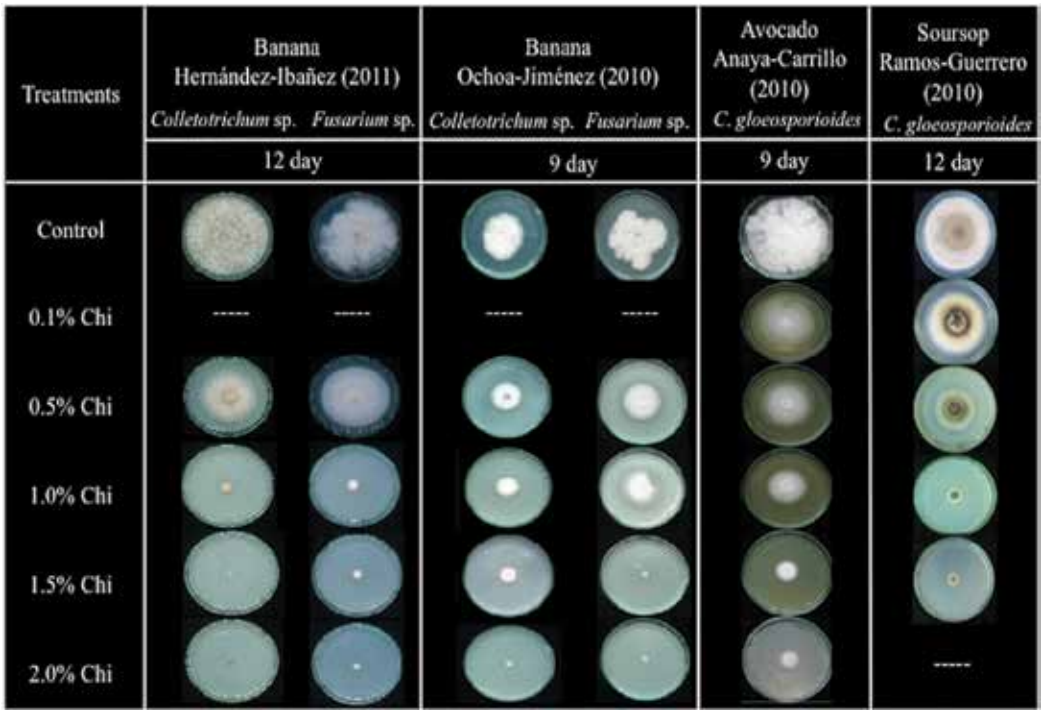

Figure 2. Inhibition of the mycelial growth of pathogens isolated from various fruits by applying chitosan at different concentrations. Control, sterile distilled water; chi, chitosan; –----, concentration not analyzed.

inhibitory effect on the viability of *Colletotrichum gloeosporioides* increased proportionally to the used concentration; the authors concluded that the antifungal activity of the biocomposites against the pathogen can be associated to a synergic effect between the chitosan and pepper tree essential oil. The principal mechanism of pepper tree (*Schinus molle*) essential oil acts against the cytoplasmic membrane, causing it to lose its integrity by assisting the chitosan to enter the interior of the cell. This leads to dissipation of the proton-motive forces and the inhibition of the respiratory enzymes responsible for the cell wall synthesis; this in turn inhibits spore germination and germ tube elongation [30]. Good results were obtained with the application of chitosan at 1.0 and 1.5% against *Colletotrichum gloeosporioides* LSC-120 isolated from soursop (*Annona muricata* L.); up to 90% was achieved using 1.5% of the chitosan ($p \leq 0.05$), whereas this strain was not totally controlled in vitro at low concentrations tested (0.1 or 0.5%) [31]. Similar results were obtained in a study with *Alternaria alternata* isolated from mango (*Mangifera indica* L.) c.v. Tommy Atkins; chitosan treatments at 0.05, 0.1, 0.5, and 1.0% inhibited the mycelial growth of the pathogen by 11.5, 23.1, 55.0, and 70.0%, respectively ($p \leq 0.05$) [32]. *Rhizopus* sp. isolated from jackfruit (*Artocarpus heterophyllus* L.) was controlled (up to 98% ($p \leq 0.05$)) with the application of chitosan in combination with H_2O_2 (peroxide hydrogen) in vitro tests (**Figure 3A**). Synergistic effects were reported with the combinations of chitosan with potassium sorbate or H_2O_2. The pathogen only was totally inhibited with the combinations of potassium sorbate and chitosan at high concentrations of the treatments (**Figure 3B**) [33].

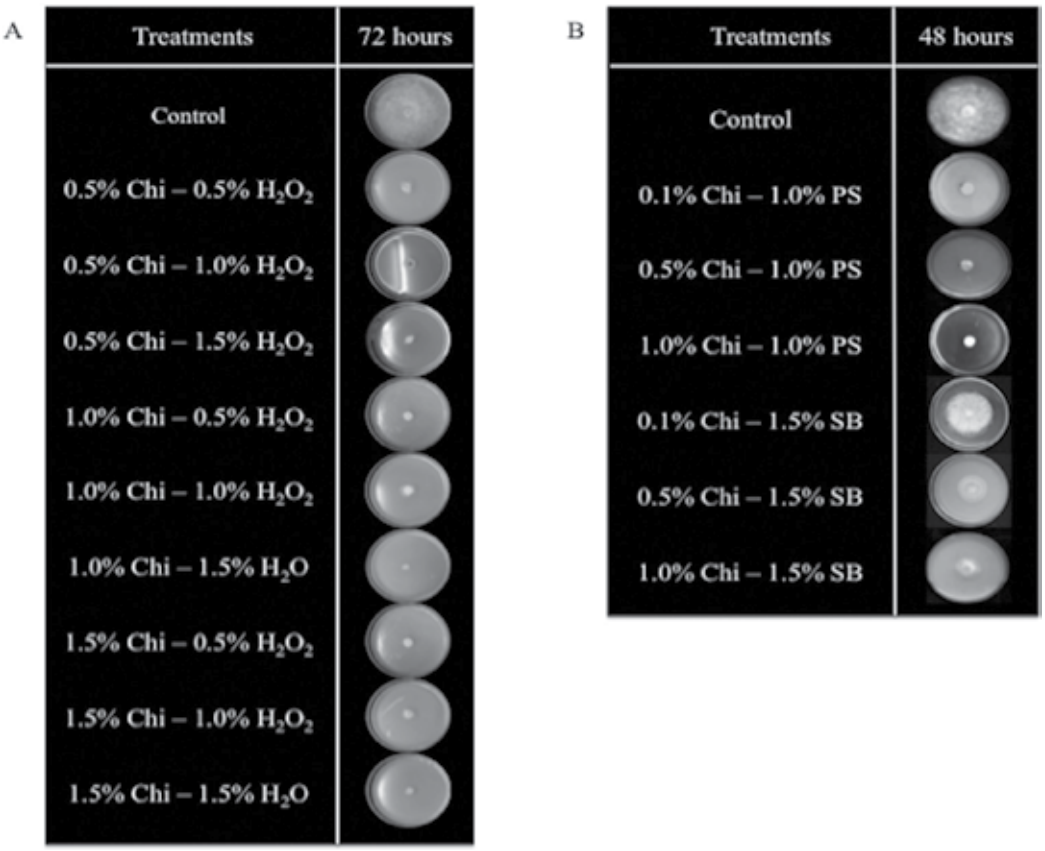

Figure 3. Effect of the interactions of chitosan, hydrogen peroxide, potassium sorbate, and/or sodium bicarbonate at different concentrations in the inhibition of mycelial growth of *Rhizopus* sp. isolated from jackfruit (*Artocarpus heterophyllus* L.). (A) Arciniega-Castro (2014) and (B) Coronado-Partida (2015). Control, sterile distilled water; chi, chitosan; H_2O_2, hydrogen peroxide; PS, potassium sorbate; SB, sodium bicarbonate.

1.2.1.2. Sporulation

In a study with fungus isolated from banana (*Musa paradisiaca*), only the application of chitosan at concentrations of 1.0% the strains of *Colletotrichum* sp. and *Fusarium* sp. showed a decrease in the final concentration of spores [26]. For the fungus of *Fusarium* sp. isolated from banana (*Musa sapientum*), chitosan treatments of 0.5 and 1.0% showed a decrease on the final concentration of the spores, and this process was totally inhibited applying concentrations of 1.5 and 2.0% of chitosan (**Table 1**) [27]. In the same way, *Colletotrichum gloeosporioides* T147 isolated from avocado (*Persea americana* mill) c.v. Hass and *Annona muricata* L. was successfully inhibited using concentrations of chitosan at 1.5 and 2.0% and 1.0 and 1.5%, respectively [28, 31]. Chitosan at concentrations of 0.5, 1.0, and 1.5% in combination with H_20_2 at 0.5, 1.0, and 1.5% affected the development of the spores; this may be due to a synergistic effect between the mechanisms of actions of both compounds that affect the sporulation conditions of the pathogens. The application of combinations of chitosan and H_20_2 at concentrations greater than 0.5% decreased the final concentration of the spores of *Rhizopus* sp. isolated from

Treatments	SoursopRamos-Guerrero (2012)	AvocadoAnaya-Carrillo (2013)	BananaHernández-Ibañez (2011)		BananaOchoa-Jiménez (2010)	
	C. gloeosporioides	*C. gloeosporioides*	*Colletotrichum sp.*	*Fusarium sp.*	*Colletotrichum sp.*	*Fusarium sp.*
Control	3.4×10^6 a	4.0×10^7 a	2.5×10^7 a	9.9×10^7 a	3.8×10^7 a	2.8×10^7 a
0.5% Chi	2.2×10^6 b	3.6×10^7 b	1.0×10^7 b	7.7×10^7 b	1.5×10^7 b	8.7×10^6 b
1.0% Chi	1.4×10^6 c	2.0×10^7 c	6.4×10^6 c	5.0×10^6 c	1.1×10^7 b	2.7×10^6 c
1.5% Chi	1.2×10^6 c	2.2×10^7 c	3.8×10^6 d	2.9×10^6 d	6.0×10^6 d	0 d
2.0% Chi	---	2.2×10^7 c	1.8×10^5 e	3.3×10^5 e	3.7×10^6 c	0 d

The values with different letters in columns are significantly different (Tukey's honestly significant difference; $p \leq 0.05$). Control = Sterile distilled water; Chi = Chitosan; --- = not determined.

