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CHITOSAN: A VERSATILE EXCIPIENT FOR VARIED APPLICATIONS

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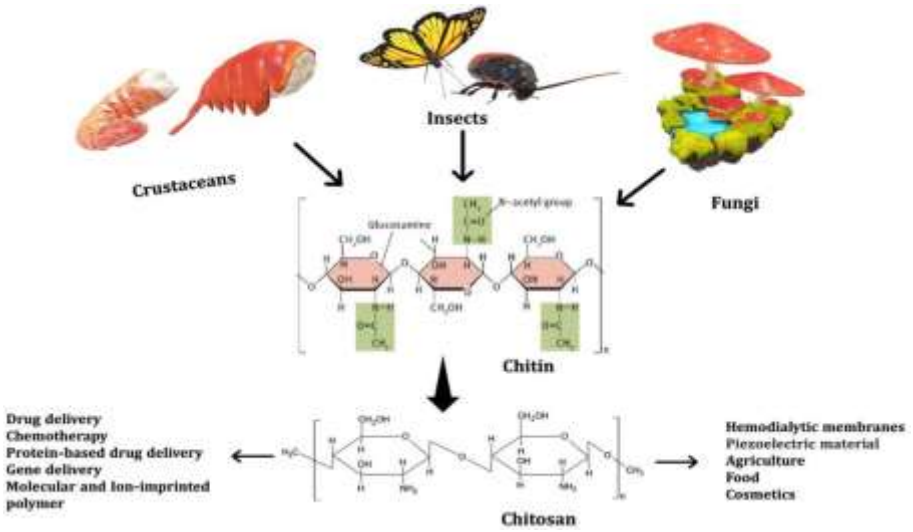
ABSTRACT

Chitosan, a deacetylated chitin derivative, has attracted significant scientific and commercial interest since the late 1970s because of its unique macromolecular structure, biocompatibility, biodegradability, and other inherent functional properties. Despite the fact that chitin is naturally abundant and can be obtained in large quantities from a variety of sources, its uses are limited due to its solubility and poor biodegradability. Deacetylation into chitosan transforms chitin into a more friendly material, influencing the performance of chitosan in numerous applications. The use of natural polymers in various formulations is very appealing to consumers, highly marketable, and is appropriate for plethora of applications. With an update in the fields of drug delivery, biomedical engineering, energy harvesting, and as hemodialytic membrane, this review aims to give readers a narrative view of the state of the art of chitosan science. It covers various aspects such as methods of chitosan preparation from chitin, physio-chemical and biological properties, and its applications in medicine, food, agriculture, and cosmetics.

Keywords: Chitosan, Chitin, Drug delivery, Piezoelectric material, Hemodialytic membrane

GRAPHICAL ABSTRACT



INTRODUCTION

Chitosan, a derivative of chitin is a copolymer of glucosamine and N-acetyl glucosamine , linked by β1–4 bonds. Chitin, the second most abundant polysaccharide after cellulose on earth is abundantly present in cell walls of crustaceans, molluscs, insects, and some fungi. Its natural abundance allows obtaining more than 1000 tons every year, of which about 70% comes from marine species. Inspite of its abundance its uses and applications are limited due to low solubility and poor biodegradability (Islam et al. 2017). Chitosan, obtained through modification of chitin either by chemical or enzymatic method on the other hand has become of great interest as a new functional material in numerous fields because of its diverse properties. The largest producers of chitosan are Japan, India, and Norway. The discovery of chitin in 1811 is attributed to Henri Braconnot while the history of chitosan dates back to 1859 with the work of Charles Rouget. The name of chitosan was, however, introduced in 1894 by Felix Hoppe-Seyler (Morin-Crini et al. 2019).