Table 1. Effect of chitosan at different concentrations on the sporulation of different pathogens isolated from different fruits.

Treatments	Jack fruitArciniega-Castro (2014)	Treatments	Jack fruitCoronado-Partida (2015)
	Rhizopus sp.		*Rhizopus* sp.
Control	4.75×10^6 a	Control	1.4×10^8 a
0.5% Chi—0.5% H_20_2	4.3×10^4 f	0.1% Chi—1.0% PS	0 e
0.5% Chi—1.0% H_20_2	2.83×10^5 b	0.5% Chi—1.0% PS	0 e
0.5% Chi—1.5% H_20_2	0 g	1.0% Chi—1.0% PS	0 e
1.0% Chi—0.5% H_20_2	8.5×10^4 d	0.1% Chi—1.5% SB	3.6×10^7 b
1.0% Chi—1.0% H_20_2	1.0×10^5 c	0.5% Chi—1.5% SB	2.0×10^7 c
1.0% Chi—1.5% H_20_2	0 g	1.0% Chi—1.5% SB	1.2×10^7 d
1.5% Chi—0.5% H_20_2	6.6×10^4 e	---	---
1.5% Chi—1.0% H_20_2	1.0×10^5 c	---	---
1.5% Chi—1.5% H_20_2	0 g	---	---

The values with different letters in columns are significantly different (Tukey's honestly significant difference; $p \leq 0.05$). Control = Sterile distilled water; Chi = Chitosan; H_2O_2 = Hydrogen peroxide; PS = Potassium sorbate; SB = Sodium bicarbonate.

Table 2. Synergistic effect between chitosan, hydrogen peroxide, potassium sorbate and/or sodium bicarbonate at different concentrations on the sporulation of ***Rhizopus*** sp isolated from jackfruit (***Artocarpus heterophyllus*** L.).

jackfruit (*Artocarpus heterophyllus* L.) [33]. The use of chitosan in combination with H_2O_2 was effective to reduce the spore's production of *Rhizopus* sp. isolated from jackfruit (*Artocarpus heterophyllus* L.) (**Table 2**) [34]. In the same sense, the synergistic effect was also evidenced with the use of organic salts (potassium sorbate and sodium bicarbonate) applied at different concentrations with chitosan, obtaining good results on sporulation as shown in **Table 2** [33].

1.2.1.3. Spore germination

Promising results have been obtained in different investigations on *Colletotrichum* sp. (banana) and *Fusarium* sp. (banana), with the total inhibition on germination applying chitosan at different concentrations (0.5, 1.0, 1.5, and 2.0%) [26, 27]. Conversely, at concentrations of 0.5 and 1.0%, only 50% of the inhibition was obtained ($p \leq 0.05$) against *Colletotrichum gloeosporioides* T147 isolated from avocado (*Persea americana* mill.) c.v. Hass [28]. Good results were reported by Chávez-Magdaleno and Luque-Alcaraz [29] with the application of biocomposites of chitosan loaded with essential oils against *Colletotrichum gloeosporioides* T147, reporting up to 95% of inhibition on spore development ($p \leq 0.05$). Phytopathogens isolated from soursop (*Colletotrichum gloeosporioides* LSC-120), mango (*Alternaria alternata*), and jackfruit (*Rhizopus* sp.) were successfully controlled by the application of chitosan alone or in combination with organic salts [31–34].

1.2.2. Effects of the application of chitosan on postharvest disease control in fruits

The use of chitosan as a coating on fresh fruits is a real alternative on the control of postharvest diseases. An important biological function of the chitosan is like an inducer of defense mechanisms in fruit and vegetable products, causing a reduction and/or inhibition of the development of diseases [35–37]. Besides, the application of chitosan with other natural methods of biological control and nanoparticles is another promising alternative for controlling postharvest diseases [38]. Chitosan nanoparticles can improve the antimicrobial activity, which is associated with the position of the amino groups favoring the binding to the cell surface and an alteration with the normal functions of the membrane, thus inhibiting the growth of the pathogen [16]. There are several studies on the application of chitosan alone or in combination with natural methods such as resistance inducer or as a disease control agent [33, 34, 39, 40]. The analysis of disease incidence, severity, and quality parameters used a completely randomized block design. Data were subjected to analysis of variance (ANOVA), and a Tukey test ($p \leq 0.05$) was used for a means of comparison.

1.2.2.1. Disease incidence and severity

In a study on banana fruits *Musa paradisiaca* and *Musa sapientum*, a total inhibition on infected fruits with *Colletotrichum* sp. and *Fusarium* sp. were reported with the application of chitosan at 1.5% compared to control (80% of incidence) [26, 27]. Related to severity, control fruits presented a damage around the crown on bananas; conversely, fruits treated do not present visible damage (**Figure 4**A, B). As shown in **Figure 4**, the absence was reported on avocado fruits c.v. Hass treated with chitosan (1.5%) and inoculated with *Colletotrichum gloeosporioides* T147. The application of biocomposites of chitosan-pepper tree (*Schinus molle*) essential oil in avocado fruits (*Persea americana* mill.) c.v. Hass infected with *Colletotrichum gloeosporioides* successfully controlled anthracnose disease in a preventive and curative way (**Figure 5**) [39]. In a study on soursop fruits (*Annona muricata* L.) artificially and naturally infected with *Colletotrichum gloeosporioides* LSC-120, a total control was obtained by the application of chitosan at 1.0% (**Figure 6**) [31]. The combination of chitosan with peroxide and GRAS substances in jackfruit (*Artocarpus heterophyllus* L.) was effective in inhibiting the

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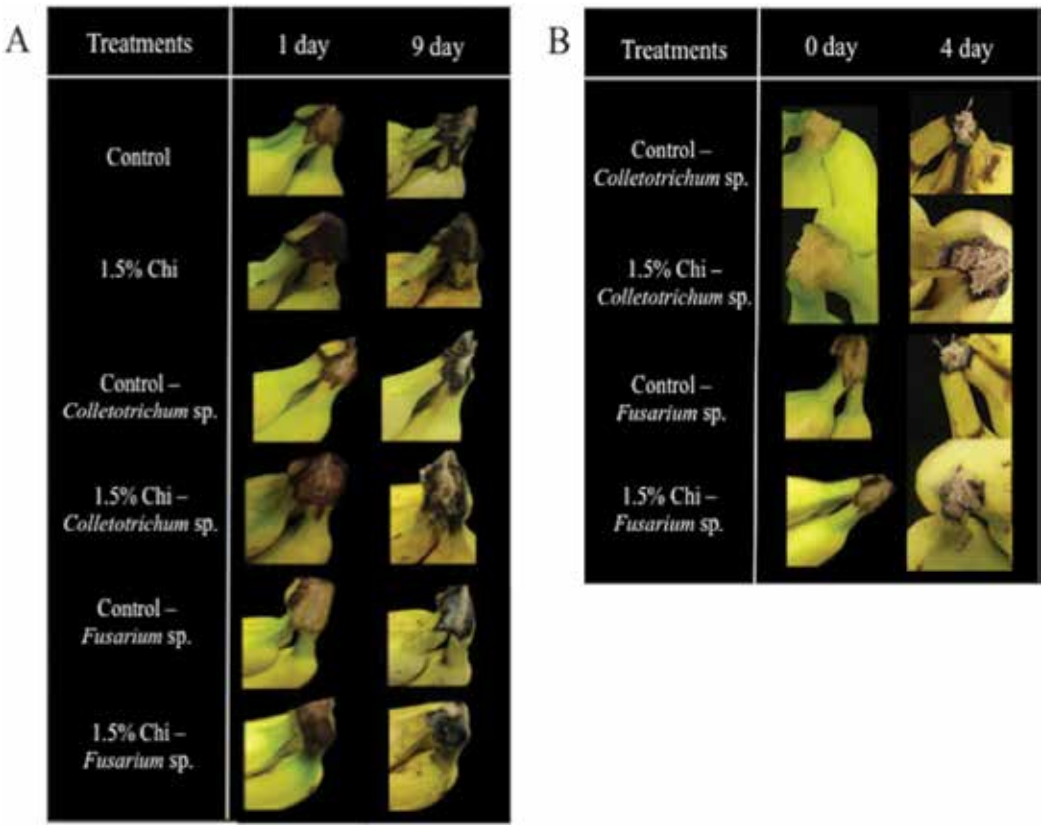

Figure 4. Crowns of banana fruits with 1.5% chitosan with and without inoculation of *Colletotrichum* sp. or *Fusarium* sp., on days 9 and 4 of storage. (A) Banana (*Musa paradisiaca*) stored at 15°C and (B) banana (*Musa sapientum*) stored at 25°C. Control, sterile distilled water; chi, chitosan.

development of soft rot disease by *Rhizopus* sp. The application of 1.0% Chi–1.0% H_2O_2 in jackfruit inoculated with the pathogen was able to totally inhibit the development of soft rot (**Figure 7**A) [34]. In other studies, the application of chitosan with potassium sorbate (SP) or sodium bicarbonate (BS) in jackfruit (*Artocarpus heterophyllus* L.) was effective for controlling the development of *Rhizopus* sp. The combination of 1.0% Chi–1.0% SP with and without inoculation of *Rhizopus* sp. fruits does not present the presence of infection (**Figure 7**B) [33]. Conversely, with the use of BS with chitosan, only 10% of severity reduction was obtained ($p \leq 0.05$). It is concluded that the combination of chitosan with SP can be an alternative to control *Rhizopus* sp. infection in jackfruit. The principal mode of action of the bicarbonate ion is through its buffering capacity, whereby an alkaline environment is sustained, and inhibition of microorganisms occurs due to the use of energy from microbial cells to produce an acidic environment [41]. The antimicrobial activity of potassium sorbate is associated to an alteration of the activity of Krebs cycle enzymes as well as the integrity of cell membranes [42]. The effective conditions for the control of diseases were for bananas *Musa paradisiaca* (15°C) and *Musa sapientum* (25°C), and avocado (*Persea americana* mill.) is 1.5% chitosan with 90–95% of relative humidity. For fruits of soursop (*Annona muricata* L.)

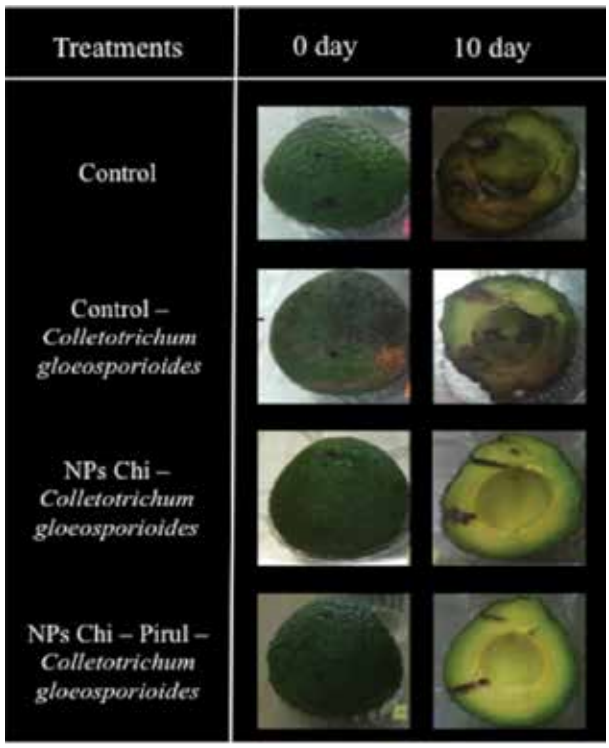

Figure 5. Avocado fruits (*Persea americana* mill.) c.V. Hass coated with chitosan nanoparticles and chitosan-pepper tree essential oil with and without inoculation of *Colletotrichum gloeosporioides* T147 for 10 days at 25°C. Control, sterile distilled water; chi, chitosan, NPs, nanoparticles.

and jackfruit (*Artocarpus heterophyllus* L.), the effective concentration was 1.0% chitosan at 20°C with 90–95% of relative humidity.

The efficacy of chitosan for controlling postharvest diseases depends not only on its antimicrobial properties. Romanazzi and Sanzani [43] reported that the arrays of defense mechanisms are activated in fruits exposed to biotic or abiotic stress, including chitosan application with or without inoculation of the pathogen. Chitosan induces the synthesis of phenolic compounds (chlorogenic acid, caffeic acid) and hydrolase antifungal enzymes (chitinases and β-(1,3)-glucanases) that hydrolyze the main components of the cell wall of fungi causing inhibition of their growth [44]. On the other hand, chitosan coating can serve not only as a protective barrier to fungal infection but also as a barrier to gaseous exchange affecting the fungal development on fruits.

1.2.2.2. Quality parameters

1.2.2.2.1. Weight loss

The ability of chitosan to form coatings is well documented; this property is useful to preserve the quality of fruits and vegetables. Chitosan applied at 1.5% on banana fruits was useful to avoid water losses compared to control [26]. The same concentration of chitosan was effective on bananas (*Musa sapientum*) with lower water losses on fruits compared to control (**Table 3**) [27].

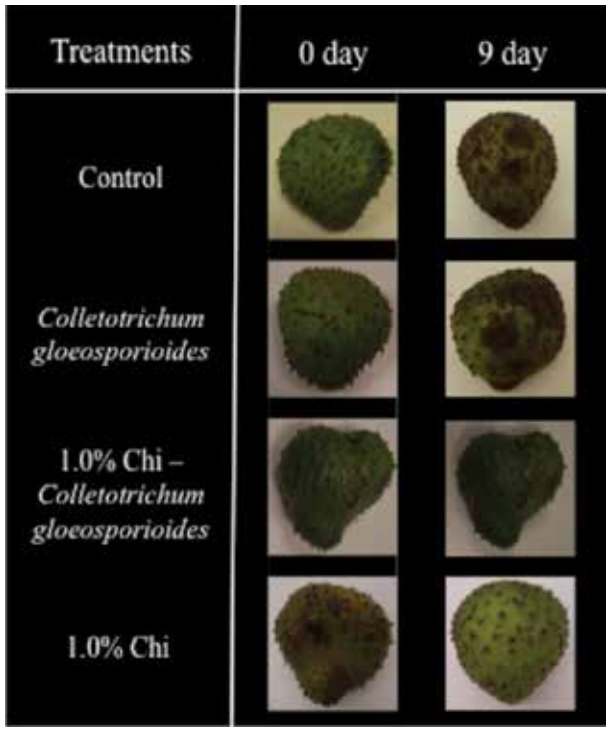

Figure 6. Anthracnose severity in soursop fruits treated with chitosan, inoculated or not with *C. gloeosporioides* LSC-120, and stored 20°C for 9 days. Control, sterile distilled water; chi, chitosan.

The ability to maintain the quality of fruits at different temperatures has been reported also for mango fruits (*Mangifera indica* L.) c.v. Tommy Atkins treated with chitosan at 1.0% and stored at 12°C. In the same sense, fruits treated with 1.0% chitosan and stored at 25°C showed the highest weight loss (10.5%) [32]. Novel formulations of chitosan like the use of nanoparticles have been applied with good results with the application of treatments (2.4%) compared to control (12.3%) [39]. The decreased weight loss in fruits is due to the presence of chitosan coating on the surface of the fruit, acting as a physical barrier to moisture loss and therefore delaying dehydration [45].

1.2.2.2.2. Firmness, pH, and total soluble solids

The loss of firmness is a factor that affects the quality of the fruits. In fruits of soursop (*Annona muricata* L.) treated with chitosan, at the end of the evaluation, fruits had a greater firmness (25 N) compared to control fruits (6.4 N) ($p \leq 0.05$). The total soluble solids (TSS) of the soursop fruits treated with 1.0% of chitosan and control showed a continuous increase (10–18% ($p \leq 0.05$)), and in the case of pH, in fruits treated with 1.0% of chitosan, an increase during storage was obtained, with a pH ranging from 3.49 to 4.10 ($p \leq 0.05$) [31]. The coating formed in banana fruits (*Musa paradisiaca*) by chitosan (1.5%) maintains the losses of firmness compared to control (data not shown). The concentration used on fruits does not affect the TSS, pH, and titratable acidity [26]. Mango fruits treated with 1.0% of chitosan no significant changes on TSS, pH, and titrable acidity were reported. The use of chitosan nanoparticles resulted effectively to

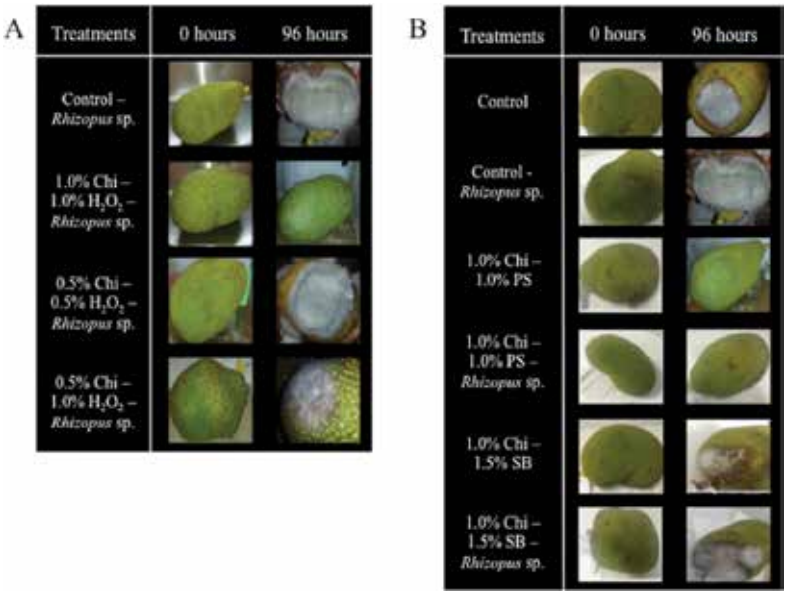

Figure 7. Severity of soft rot infection in jackfruit (*Artocarpus heterophyllus* L.) treated with the combination of chitosan, potassium sorbate, and sodium bicarbonate inoculated with *Rhizopus* sp. at 25°C. (A) Arciniega-Castro (2014) and (B) Coronado-Partida (2015). Control, sterile distilled water; chi, chitosan; H_2O_2, hydrogen peroxide; PS, potassium sorbate; SB, sodium bicarbonate.

Fruits	Temperature	Treatments	% Weight loss
Banana (*Musa paradisiaca*)	15°C	Control	8 ± 0.23^a
		1.5% Chi	2 ± 0.12^b
Banana (*Musa sapientum*)	25°C	Control	9.8 ± 0.34^a
		1.5% Chi	8 ± 0.47^a
Mango (*Mangifera indica* L.) c.v. Tommy Atkins	12°C	Control	5 ± 0.25^a
		1.0% Chi	3 ± 0.39^b
	25°C	Control	15 ± 0.5^a
		1.0% Chi	10.5 ± 0.46^b
Soursop (*Annona muricata* L.)	20°C	Control	18 ± 0.7^a
		1.0% Chi	13.3 ± 0.55^b
Avocado (*Persea americana* mill.) c.v. Hass	25°C	Control	12.3 ± 0.5^a
		NPs Chi	2.4 ± 0.43^b
		NPs – Chi - P	1.5 ± 0.51^b

Data are means ± standard deviation. The values with different superscripts are significantly different (Tukey's honestly significant difference; $p \leq 0.05$). Control = Sterile distilled water; NPs = Nanoparticles; Chi = Chitosan, P: Pepper tree essential oil.