EXTRACTION OF CHITIN

Chitin is a poly (β-(1-4)-*N*-acetyl-D-glucosamine) with β(1→4) linkages **(Fig.1),** where the hydroxyl group in the C2 position has been replaced by an acetamido group (Dhillon et al. 2013). Chitin in its native form is a semi-crystalline biopolymer formed through hierarchical organization; basically the polymer chains assemble into alternating amorphous and crystalline regions, which are stabilized by hydrogen bonds and van der Waals forces (Ling et al. 2018). The three allomorphic crystalline forms are designated as α, β and γ, based on the orientation of polysaccharide chains (Hou et al. 2021). The characteristics of the tissue and the physiological function of chitin is determined through its molecular and crystalline order. Chitin provides favorable sites for nucleation, regulates the placement and orientation of mineral phases, and acts as a template for bio- mineralization processes including calcification and solidification. This process explains why chitin may be found in solid forms in a range of biomass, including the exoskeletons of crustaceans and the cell walls of fungus and diatoms. In addition to being found in bacteria, fungus, and algae, chitin may be found in various forms in at least 19 different animal phyla (Ahmad et al. 2020). The extraction of chitin from fungal sources is gaining momentum due to the greater advantages of using fungal sources over traditional sources. The synthesis of chitin is a highly complex and sequential process that shows variations according to the species includes various steps including demineralization, deproteinization and decolorization. The deproteinization is carried out by an alkaline treatment, whereby lipids and proteins are hydrolyzed. The demineralization stage is generally performed in the presence of acids, whereas the decolorization requires an oxidative treatment. Due to the high acetyl content, chitin is hydrophobic in nature. Deacetylation of chitin by basic treatment into chitosan transforms it into a more water and organic solvents friendly material, influencing the performance of chitosan. Depending on the production method and species used, the degree of deacetylation ranges from 56 to 99%, but at least 85% deacetylation is required for a good solubility of chitosan (Younes and Rinaudo 2015). Apart from chemical methods chitin extraction can also be carried out through biological methods which can be classified as enzymatic or fermentation methods where the former used enzymes to break down shells and the later uses bacteria to digest until only chitin remains (Hossin et al. 2021). In enzymatic methods, proteinases are used to remove shell proteins and isolate chitin. This method requires specificity of an enzyme and mild reaction conditions (commonly 25–59 °C) to remove proteins with minimal deacetylation and damage to the chitin chain (Hamdi et al. 2017). The fermentation method involves demineralization through the use of acid-producing bacteria, most commonly used cultures for this process are lactic -acid producing bacteria. It can result in complete extraction of chitin and fermentation with protease-producing cultures can improve the purity of chitin produced (Mohan et al. 2022). The major limitation of enzymatic process is the optimization of reaction time and cost , particularly when scaling up the production. Even with longer reaction times it is rare for deproteination to exceed 90% which attributes to the loss of active sites to which enzymes can bind as proteins are removed from chitin

(Kou et al. 2021). For fermentation process, preparation of media to support culture growth and source of carbon is the major requirement for achieving optimal chitin extraction. The major drawback is the long fermentation time which can last for three to four days (Cahyaningtyas et al. 2021).

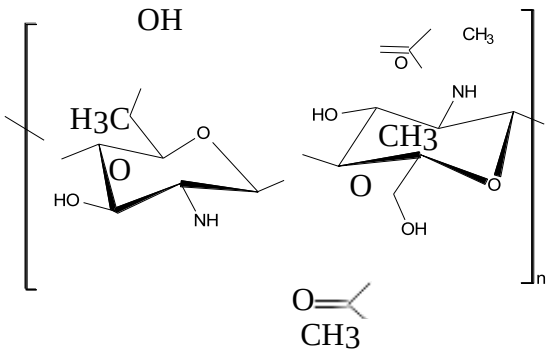


Figure: 1 Structure of Chitin PREPARATION OF CHITOSAN

FROM CHITIN

Chitosan, an important biopolymer can be obtained from animal/marine sources and can be extracted or produced from various fungal sources after the chitin deacetylation. The degree of deacetylation in chitin is typically 0.90, which indicates presence of certain amine groups. Chitin can be converted to chitosan through this step, wherein N-acetyl groups are converted into amine **(Fig.2).** It has a typical degree of deacetylation of less than 0.35 and is thus a copolymer composed of glucosamine and N-acetylglucosamine (John Kasongo et al. 2020). Deacetylation can be done using traditional chemical approach at temperatures usually above 100
°C. These reactions can last between 30 minutes and 5 hrs; some reactions can last up to 24 hrs (Kou et al. 2021). However, it can be carried out using biological methods through enzyme deacetylase, sourced from fungi. The major limitation to enzymatic method is that chitin deacetylase is not commercially available and those extracted form biomass suffer from low activity (Tan et al. 2020). Longer reaction times, higher temperatures, and higher alkali concentrations correspond to higher degrees of deacetylation at the cost of molecular weight (John Kasongo et al. 2020). The charge density of chitosan depends on the degree of deacetylation and pH of the media. The oligomers of chitosan are soluble over a wide range of pH, contrary to this chitosan with higher molecular weight is soluble in acidic media inspite of high deacetylation degree (Kaczmarek et al. 2019). Recent advancements in fermentation technology suggest that fungal biomass could serve as a potential source of chitosan to meet its growing requirement for varied applications.

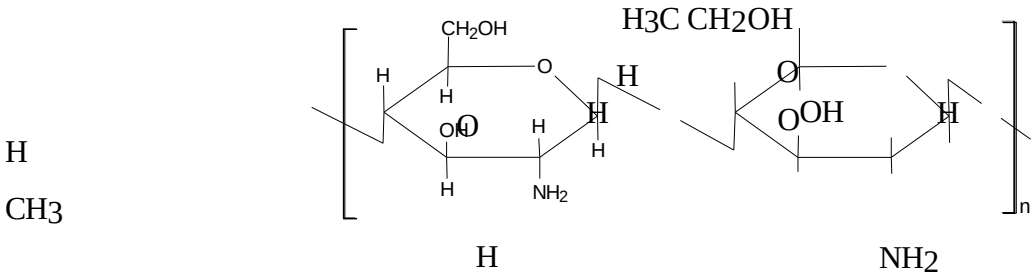


Figure: 2 Structure of Chitosan

CHITOSAN: PROPERTIES AND APPLICATIONS

Physio-chemical properties

Chitosan is a linear aminopolysaccharide with high nitrogen content and has attracted major scientific and industrial interest as a new functional material because of its structure, biocompatibility, biodegradability and emerged as a very important raw material for the synthesis of wide range of products in various industries (Dhillon et al. 2013). The functional groups found in chitosan are a primary amino group, primary and secondary hydroxyl groups, glycosidic bonds and the acetamide group. These functional groups present in the polymer allow for a great number of modifications, producing polymers with new properties and behaviors (Aranaz et al. 2021). It is characterized by molecular weight in the range of 100-500 kDa which is determined by measuring the viscosity. The choice of molecular weight of chitosan depends on the nature of desired application. Generally, chitosan is highly soluble in acid solution (mainly below pH = 6.0), due to the presence of amine groups. At low pH values, the amine groups are positively charged due to protonation, so chitosan can be a water-soluble cationic polyelectrolyte. However, when the pH increases above 6, the amine groups of chitosan residues are deprotonated, and the biopolymer loses its charge leading to an insoluble polymer (Jiménez-Gómez and Cecilia 2020). The solubility of chitosan depends on different factors such as polymer molecular weight, degree of acetylation, pH, temperature, and crystallinity. The stability of the polymer is determined through its viscosity and viscosity in turn depends on the molecular weight and deacetylation degree. It decreases as the molecular weight of chitosan is reduced (Wang and Xu 1994).

Biological Properties

Chitosan and its derivatives exhibit numerous important biological properties including antitumoral, antimicrobial, antioxidant, anti-inflammatory, non-toxic, biodegradable, biocompatible, anticoagulant, and anti-allergic which allows chitosan to be used in a multitude of applications **(Fig:3)**. Chitosan, and its derivatives exert antimicrobial activity against different microorganisms, including bacteria, filamentous fungi, and yeast. It seems to have a growth-inhibitory activity since bacteria is able to grow after the polymer is removed from the media. This is of importance since resistant populations might emerge if the cells adapt to chitosan (Raafat et al. 2008). Due to the presence of amino and several hydroxyl groups, chitosan can react with free radicals exhibiting scavenging ability demonstrating anti-oxidant potential (Zhou et al. 2021). Interestingly, they can be modified to improve their antioxidant activity, for instance modifications with gallic acid (Ngo et al. 2011) or phenolic compounds (Eom et al. 2012). It also increases the healing for open

wounds and reconstruction if tissue by prevention microbial infections. It can be easily biodegradable by the effect of bio enzymes that can hydrolyze chitosan into its oligomers. The degradation products are *N*-acetyl glucose and glucosamine, which are nontoxic to human body and have no immunogenicity. In addition to these characteristics, chitosan-based nanoparticles have a high binding power with empty orbitals and a large number of lone-pair electrons, which are used in a variety of applications that are discussed further. The uniformity and particle size of the nanoparticles serve as the foundation for this function. These microspheres can be created by crosslinking emulsions, ionically crosslinking materials, evaporating solvents, spray drying, precipitation, or flocculation, and coating them with chitosan solutions (Zhao et al. 2018).