Table 3. Weight loss of fruits at different temperatures treated with chitosan.

maintain firmness (29 N) on avocado fruits (*Persea americana* mill.) c.v. Hass compared to control (15 N) ($p \leq 0.05$) [39]. In terms of firmness, it decreases as maturation progresses due to changes occurring at the level of the cell wall, where there is hydrolysis of the pectic compounds due to the action of the enzymes cellulase, pectin methylesterase, and polygalacturonase, which in turn degrade high molecular weight polymers such as cellulose and hemicellulose [46]. The coating of chitosan at different concentrations on fruits does not change the quality parameters (TSS, pH, and titratable acidity). This may be due to the fact that chitosan does not interfere in the metabolism cycles (synthesis of sugars, synthesis of organic molecules) [47].

2. Conclusions

The use of chitosan in agriculture commodities can be a suitable alternative to the use of fungicides for controlling postharvest diseases, as well as to preserve the quality of fruits.

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Conflict of interest

Replace the entirety of this text with the "conflict of interest" declaration.

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Section 3

Chitosan in Agriculture

Chapter 16

Chitin/Chitosan's Bio-Fertilizer: Usage in Vegetative Growth of Wheat and Potato Crops

Boukhlifi Fatima, Mamouni Fatima Zahrae and R. Razouk

Additional information is available at the end of the chapter

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Abstract

This chapter consists of valuing the chitosan to create bio-fertilizers as fertilizers without going through the composting process because of their richness in the nutrient base elements of plants: nitrogen and phosphorus. Physicochemical analyses of the chitosan focused on pH, dry matter, organic matter, nitrogen, phosphorus and potassium as well as IR and XRD. The samples thus prepared were monitored for 15 days. PH, temperature and conductivity were monitored daily. According to the physicochemical analyses of waste (nitrogen, phosphorus and potassium) and the nutritional needs of our selected crop (soft wheat, Arrehane variety which are 90-90-50 U/ha), several doses are then determined for the purpose of the optimal formula after their application on the crop. An application of bio-fertilizer on the potato was also undertaken. Follow-ups were carried out during this study, such as the monitoring of the vegetative growth of wheat and the mineralization of the soil via its physicochemical analyses. The results show that our bio-fertilizer is rich in nitrogen with 4.98% and phosphorus with 1.42% and mineralizes quickly on the ground while leaving the soft wheat to absorb its nutrients effectively and improving its growth properties, then giving good yields.

Keywords: chitosan, vegetative growth, wheat, potato, crops, bio-fertilizer

1. Introduction

The consumption is always higher and more diverse, which leads to a growing production of wastes in quality and quantity. This growth causes huge danger on the environment and hence on the human health [1]. So many organic wastes are generated then constantly to the world by the domestic and the halieutic industry [2]. The sector of fishing is a part of the strategic

sectors in the world. It plays an important role in the global economy [3, 4]. The development of this sector is related to environmental issues, in particular to waste management. Indeed, the quantities of the halieutic waste are considered at several thousand, tons of waste a year as researched by Chabbar [5] and Afilal et al. [6].

These are randomly put in the existing systems of evacuation causing big problems [7]. One of these problems is the negative effect on the environment and human health El Moutawakila [8] and Benzakour et al. [9]. However, several studies have been interested in the evaluation and in the treatment of this waste. Some of them had studied the evaluation of their potential polluting [10].

Thus, the process of biotransformation for this type of waste seems to be the most suitable to resorb these problems. It corresponds to the elaboration of beneficial products, of natural origin, usable as bio-fertilizing for grounds [4] in substitution of artificial fertilizers. Moreover, the excessive application of artificial fertilizers for one of the most important agricultural processes in the world, the volatilization of ammonia in the air, the pollution of water resources causing their eutrophication, the degradation of the ground by their pollution attack of the cultures by phytopathogenic diseases [11–13].

This biotransformation consists then of the spreading of bio-fertilizers, which are in the form of dried, crushed and spread waste in agricultural plots.

Indeed, direct spreading is the simplest method of valuation requiring the least investment; it provides nutrients, improves soil quality with water retention and stimulates microbial activity. However, composting does not seem to be favorized in the logistics (time and local manufacturing, odors, etc.), due to the limited supply of carbon materials and environmental constraints [14] such as the attraction of insects and plants, pests and the risk of weeds in crops [15].

In this context, the bio-fertilizing potential of seafood, that is, chitin/chitosan, is explored for wheat and potato crop. They represent in fact a rich organic source for organic farming. The objective of this chapter is to valorize seafood waste, considering it as a source of bio-fertilizers and not only as a source of pollution, thanks to a simple and inexpensive process by spreading them directly in the agricultural environment. Their valorization always allows the protection of the environment and the acquisition of a new economic source.

2. Study of the use of raw chitin-chitosan as a bio-fertilizer

2.1. Inconveniences of nitrogenous chemical fertilization

In agriculture, chemical fertilizers are administered to increase crop yield. They provide the nutrients that plants need to grow. There are several chemical forms of nitrogen fertilizers in the market and the distinction between them is made possible through the various conventional chemical tests. The choice of one form over another is often difficult because of the contradictions in the published results on the composition of measures of agronomic efficiency [16].

The trio nitrogen, phosphate and potassium (NPK) is the basis of all these products, and they are also responsible for massive soil pollution but are especially the major cause of pollution of groundwater, the main reservoirs of drinking water. If they change their environment and make the water unsafe, there are certain dangers listed in **Table 1** [17].

The dangers of nitrogen fertilizers (nitrates)
- When they are not consumed by plants, they easily seep into the soil and progressively reach the groundwater.
- They are soluble in water.
- Before the 1950s, the nitrate content per liter of water did not exceed 1 mg. Nowadays, it easily exceeds 50 mg/l.
- Contribute to the phenomena of eutrophication [18].
- They are degraded by a bacterium and turn into nitrites that can poison the blood by oxidizing hemoglobin. The fluid then poorly fixes oxygen and causes respiratory problems.

Table 1. Some dangers of chemical fertilizers.

2.2. Contribution of waste to soils

The return to the ground of waste has been practiced by man since always. There are two reasons to explain this ancestral practice: first, the fertilizing value of this waste and then the capacity of the soil to purify the effluents, in particular, liquids, which makes it possible to protect the deep and surface waters against all risk of pollution. Strengthening regulations on the protection of the environment should make it possible to sustain this agricultural recycling while preserving the quality of the receiving soils, crops and water [13]. Waste spread in agricultural fields comes primarily from agriculture itself or from industries directly linked to it. Depending on their chemical composition, waste can be brought to the soil to provide fertilizer amounts equivalent to mineral fertilization (**Table 2**).

Some residual products are brought to the soil as an amendment. An amendment is a contribution to soils for the main purpose of improving their physico-chemical and biological properties. The organic matter content of a soil is one of the key elements leading to a stable structure and helping to limit the risk of soil erosion, especially in soils with a silty texture [19, 20]. However, the use of waste as fertilizer or amendment can only be accepted if their negative environmental impacts are minimal compared to their positive effects. With increasing public awareness of the need to preserve soil quality as well, the risks associated with land application now include not only the plant and water aspects but also those of soil and even air.

2.3. Physico-chemical preparation and characterization of the raw chitin

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Foams of sugar refinery	29.0	7.4	4.0	0.9
Liquid manure of poultry	13.3	10.2	1.8	5.4
Fertilizer of cattle	28.4	6.2	1.4	5.9
Fertilizer of sheep (mutton)	29.3	8.6	1.8	11.0
Compost of fertilizer of bovine	35.2	7.6	1.3	6.1

Table 2. Average contents of fertilizing elements in waste spread in agriculture [18].

Figure 1. Shrimp waste after drying and grinding "DC".

The analyses focused on pH, nitrogen (N), phosphorus (P), potassium (K) and organic matter [17]. The characterization of raw chitin is performed using IR spectroscopy and X-ray diffraction (XRD).

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MS ≥ 30% MB and MO ≥ 20% MB depending on the type designation and C/N > 8.

MB: raw material; MS: dry matter; MO: organic matter.

According to the results obtained, our bio-fertilizer ratifies almost all the values of the AFNOR standard quoted above.

Indeed, the sum of the percentages on N, P and K is less than 7%, it is 6.45%. The percentages of phosphorus and potassium are 3% lower except that in nitrogen, the MO content is

Waste/parameter	pH	DM (%)	OM (%)	C (%)	N (%)	P (%)	K (%)	C/N
SW	8.55	26.13	56	28	4.98	1.42	0.05	5.62

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According to the analyses, shrimp waste is rich in nitrogen. The composition of its waste (carapaces, viscera, small portions of flesh attached and associated water) is characterized by its high nitrogen content, which is granted with several previous works [15, 23, 24].

This richness is due to the high crude protein content; a factor of 6.25 was used to convert total nitrogen into protein. The percentage of protein in our sample is therefore 31.12%; this value is approximate to those of other research: protein contents of 52 [25], 44.12 ± 0.79 [26] and 47, 43 and 47.75% [23].

Ravichandran et al. also reported [27] that the percentage of crude protein in the dry matter of raw chitin is 24.03%. Similarly, a percentage close to these values of 29.3% was found by Prameela et al. in 2010 [28]. Also, Khan and Nowsad [29], in 2012, found high percentages ranging from 40 to 50% of proteins in shrimp shells.

These results showed that shrimp waste is high in phosphorus, which is in agreement with many authors who have found that this content in the head and shell is, respectively, 0.017 and 0.029% [23]. This phosphorus richness is attributed to its contribution to the formation of crustaces structures and their strengthening when phosphorus is combined with calcium.

The shrimp waste had an alkaline pH (8.55); this value was found by Mohan et al. which is 8.10 ± 0.10 [28, 30, 31].