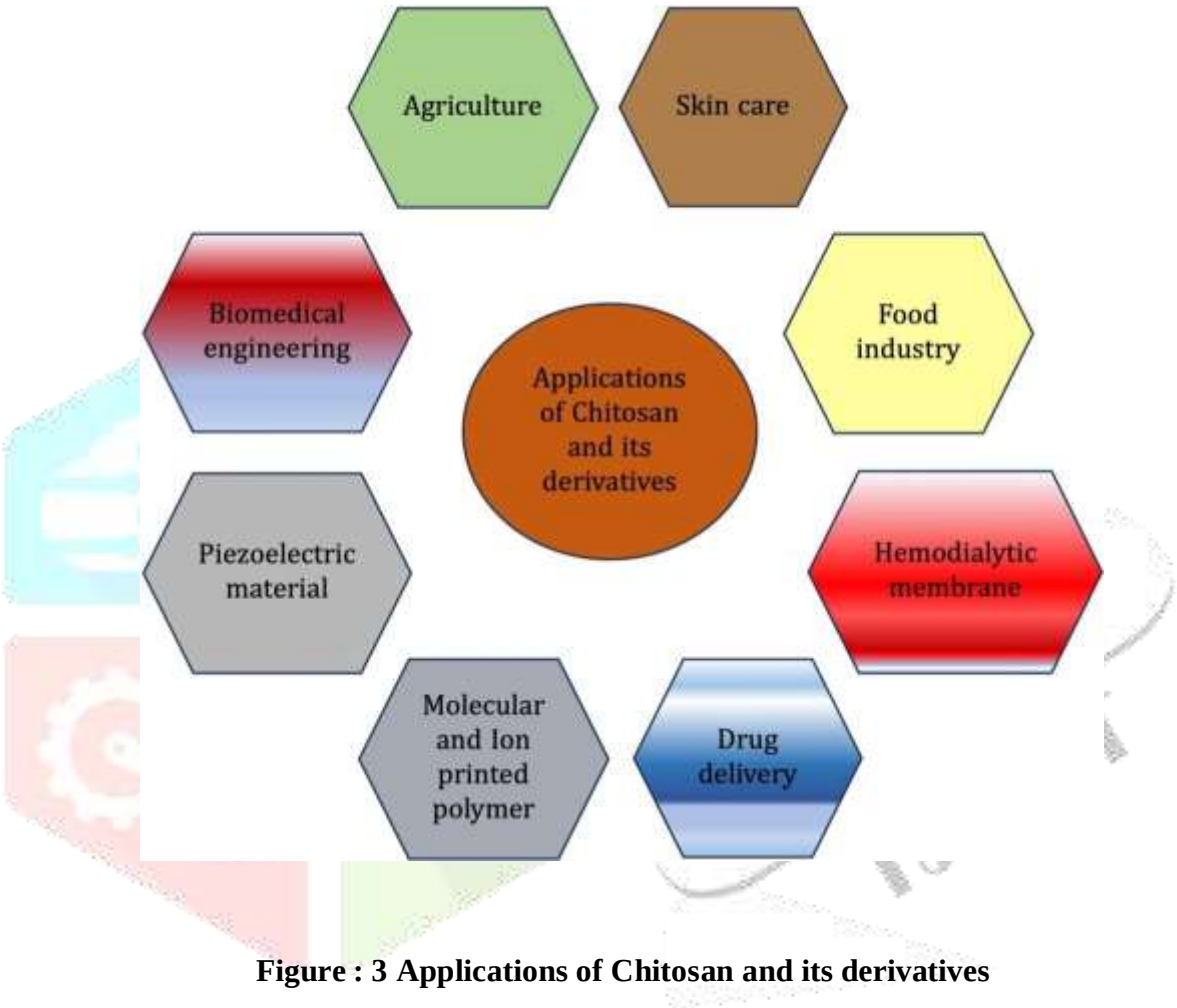


Figure : 3 Applications of Chitosan and its derivatives

APPLICATIONS OF CHITOSAN

Drug delivery

The technological property of chitosan makes it an excellent candidate for controlled drug delivery systems this is due to the presence of amino groups which results in the cationic character of this biopolymer. These amino groups are responsible for numerous properties such as controlled drug release, in situ gelatin, transfection, permeation enhancement, and efflux pump inhibitory properties (Bernkop-Schnürch and Dünnhaupt 2012). It is widely used for the encapsulation of a large variety of molecules such as growth factors, anti-microbials, anti-tumor or anti-inflammatory drugs. Its physical and biological properties makes it useful for the fabrication of microneedles making it suitable for transdermal drug delivery (Aranaz et al. 2021).