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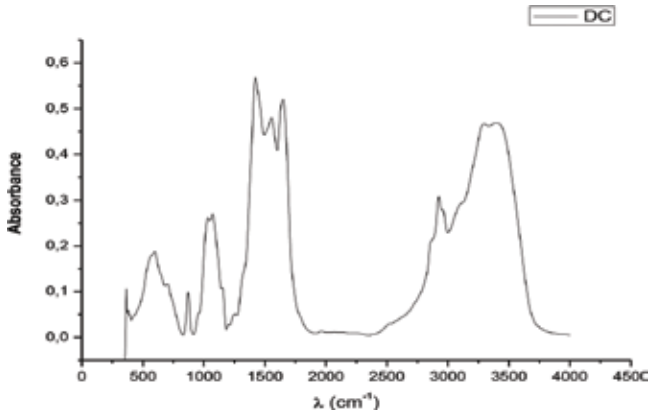
The observation of the X-ray diffraction spectra of shrimp waste shows the presence of two intense peaks at $2\theta = 9.9°$ and at $2\theta = 19.9°$; these results are identical to those obtained by Ahlafi et al. [31], with pure chitin; these researchers showed that α-chitin has two peaks of diffraction, $2\theta = 9.3°$ and $2\theta = 19.4°$. Other authors, Liu S at al. 2012, [32] showed that chitin



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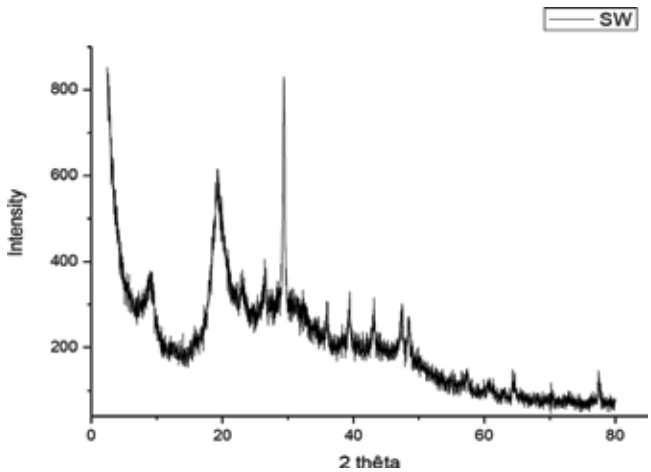

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Soft wheat, variety Arrehane, was sown at a rate of 15 seeds per pot with a surface area of 0.07 m^2, simulating a seeding rate of 214 seeds per square meter. The sowing was in November 2014, the growth period started and ended at the end of June and the harvest took place in July 2015. The temperature and lighting are natural ambient. Watering was done as needed with well water.

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The monitoring of the crop is carried out there after measuring the growth parameters of the wheat until it reaches maturity. Each sample then consists of the ears of wheat harvested in

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Soil samples of 0–20 cm were taken three times during the wheat growth cycle at the time of spawning, tillering and at maturity in all pots of the experimental set using a stainless steel tool. The samples were put in the laboratory for measuring the fresh weight, then are dried in an oven for 48 h at a temperature of 60°C for carrying out analyses of the elements P, K, organic matter and pH and conductivity measurement according to the internal protocols of INRA.

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$$K_2O\ (ppm) = ppm \times 10 \times 1.2 \quad (1)$$

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Phosphorus analysis was performed using the 0.5 M sodium bicarbonate ($NaHCO_3$) extraction solution at pH = 8.5. The same filtration process is thus carried out, extract was taken and put in Erlenmeyer flasks and then sulfuric acid (5 N) was added to acidify the solution. The staining

Section 3

Chitosan in Agriculture

Chapter 16

Chitin/Chitosan's Bio-Fertilizer: Usage in Vegetative Growth of Wheat and Potato Crops

Boukhlifi Fatima, Mamouni Fatima Zahrae and R. Razouk

Additional information is available at the end of the chapter

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Abstract

This chapter consists of valuing the chitosan to create bio-fertilizers as fertilizers without going through the composting process because of their richness in the nutrient base elements of plants: nitrogen and phosphorus. Physicochemical analyses of the chitosan focused on pH, dry matter, organic matter, nitrogen, phosphorus and potassium as well as IR and XRD. The samples thus prepared were monitored for 15 days. PH, temperature and conductivity were monitored daily. According to the physicochemical analyses of waste (nitrogen, phosphorus and potassium) and the nutritional needs of our selected crop (soft wheat, Arrehane variety which are 90-90-50 U/ha), several doses are then determined for the purpose of the optimal formula after their application on the crop. An application of bio-fertilizer on the potato was also undertaken. Follow-ups were carried out during this study, such as the monitoring of the vegetative growth of wheat and the mineralization of the soil via its physicochemical analyses. The results show that our bio-fertilizer is rich in nitrogen with 4.98% and phosphorus with 1.42% and mineralizes quickly on the ground while leaving the soft wheat to absorb its nutrients effectively and improving its growth properties, then giving good yields.

Keywords: chitosan, vegetative growth, wheat, potato, crops, bio-fertilizer

1. Introduction

The consumption is always higher and more diverse, which leads to a growing production of wastes in quality and quantity. This growth causes huge danger on the environment and hence on the human health [1]. So many organic wastes are generated then constantly to the world by the domestic and the halieutic industry [2]. The sector of fishing is a part of the strategic

sectors in the world. It plays an important role in the global economy [3, 4]. The development of this sector is related to environmental issues, in particular to waste management. Indeed, the quantities of the halieutic waste are considered at several thousand, tons of waste a year as researched by Chabbar [5] and Afilal et al. [6].

These are randomly put in the existing systems of evacuation causing big problems [7]. One of these problems is the negative effect on the environment and human health El Moutawakila [8] and Benzakour et al. [9]. However, several studies have been interested in the evaluation and in the treatment of this waste. Some of them had studied the evaluation of their potential polluting [10].

Thus, the process of biotransformation for this type of waste seems to be the most suitable to resorb these problems. It corresponds to the elaboration of beneficial products, of natural origin, usable as bio-fertilizing for grounds [4] in substitution of artificial fertilizers. Moreover, the excessive application of artificial fertilizers for one of the most important agricultural processes in the world, the volatilization of ammonia in the air, the pollution of water resources causing their eutrophication, the degradation of the ground by their pollution attack of the cultures by phytopathogenic diseases [11–13].

This biotransformation consists then of the spreading of bio-fertilizers, which are in the form of dried, crushed and spread waste in agricultural plots.

Indeed, direct spreading is the simplest method of valuation requiring the least investment; it provides nutrients, improves soil quality with water retention and stimulates microbial activity. However, composting does not seem to be favorized in the logistics (time and local manufacturing, odors, etc.), due to the limited supply of carbon materials and environmental constraints [14] such as the attraction of insects and plants, pests and the risk of weeds in crops [15].

In this context, the bio-fertilizing potential of seafood, that is, chitin/chitosan, is explored for wheat and potato crop. They represent in fact a rich organic source for organic farming. The objective of this chapter is to valorize seafood waste, considering it as a source of bio-fertilizers and not only as a source of pollution, thanks to a simple and inexpensive process by spreading them directly in the agricultural environment. Their valorization always allows the protection of the environment and the acquisition of a new economic source.

2. Study of the use of raw chitin-chitosan as a bio-fertilizer

2.1. Inconveniences of nitrogenous chemical fertilization

In agriculture, chemical fertilizers are administered to increase crop yield. They provide the nutrients that plants need to grow. There are several chemical forms of nitrogen fertilizers in the market and the distinction between them is made possible through the various conventional chemical tests. The choice of one form over another is often difficult because of the contradictions in the published results on the composition of measures of agronomic efficiency [16].

The trio nitrogen, phosphate and potassium (NPK) is the basis of all these products, and they are also responsible for massive soil pollution but are especially the major cause of pollution of groundwater, the main reservoirs of drinking water. If they change their environment and make the water unsafe, there are certain dangers listed in **Table 1** [17].

The dangers of nitrogen fertilizers (nitrates)
- When they are not consumed by plants, they easily seep into the soil and progressively reach the groundwater.
- They are soluble in water.
- Before the 1950s, the nitrate content per liter of water did not exceed 1 mg. Nowadays, it easily exceeds 50 mg/l.
- Contribute to the phenomena of eutrophication [18].
- They are degraded by a bacterium and turn into nitrites that can poison the blood by oxidizing hemoglobin. The fluid then poorly fixes oxygen and causes respiratory problems.

Table 1. Some dangers of chemical fertilizers.

2.2. Contribution of waste to soils

The return to the ground of waste has been practiced by man since always. There are two reasons to explain this ancestral practice: first, the fertilizing value of this waste and then the capacity of the soil to purify the effluents, in particular, liquids, which makes it possible to protect the deep and surface waters against all risk of pollution. Strengthening regulations on the protection of the environment should make it possible to sustain this agricultural recycling while preserving the quality of the receiving soils, crops and water [13]. Waste spread in agricultural fields comes primarily from agriculture itself or from industries directly linked to it. Depending on their chemical composition, waste can be brought to the soil to provide fertilizer amounts equivalent to mineral fertilization (**Table 2**).

Some residual products are brought to the soil as an amendment. An amendment is a contribution to soils for the main purpose of improving their physico-chemical and biological properties. The organic matter content of a soil is one of the key elements leading to a stable structure and helping to limit the risk of soil erosion, especially in soils with a silty texture [19, 20]. However, the use of waste as fertilizer or amendment can only be accepted if their negative environmental impacts are minimal compared to their positive effects. With increasing public awareness of the need to preserve soil quality as well, the risks associated with land application now include not only the plant and water aspects but also those of soil and even air.

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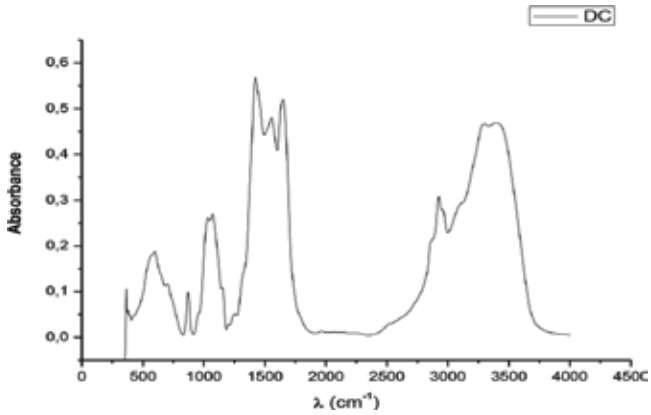

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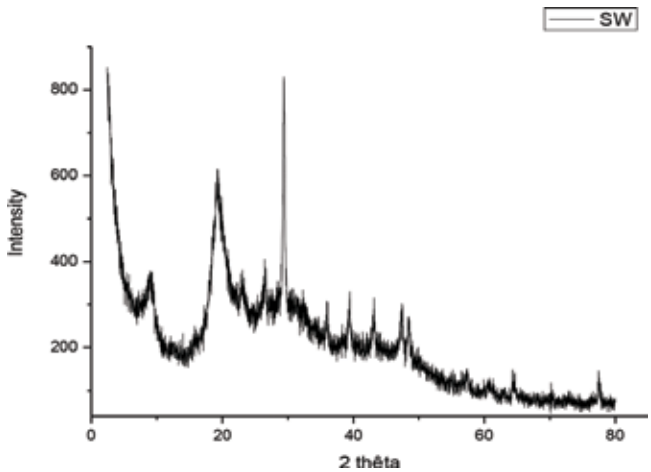

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Phosphorus analysis was performed using the 0.5 M sodium bicarbonate ($NaHCO_3$) extraction solution at pH = 8.5. The same filtration process is thus carried out, extract was taken and put in Erlenmeyer flasks and then sulfuric acid (5 N) was added to acidify the solution. The staining

solution was then added. This supplemented with distilled water; the solutions of the calibration range were also prepared in order to plot the calibration curve to deduce the phosphorus concentrations. The intensity of the blue color of the solutions is read at 820 nm after 15–30 min.

The calculation formula is as follows:

$$P\ (\text{ppm; soil}) = \text{reading}\ (\text{ppm}) \times 20 \tag{2}$$

c Soil content of organic matter

To know the amount of organic matter present in the soil, 1 N of potassium dichromate, sulfuric acid (H_2SO_4), distilled water and concentrated phosphoric acid (H_3PO_4) were used.

In the last step, a few drops of the indicator (diphenyl amine) were added. After homogenization, the excess of $K_2Cr_2O_7$ with 1 N $FeSO_4$ was titrated to bright green and the volume of $FeSO_4$ was recorded under the same conditions as a blank solution without a soil sample.

The calculation is done by the following formula:

$$\text{OOC}\,\% = \frac{((V(\text{white}) - V(\acute{e}ch.titr\,\hat{e})) \times 2 \times 0.3 \times 0.5}{\text{weight of the ground}} \tag{3}$$

$$\text{TOC}\,\% = 1334 \times \text{OOC}\% \tag{4}$$

$$\%\text{M.O} = 1724 \times \text{TOC} = 2.3 \times \text{OOC} \tag{5}$$

d pH analysis

A quantity of soil is suspended in a double volume of distilled water (5 g of soil/10 ml of water). The mixture is stirred with a glass rod. The mixture was allowed to stand for 30 min, stirred 5 or 6 times during this period, and then the pH was measured.

2.6.3 Results of growth monitoring of common wheat and comparison of root volume and weight

At the end of the test, roots and residues were extracted by a stream of water that caused the soil to settle downward and the exclusion of floating roots upward. The roots of the wheat were then removed from the soil, washed and cleared of soil debris and then oven-dried at a temperature of 60°C for 48 h.

The growth of common wheat was followed throughout the season by length measurement. At harvest, several parameters were measured, namely:

- final length of plants (cm)
- weight of grains/pot (g)
- grain yield estimate (ton)/ha
- root volume and weight

a Wheat growth for each dose

Figure 5 shows the growth of wheat, based on the doses of treatments that are 100, 150, 200 and 300%. In general, the average growth data showed that wheat grew well in all the increasing doses provided by the biological treatment (raw chitin) ending up to almost 99 cm in length (150% treatment) and exceeds the growth of wheat receiving the chemical fertilizer at all these doses and the witness which did not undergo any contribution. For the 100% dose, the wheat amended by the raw chitin exceeds in length that is amended by the chemical fertilizer with 6.33 cm and the control with 13.53 cm, which is the same for the other doses of 150, 200 and 300%, successively, with 10.27, 5.37 and 1.1 cm for the chemical fertilizer and with 20.73, 19.5 and 12.16 cm for the witness.

These results do not corroborate those found in the study of the effect of raw chitin on radish cultivation [15] where the length of radishes fertilized by mineral fertilizers exceeds that of radishes amended by shrimp residues with 1.1 cm. On the other hand, they are corroborated with the work of Taiek et al. where they demonstrated that the fish waste allied to malting releases allowed for an optimal growth of barley and tomato, better even than the commercial fertilizers [5].

b Comparison between doses

The growth of wheat fertilized by doses 150 and 200% of the gross chitin exceeds that of the other two doses; the wheat fertilized by the 300% dose is good growth but the latter has declined toward the end of the growth cycle (from April 29 to May 06, 2015). This is due to the excess of nitrogen in the soil and therefore is absorbed by the wheat plants, which causes a delay in the maturity phase [33] unlike, the others; it is concluded that the 300% dose is limited to growth. From there, we can recommend that the following doses corresponding to treatments 150 and 200% of our bio-fertilizer are equivalent successively to 3 and 4 t of N/ha which is equivalent to 135 and 180 U N/ha (**Figure 6**).

The wheat grows very closely with regard to all the doses of the chemical fertilizers brought. At the end of the growth cycle, the wheat amended with the 200% dose outgrows the other wheat plants. That is the dose that corresponds to 180 U of nitrogen per hectare, and this is the recommended dose per hectare by the INRA in Morocco which varies between 160 and 200 U/ha [34].

c Yield results of soft wheat

At maturity, soft wheat was harvested manually from ground level, and the harvested biomass was weighed later. The grains were separated from the straw with a drummer, and the grain yield was recorded after weighing.

Applying our only bio-fertilizers significantly increased wheat yields compared to chemically fertilized wheat; (**Figure 6**) it is a maximum yield of 30 q/ha for bio-fertilizers against 16.18 q/ha for commercial fertilizer treatment 200% corresponding to the contribution of 180 U of

http://dx.doi.org/10.5772/intechopen.75208

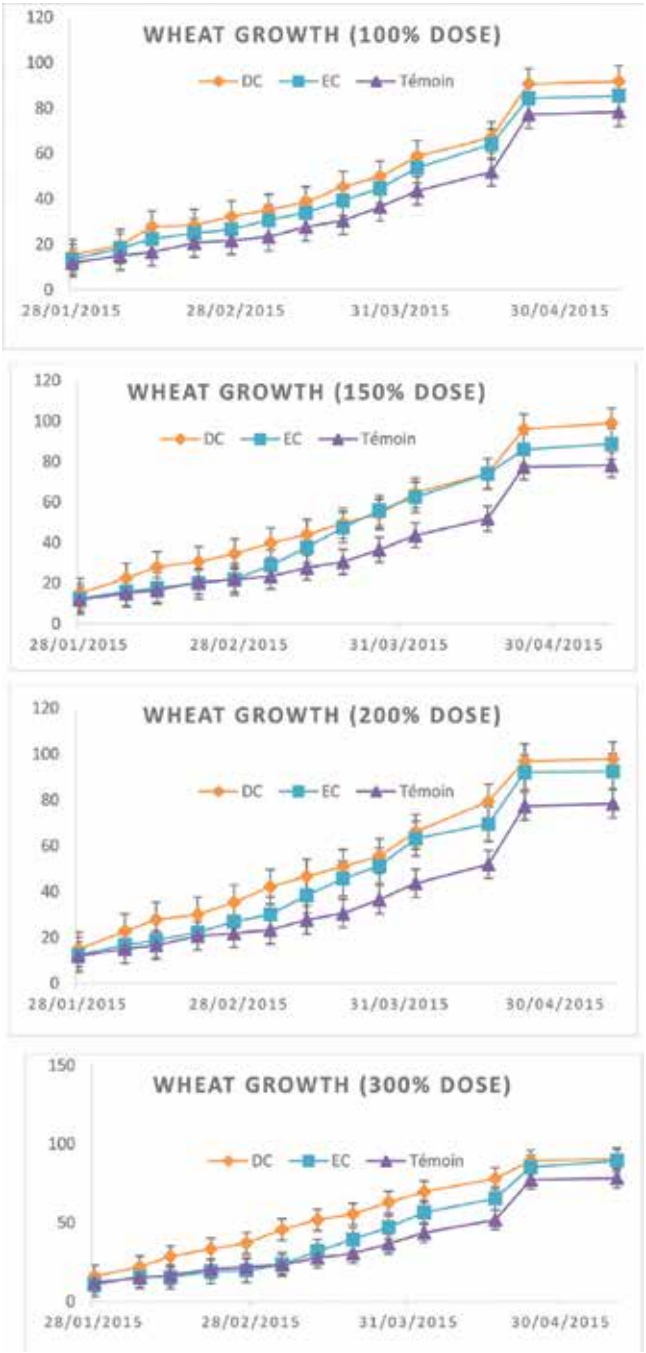

Figure 5. Comparison between bio-fertilizer and chemical fertilizer in terms of wheat growth for each dose: (a) 100, (b) 150, (c) 200, and (d) 300%.