Chemotherapy

Chitosan can be used to deliver both hydrophilic and hydrophobic anti-cancer drugs. The presence of free amine groups can be easily functionalized for conjugation of chemotherapeutic drugs. Doxorubicin is a water- soluble drug used for cancer treatment. The most common side effect is cardiotoxicity. In order to reduce these side effects, the drug has been encapsulated in chitosan nanoparticles which can improve the absorption in small intestine. This can improve the absorption and also increases the survival rate of drug conjugate and reduces adverse reaction of drugs (Feng et al. 2013). Chitosan and its derivatives have been synthesized with suitable characteristics that can support the delivery of poorly water-soluble drugs. Paclitaxel, a hydrophobic chemotherapeutic, showed enhanced activity when encapsulated in a glyceryl monooleate-chitosan core-shell nanoparticle prepared using an emulsification-evaporation technique. Under acidic tumor conditions chitosan nanoparticles containing paclitaxel become smore aggressive and interact strongly with negatively charged tumor cells (Yang et al. 2009).

Protein based drug delivery system

Peptide/protein-based drugs are structurally and chemically labile compounds. They are easily hydrolyzed by enzymes in the gastrointestinal tract. Due to their inherent physical, chemical, and proteolytic instability and the large size these biotherapeutics are poorly absorbed across mucosal surfaces. Thus, non-invasive mucosal routes have attracted interest for administration of Peptide/protein-based drugs (Amidi et al. 2010). Chitosan based delivery system can significantly enhance the stability, absorption and/or cellular uptake. Insulin-based chitosan nanoparticles have been synthesized through membrane emulsification and crosslinking. The resulting chitosan nanoparticles exhibited high drug entrapment efficiency, good stabilization, low outbreak, and steady release of insulin (Wang et al. 2006). Chitosan is a mucoadhesive polysaccharide capable of opening the tight junctions between epithelial cells and thus enhance the assimilation of hydrophilic substances through the epithelial layer. Due to the presence of various functional groups, large variety of chitosan derivatives can be obtained with tunable properties aimed for the applications (Garg et al. 2019).

Gene delivery

The development of a dependable and secure delivery method continues to be a significant barrier to the success of gene therapy. The development of novel carrier systems for gene delivery is an enabling technique for the treatment of many genetic diseases. Chitosan has been promoted as highly attractive biopolymer to deliver nucleic acids intracellularly and induce a transgenic response resulting in either upregulation or down reregulation of protein expression due to its potential adjuvant properties and reduced hazardous effects that come with other synthetic vectors. It can bind DNA and prevent it from being degraded by nucleases, thereby increasing the resident time of DNA in the gastrointestinal tract and can transport genes in vivo with comparable effectiveness (Masotti and Ortaggi 2009). The degree of acetylation and polymerization affects the biophysical characteristics and biological functionality of chitosan-based systems(Huang et al. 2005). An intricate coagulation process was used to produce plasmid DNA that was enclosed in chitosan nanoparticles, and the results demonstrated that the plasmid DNA was successfully expressed in vivo (Guliyeva et al. 2006). There have been numerous attempts to establish a relationship between different parameters which can affect the efficiency of transfection with plasmid DNA in vitro and to determine the intracellular trafficking routes underlying their mode of action (Thibault et al. 2010).

Molecularly and Ion imprinted polymer

Molecular imprinted polymers (MIP) are synthetic materials containing specific cavities (template) to a target molecule. Once the template molecule is extracted from the resulting polymer, a specific three-dimensional cavity is created which is responsible for the recognition and specific integration of the target molecule in the cavities. The mechanism of MIP to bind to template molecule is similar to that of antibody to recognize specific antigen in the biological system. Chitosan is extensively used as imprinting polymer with a selected crosslinking agent which allows the creation of three-dimensional networks that facilitate the incorporation of the template and as an additive material for MIP nanocomposite preparation. The presence of hydroxyl and amine groups in chitosan facilitates its modification to react with different cross-linking agents (Karrat et al. 2020).