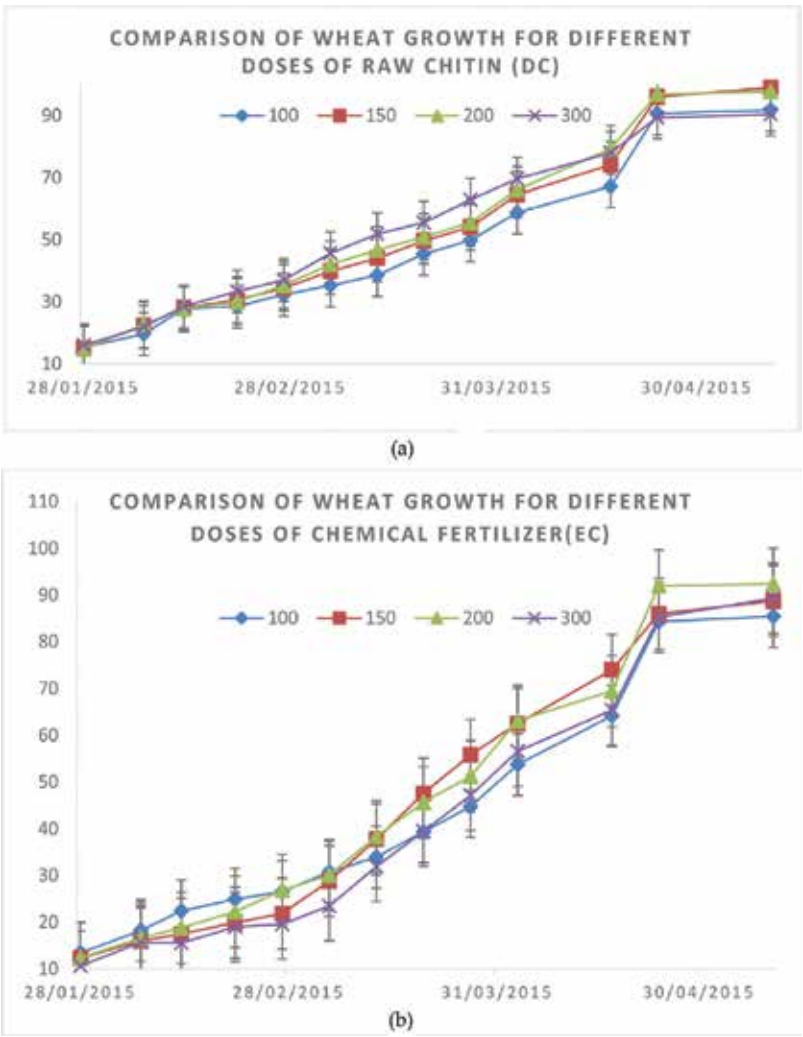

Figure 6. Comparison of growth of the wheat for all the doses of the used fertilizers (a) DC and (b) EC.

nitrogen per hectare, which approves a correlation with growth well developed in wheat fertilized by the gross chitin with the same treatment above. According to the Ministry of Agriculture at the national level, the average yield of common wheat has increased, between the periods 2000–2007 and 2008 and 2015, from 14.3 to 19.2 q/ha, a value lower than that found for our organic fertilizers [35].

In addition, application of marine residues has been shown to have positive effects on crop yields. Abdel-Mawgoud [36] has shown that a foliar application of chitosan on strawberry plants helps to increase the height of the plants, the number of leaves and even the yield of strawberries. Abdel-Mawgoud et al. [37] also noted an increase in fall triticale yield following the early spring application of mussel residues. Also, Karine has shown that treatment with pure marine residues, dried shrimp waste, has produced the best radish yields

[14]. In addition, the authors state that composting reduces the availability of nitrogen from marine residues. Indeed, some of the nitrogen is lost through volatilization during the composting process. After fermentation, the decomposition of the fresh residues generates toxic compounds (volatile fatty acids, lactic and acetic acids, etc.). Some studies have highlighted another benefit that corresponds to the safener effect of marine residues or marine residue extracts on various diseases. In 2006, ADAS [38] stated that the addition of crustacean residues stimulates soil microbial activity, which can promote competition between soil microorganisms at the expense of pathogenic microorganisms.

d Results of comparison between volume and weight racinaires

The role of roots is very important for the absorption and the transport of the water and the mineral elements toward the air parts of plants [39]. Their development intervenes in the evolution of the properties of the ground and more particularly its structure and its content in organic matter [40]. The roots of the wheat fertilized by the bio-fertilizer exceed in weight and volume, though some wheat is fertilized chemically; (**Figures 7**, **8** and **9**) our results confirm the results

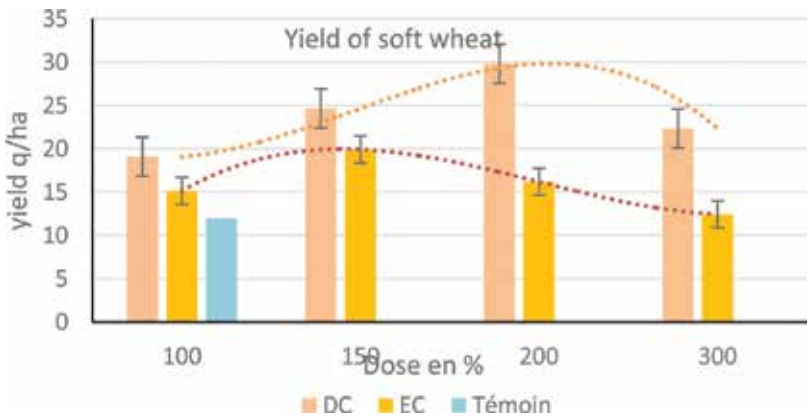

Figure 7. Yield on the common wheat.

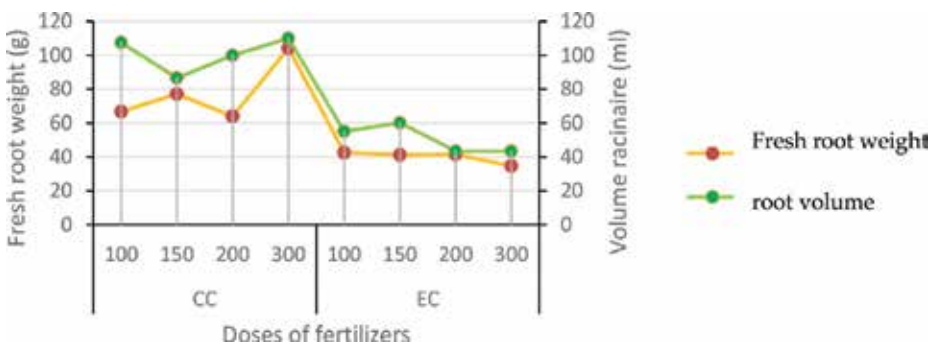

Figure 8. Comparison between the bio-fertilizer and the fertilizing in terms of volume and weight racinaires some common wheat.

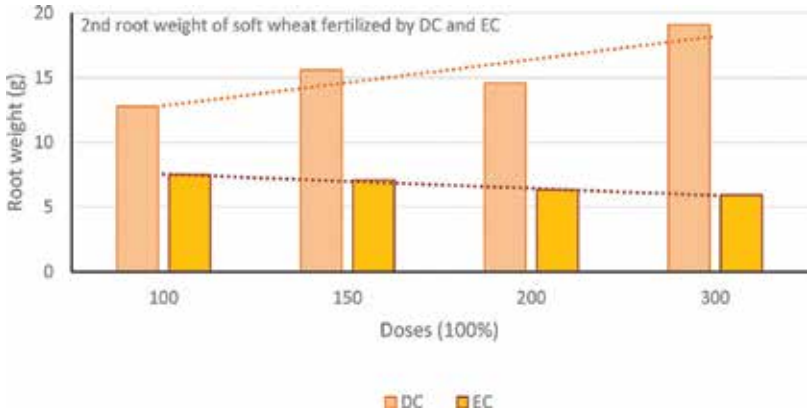

Figure 9. Secondary root weight of soft wheat.

of Karine [14] where the diameter and the biomass of roots for radish fertilized by the waste of shrimps are superior with regard to those of radish which is fertilized by the artificial fertilizer.

e Evolution of soil mineralization

Table 6 represents the mineralization of total organic carbon (percentage) during the growth phases of soft wheat.

The mineralization of organic matter is a process of degradation. Its main consequences are the decrease of the organic matter content in a soil and the selective disappearance of certain compounds [41, 42]. The decomposition of organic matter is expressed in terms of mineralized carbon and types of molecules present in the soil over time [43].

However, the effects of bio-fertilizers on biological activity and mineralization of nitrogen in the soil depend in particular on their nitrogen concentration and their C/N ratio [44], which further confirms the results found for our bio-fertilizer from shrimp waste where the nitrogen content is 4.98%. The bio-fertilizers with lower C/N ratios are mineralized rapidly in the soil, releasing significant amounts of nitrogen absorbed by subsequent crops [14, 45–47] that also

Dose	DC			EC		
	After lifting-tallage	Tillering-montaison	Bolting-heading	After lifting-tallage	Tillering-montaison	Bolting-heading
100	23.84	96.28	−48.14	17.91	98.95	−66.86
150	28.20	113.66	−53.49	24.88	98.95	−48.14
200	31.22	108.31	−45.47	27.62	100.29	−50.81
300	35.17	100.29	−56.16	18.49	82.91	−34.77
Témoin	40.87	73.55	−34.77	40.78	73.55	−34.77

Table 6. Total organic carbon mineralization (%).

states, for this study, the C/N ratio which is 5.62. Besides the carbon decomposition rate has peaked at 31.22 and 35.17% at post emergence-tillering phase and 113.66 and 108.31% at tillering-stem extension phase for bio-fertilizer doses used, respectively, at about 200 and 300% which explains the high content of the soil in this element following its total decomposition. This increase is also explained by the decrease in the mineralization potential, which corroborates the work of Martel et al. [48] and Karine [14]. The regular supply of residues or manure can increase the total organic C of the soil to a higher equilibrium level, related to the balance between C inputs and decomposition processes [48]. And it is the same—the decomposition of the carbon element in the soil for the wheat fertilized by the chemical fertilizer is maximum for the doses of 150 and 200%, successively at 24.88 and 27.62% (after the emergence-tillering phase) and 98.95 and 100.29% (tillering-stem extension) for doses of successively 100 and 200%.