Biomedical engineering

A substantial body of evidence suggest that chitosan and its derivatives are promising candidates for supporting materials in tissue engineering. The implantation of either an autologous or synergistic graft in place of the damaged area of the body is the contemporary practice of tissue engineering. The implant must satisfy the requirements relative to biocompatibility as well as functional and mechanical stability(Aranaz et al. 2021). This involves combining different scaffolding material with modulated cells, including natural and synthetic polymers. A scaffold should possess high porosity, biodegradability, structural integrity and importantly should be non-toxic to cells and biocompatible (Jayakumar et al. 2010). Several researchers have developed chitosan-based scaffolds using lyophilization technique and found these composite scaffolds to be compatible with adequate porosity, degradation properties and that chitosan alone or in combination with other polymers have demonstrated the significant promise of these biopolymers for tissue engineering that can be fine-tuned to suit the increasing focus that this field requires (Peter et al. 2010; Chung et al. 2002).

Hemodialytic membranes

Chitosan holds promise for being used as an artificial kidney membrane due to its intrinsic high mechanical strength in addition to permeability to urea and creatinine and impermeability to serum proteins (Radhakumary et al. 2005). It can be modified by blending with water-soluble polymers and by graft copolymerization to develop dialysis properties such as adsorption and diffusion through the pore membrane (Saiful et al. 2022)

Piezoelectric material

The physical and chemical properties of chitosan make it advantageous for its use and application in sensors. Piezoelectric materials are known as smart materials as they can transduce the mechanical energy applied to it into electrical signals, and this is a reversible process and hence can be used for force/pressure-sensing applications. Glycine and chitosan are used in fabrication of the films consisting of a stable spherulite structure of beta glycine embedded in an amorphous chitosan polymer. This can be used as a promising biodegradable sensor for applications in biomedical diagnosis (Hosseini et al. 2020). Also, this piezoelectric property of chitosan is exhibited due to its non-centrosymmetry nature (Praveen et al. 2017). The piezoelectric phenomenon is highly dependent on the crystal lattice structure of the materials used and the presence of a centre of symmetry in the structure. The most famous piezoelectric polymer used is poly vinylidene fluoride (PVDF) which is used in various biomedical applications due to its high piezoelectric coefficient. Because of solubility of chitosan in acids, biocompatibility, non-toxic, anti-bacterial and easily

formed into films, it is very favorable as piezoelectric biomaterial (Hazmi et al. 2023).

Agriculture

Due to the presence of hydroxyl and amino groups in the crosslinked structure of chitosan, microstructures, nanoparticles, and nanocomposites of chitosan have been widely used as an absorbent to remove various inorganic and organic pollutants from wastewater for sustainable agricultural practices (Olivera et al. 2016). Chitosan-based nanoparticles (CNPs) has been used in agriculture as pesticides, herbicides, insecticides, and to produce better-quality food with a higher yield. (Kumaraswamy et al. 2018). Additionally, due to its cationic nature, biodegradability, non-toxicity, and adsorption properties, chitosan can be used by itself or in conjunction with other substances to serve as an encapsulating agent in the creation of slow-release fertilizers (Bandara et al. 2020). Salinity is a significant stress factor affecting plant growth throughout the world. Nano chitosan helps in controlling salinity stress due to its high surface to volume ratio resulting in higher penetrability and the ability to form more interactions (Sen et al. 2020). Also, nano chitosan is able to combat drought stress, heavy metal stress and abiotic stress (Arif et al. 2021). It is known that chitosan NPs induce plants to respond with innate immunity by up-regulating defense-related genes and elevating secondary metabolites.

Food

Since synthetic compounds are becoming less popular in food applications, chitosan and its derivatives have attracted a lot of attention in recent years. The broad-spectrum antimicrobial activity of chitosan offers great commercial potential for this product as natural antimicrobial. This activity is associated with its physicochemical characteristics and depends on the type of microorganism. The mode of action can be classified as extracellular and intracellular, or both based and is highly dependent on the targeting site and type of microorganism (Ke et al. 2021). Also due to their biodegradability and semi-permeability, several approaches have been used to utilize chitosan along with other polysaccharides to form edible films and coatings to extend the shelf life and improve the quality of food products. These films are tough, long-lasting, flexible and very difficult to tear (Shahbaz et al. 2023). Processing of clarified fruit juices commonly involves the use of clarifying agents, including gelatin, bentonite, silica sol, tannins, potassium caseinate and polyvinyl pyrrolidone. Chitosan demonstrated powerful clarifying capability without affection nutritional value of fruit juices (Abdelmalek et al. 2017). Recently the effectiveness of chitosan as a dietary supplement in lowering cholesterol in murine models has been investigated. Lifestyle disorders, which pose a serious threat to cardiovascular health, have increased considerably. According to a meat analysis done by Ahn et al it was found that chitosan administration decreased triglyceride and cholesterol levels and that bioavailability of dietary fat was also decreased (Ahn et al. 2021).

Cosmetics

Many of the potential uses of chitosan in tissue engineering involve skin or mucous membranes, which can serve as inspiration for the development of efficient and safe cosmetics. Despite being less popular than other polymers like collagen or hyaluronic acid, chitosan serves two crucial cosmetic functions: film formation and hair fixing (Aranaz et al. 2018). It has the ability to bind water, which hydrates the skin and forms a hydrophilic film that prevents water loss from the skin. It can also be used as a thickener. In order to create clear, elastic films over the hair fibres, chitosan and its cationic derivatives interact with keratin. These films strengthen and soften hair while preventing hair damage. When hair was covered in chitosan, the mechanical properties of the hair improved, increasing hair thickness while also improving overall appearance and conditioning of the hair (Sionkowska et al. 2017). Chitosan and its films show UV absorption below 400nm making them useful in sunscreens and prevent skin photoaging. It is possible that origin of chitosan and its properties may contribute to the capacity of UV resistance (Kong et al. 2017). 0.1% chitosan increases the

availability of lipophilic components of the creams favoring better penetration of vitamins and minerals. Vitamin C, which is widely used in cosmetics, is prone to degradation, thus new formulations are required to keep it stable. It was encapsulated using electrostatic interaction with glycidyl trimethylammonium chloride-chitosan and then cross-linking with phosphorylated cellulose to form nano capsules. The resulting nano-formulation has improved antioxidant and antibacterial properties and can control the release of active compounds in food and nutraceutical applications (Baek et al. 2021). Chitosan's antimicrobial property also makes it possible to reduce the quantity of preservatives used in the formulation (Kulka and Sionkowska 2023).

CONCLUSION

Chitosan and its derivatives are promising materials and have been used in a myriad of applications for a long time despite having few limitations. The presence of primary amino groups on the chitosan backbone offers opportunities for chemical modification, leading to unique physio-chemical properties that make them versatile biopolymers for applications in tissue engineering, drug delivery, gene delivery, agriculture, skin care, food industry, water purification, and other fields. However, thorough research on the biological and physiochemical properties of chitosan derivatives should be carried out, particularly the research on their toxicity to human being should be comprehensively studied. Although there are countless potential uses for chitosan-based nanoparticles, it is important to take environmental sustainability and green manufacturing into account when developing high-tech chitosan-based products for the benefits of humans. The physiochemical properties of chitosan are also influenced by its source, and it has been discovered that chitosan derived from fungi is more consistent and has the requisite properties than chitosan derived from other sources such as insects, crustaceans. In contrast to chitosan derived from crustacean sources, the physicochemical properties of fungal chitosan, such as molecular weight and degree of deacetylation, can be controlled. However, more research is required to determine its toxicity and stability at various temperatures. Furthermore, its antimicrobial property can be used to develop water disinfection devices and find its use as a preservative in various foods. However, more research is required into toxicity and stability at various temperatures. Particularly in the food industry and nutraceuticals, the molecular weight and chain length of chitosan and its derivatives determine the majority of their physiological and functional properties. Since the human intestine lacks the enzyme chitosanase, chitosan cannot be degraded, its beneficial effects on metabolic disorders should be further investigated. As a result, it can create a large amount of dietary fiber that is excreted without any degradation. Since dietary fiber is known to lower cholesterol and regulate other factors that can improve cardiovascular disorders, chitosan as a nutraceutical may have an impact on the physiological functions or metabolism in human body.

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