Subsequent to the cycle, we find that 53.49 and 56.16% of the carbon element was mineralized during the stem extension-heading period; those are maximum values for the bio-fertilizer doses of 150 and 300%, respectively. For the chemical fertilizer, 66.86 and 50.81% values of the mineralized carbon (maximum values) are determined for doses of 150 and 300% too. This is where the microbial communities convert the carbon element provided by the bio-fertilizer and the chemical fertilizer into stable C in the soil, as studied by Kallenbach et al. [49].

2.7. Application of the bio-fertilizer on potatoes

2.7.1. Preparation of the ground

The slightly acidic pH (pH = 5.5 or 6) of the soil can give good yield. Excess alkalinity of the soil can cause development of the common tuberous gall.

The high salinity of the soil can block the absorption of water by the root system, so to complete in combination with these conditions, the soil preparation is an essential step for the best fertilizer application. The preparation techniques consist of ensuring good contact between the tubers and the soil because the development of the root system will usually be delayed if the soil is poorly prepared. The soil should be prepared to a depth of at least 25–30 cm; such a loose layer promotes aeration of the soil, ensures good root development and facilitates ridging.

A good seedbed can be done as follows:

- medium plowing (25–30 cm) with plow;
- spreading of dry waste and phospho-potassium fertilizers with doses well respected according to the needs of the potato/ha;
- a good preparation of the first 10 cm of the soil allows a good cover of the plant.

Fertilization remains one of the most important factors for good potato production. For a production of 25 tons/ha (tubers + haul), the requirements of the amount of essential elements for the plant include 160 kg (N)/ha, 45 kg (P_2O_5)/ha, 275 kg (K_2O)/ha, 50 kg (MgO)/ha and 70 kg (CaO)/ha.

The potato is very demanding in organic manure, the needs are of the order of 30 T/ha and this dose can be doubled in soil low in organic matter. The maximum nitrogen absorption takes place at the time of 50–80 days after planting. Nitrogen can be applied in the form of sulfate of ammonia; the phosphorus is hardly absorbed by the plant for it must be applied before planting and in the most assimilable form. The doses should be divided into three periods; emergence, first hump and second hump.

The recommended doses are only average and must be adapted according to the richness of the soil. A preliminary soil analysis is necessary to evaluate the level of soil fertility. Nitrogen should be located at the ridges while avoiding direct contact between the plant and the fertilizer.

2.7.2. Plantation of the potato

The tubers of the potato are classified according to the following sizes: from 28 to 35 mm, from 35 to 45 mm, from 45 to 55 mm and calibers greater than 55 mm. Planting density of 15 to 20 stems/m^2 gives good land use; pre-germinated 35–55 mm of plant produces approximately 5–6 main stems, usually 4 plants/m^2 with a distance of 70 cm between lines and 30 cm between plants; the seed requirements/ha equals about 2000 up to 2500 kg. Planting tubers at a uniform depth that depends on the type of soil, climatic conditions, and the physiological age of the plants gives a homogeneous culture.

The choice of planting at 5–6 cm of depth is applied in moist, heavy soil as the mother tubers may run out before the germs reach the soil surface, but for a textured soil, where a slight or a risk of drying out is to be feared, the planting is often done at a depth of about 10 cm.

2.7.3. Irrigation of the potato

The organization of activated irrigation promotes and ensures the mechanisms of transport of mineral elements, synthetic products, transpiration and thermal regulation at the leaf level because the potato is very sensitive to both deficit of water and excess water; it requires water which is evaluated between 400 and 600 mm according to the climatic conditions, the type of soil and the length of the cycle. According to the following frequency:

- During germination, the amount of water needed is low.
- For 60 days up to 90 days after planting; irrigation should be carried out at very short intervals (6 or 7 days) in light soil and for 12 or 15 days in heavy soil up to 10–20 days before harvest.

The quality of irrigation is measured by the rate of salts which must be less than 4 g.

2.7.4. Earthing-up

The operation of earthing-up consists of returning the earth to drive the mound; the purpose of this operation is of cover the superficial roots of the plant, the nitrate and potassium fertilizers applied during the culture, and prevent the culture of the moth (decreased the attack of insects).

The first planting is done 2 to 3 weeks after the late emergence of the plant, the length of the plant must be at least 10cm above the ground. It is necessary to stay up during harrowing not to affect the system racinaire and the recently formed tuber, this operation is important because it consists in taking all the weed. The yellowing of the lower leaves, the drying of stalks and the firmness of the skin of tubers are factors of the harvest after 3–4.5 months. The lifting must be made in dry weather and tubers should not be left exposed too much to the sun to avoid the development of the black spots and the attack by the moth.

2.7.5. Hilling

The humping operation is to bring the previously loosened earth to the ridge to form the hill; the purpose of this operation is to

cover the superficial roots of the plant, the nitrogenous and potassic fertilizers applied during the cultivation and prevent the cultivation of ringworm.

The first hilling is done 2–3 weeks after the lifting, after a hoeing operation, so as to cover at least 10 cm of soil, and then the operation is repeated every 2–3 weeks.

Care should be taken during hoeing not to touch the root system and newly formed tubers, this is important because it involves taking all the weeds.

Yellowing of the lower leaves, drying of the stems and firmness of the tuber skin are factors in the harvest after 3–4.5 months.

Tearing should be done in dry weather and care should be taken not to leave the tubers too exposed to the sun to avoid the development of black spots and ringworm attacks.

2.7.6. Application of bio-fertilizer: raw chitin

The application of raw chitin on the potato in this study is performed qualitatively in bags.

- initialization of the bottom of the bags by 2 cm sand sole to seeds of large pores to facilitate the permeability of excess water during irrigation;
- preparation of a mixture of sand-clay-soil (one-third of the sand + two-thirds of the clay soil);
- addition of a 5 cm layer of soil that we have prepared so as to not lose fertilizer doses by the permeability of water;
- tuber planting in another layer of soil mixed with 50% of the initially determined doses to a depth of 7 cm;
- monitoring of buddies by irrigation according to the needs of the water needed for the growth of the potato during planting and after so as to keep the soil moist;
- the soil after the first hilling must be mixed with one of the 25% of the doses;
- the same operation for what concerns the second hilling with the last 25% of the doses, after having the germs on the surface and the formation of the stems.

Time (J), after survey	Number of main stems (petioles)	Length of petiole max (cm)	Length of compound leaf max (cm)	Number of compound leaves	Length of secondary leaf (leaflet) max (cm)	Width of secondary leaf (leaflet) max (cm)	The length of the plant (cm)	Number of secondary petioles
9	3	5.5	9266	7666	4166	3233	9.5	
12	3	8	14.333	13.666	6566	5733	13.333	
16	3333	13.666	25.666	23	8	6266	23.5	
19	3333	19.666	28	24	9.5	6,4	31.666	
23	3333	28	29	27	10.433	9666	38	
26	3333	31.333	30	35.333	10.93	9,5	40.666	14
30	3333	33.833	30.5	32.333	10.933	9633	41.833	15
33	3333	35.666	30.666	33.666	11	9833	45.5	17
37	3333	37	31.5	35.333	11.133	9,9	46.666	19
40	3333	39.666	33	37.666	11.333	10	48	19

Table 7. Potato monitoring in soil contains 300% of N requirements in raw chitin.

Figure 10. Growth of the potato according to the fertilizer used.

2.7.7. Study and follow-up of the factors indicating the good growth of the potato

The length of the plant, the length of the petioles, the length of the compound leaves and the length and width of the leaflets are all more important factors that promote photosynthesis that allows the transformation of mineral matter into plant tissue. They increase the volume of the flowering and improve its precocity, help to increase the weight of the seeds, lengthen the plant and increase the protein content of the seeds, so all these factors are important to obtain at the end of the harvest a good performance. Thus, in **Table 7**, the analyses and measurements performed on these growth factors are grouped together. We also give pictures (**Figure 10**) of some pots on which we made our measurements.

Figure 10 illustrates well that the potato in the case of the fertilization by the raw chitin is well pushed compared to the case of fertilization by the chemical fertilizer. This proves that there is a significant degradation of chitin which brings a significant amount of nitrogen to the plant.

3. Conclusion

As far as this study is concerned, the dehydrated marine residues, such as the shrimp waste, mainly raw chitin, represent an interesting source of nitrogen and carbon for the agricultural valorization. Consequently, their treatment produces wheat yields significantly higher than those fertilized with chemical fertilizers. The raw chitin is more effective than chemical fertilizer and more effective than the test.

So, we recommend the dose to bring from 200% corresponding to 180 U of nitrogen of our bio-fertilizer which is the raw chitin for the cultivation of soft wheat, variety Arrehane. The richness of shrimp was also proved by the quality and quantity of potatoes obtained after fertilization with raw chitin. The biological degradation of chitin and the important contribution of nitrogen have been proved by vegetative growth.

The use of bio-fertilizers from organic waste as chitin/chitosan remains a useful and sustainable practice. It is necessary for the reduction of these environmentally harmful wastes' rate to increase the agricultural yields and to have an effective maintenance of soil fertility.

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Chitin is the second most abundant biopolymer after cellulose and is a resourceful copious and cheap biomaterial discovered in 1859 owing to significant industrial and technological utility. Raw chitin-chitosan resembles keratin in its biological functions. Chitin chemistry vastly developed via innate unparalleled biological features and exceptional physicochemical characters. Chitosan endures assorted chemical/physical modifications easily at free proactive functionalities, yet intact bulk properties are achieved through processing, viz., film, membrane, composite, hybrid, nanofibre, nanoparticle, hydrogel and scaffolds. Rapidly lessen bioresources signify chitosan as an option due to renewable eco-friendliness and drive embryonic myriad applications in S&T. Controlled surface modification in its flexible framework imparts advanced functionalized applications in science and technology developments. Chitosan-matrix is advantageous over biopolymers due to inherent economic, versatile and unequivocal portfolio from bio-molecule to quantum dots which traced its great journey in modern S&T. Overall, chitosan chemistry boosted R&D in countless domains like agriculture, biochemical, medicine, pharmaceutics, nanotechnology, biotechnology, material/food science, microbiology, biomedicine, bioengineering, biochemistry, bioprocessing and environment.

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