Chitin and chitosan nanoparticles: Sustainable solutions for agriculture, aquaculture, environmental pollution and food industry

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Abstract

Chitin and chitosan, naturally derived biopolymers from crustacean shells and fungal cell walls, have gained significant attention due to their biodegradability, biocompatibility and eco-friendly properties. Their application in agriculture has demonstrated promising results in crop protection, nutrient delivery and soil health improvement. In aquaculture, chitin and chitosan nanoparticles have been explored for disease management, water quality enhancement and sustainable feed supplementation, offering eco-friendly alternatives to conventional treatments. Additionally, their strong adsorption capabilities make them effective in wastewater treatment and pollution control by removing heavy metals and organic pollutants. In the food industry, their antimicrobial, antioxidant and barrier properties contribute to food preservation, extending shelf life and ensuring quality. This review highlights recent advancements in the utilization of chitin and chitosan nanoparticles across these domains, emphasizing their potential as sustainable and multifunctional materials for diverse applications.

Keywords: Chitin, Chitosan nanoparticles, Agriculture, Aquaculture, Environmental pollution and Food science and Technology

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Introduction

Chitin, a naturally occurring polysaccharide, was first identified by Henri Braconnot in 1811 and initially termed fungine. Later, in 1823, Odier discovered the same compound in insects and plants, renaming it chitin. As the second most abundant polysaccharide after cellulose, chitin plays a crucial role in providing structural integrity in various biological systems. While cellulose strengthens plant cell walls, chitin is essential for the mechanical support of fungal cell walls and arthropod exoskeletons (Rudall and Kenchington, 1973). The estimated global presence of chitin in the biosphere is around 1.1 ×10^{13} kg. However, its industrial and commercial applications are restricted due to its insolubility in most solvents and the difficulty of obtaining it in a pure and cost-effective manner. Chitin is a rigid, nitrogenous polysaccharide, white in appearance and inelastic in nature, found in invertebrate exoskeletons and internal structures. Structurally, it is a linear polymer composed of N-acetylglucosamine residues linked via β-1,4 glycosidic bonds. This biopolymer exists in three polymorphic forms α, β and ϒ distinguished by differences in their molecular arrangements (Khoushab and Yamabhai, 2010). Among these, α-chitin is the most widespread, found in fungal and yeast cell walls, insect cuticles, nematode and rotifer eggshells and various marine organisms such as krill, lobsters, crabs and shrimp. It is also present in specialized structures such as mollusk radulae, cone snail harpoons and Phacocystis seaweed filaments. In contrast, β-chitin is predominantly located in squid cuticles, cephalopod pens and diatoms such as *Thalassiosira fluviatilis*. The structural distinction between these forms lies in their molecular arrangement: α-chitin consists of parallel chains held together by intermolecular hydrogen bonding, making it highly rigid with low water swelling capacity, whereas β-chitin lacks inter-sheet hydrogen bonding, which allows for higher water absorption and swelling properties (Minke and Blackwell, 1978). The ϒ-chitin variant is considered a hybrid of α and β forms rather than a distinct polymorphic structure (Roberts, 1992).

Chitin's biosynthesis is facilitated by chitin synthase, an enzyme belonging to the glycosyltransferase family. This enzyme catalyzes the polymerization of N-acetylglucosamine monomers using UDP–N-acetylglucosamine as a precursor, with divalent cations acting as cofactors. The biosynthetic pathway follows three key stages: first, chitin synthase, embedded in the cytoplasmic membrane, initiates polymer formation. Next, the growing polymer is translocated across the membrane and released into the extracellular matrix. Lastly, individual polymer chains spontaneously assemble into crystalline microfibrils of varying dimensions (Merzendorfer, 2006). The crystalline structure of chitin is primarily stabilized by extensive hydrogen bonding, which occurs between carbonyl (C=O) and amine (H-N) groups, as well as between adjacent sugar rings (Minke and Blackwell, 1978). With the exception of

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chitin found in diatoms, natural chitin is often cross-linked with other biomolecules. In fungal septa and cell walls, it integrates with other polysaccharides such as glucans and mannans, either directly or through peptide bridges (Roberts, 1992). In arthropods, chitin interacts with structural proteins via covalent and non-covalent bonds, forming complex architectures that contribute to its functional properties (Meyers and Kozloff, 1990).

Chitosan

Chitosan, a derivative of chitin, is predominantly obtained from the exoskeletons of crustaceans like crabs, shrimp and crawfish (Raafat and Sahl, 2009). Commercially, it is produced through the deacetylation of chitin, with the degree of deacetylation influencing its properties (Kumar, 2000). Recognized for its biocompatibility, biodegradability and non-toxic nature, chitosan possesses remarkable film-forming capability, chelation and absorption characteristics. These properties have led to its widespread application in pharmaceuticals, cosmetics, medicine, food processing and agriculture (Kurita 2000; Raafat and Sahl, 2009). Chitosan is a cationic polysaccharide composed of β-(1,4)-linked 2-acetamino-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose, with reactive amino groups at the C2 position and hydroxyl groups at C3 and C6 that facilitate diverse industrial applications (Pradip *et al*., 2004). It has a significantly higher nitrogen content (7.21%) than synthetically substituted cellulose (1.25%) (Muzzarelli, 1973).

Chitosan nanoparticles

Due to its biodegradability and biocompatibility, chitosan has been extensively used in nanoparticle systems (Muhammed Rafeeq *et al*., 2010). These nanoparticles are advantageous for their ability to regulate the release of active agents, their non-toxic nature and their free amine groups that allow cross-linking (Agnihotri *et al*., 2004). Chitosan nanoparticles are used in a variety of applications, including drug delivery systems, enzyme encapsulation and wound healing coatings (Wang *et al*., 2011). The synthesis of chitosan nanoparticles involves several techniques, such as emulsion cross-linking, coacervation/precipitation, ionotropic gelation and molecular self-assembly, each affecting the final properties of the nanoparticles (Mitra *et al.*, 2001). When properly synthesized, these nanoparticles exhibit strong affinity for negatively charged biological membranes, facilitating site-specific targeting in vivo (Sadeghi *et al*., 2008).

Economic aspects of chitin and chitosan

The global commercial production of chitin and chitosan occurs in countries such as India, Japan, Poland, Norway and Australia (Madhavan *et al*., 1974). The main raw material for production is shrimp shell waste from seafood processing industries, making the process economically viable, especially when coupled with carotenoid recovery. Shrimp shells contain astaxanthin, a

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highly valuable carotenoid primarily used as a fish feed additive in aquaculture, particularly for salmon farming. Producing 1 kg of 70% deacetylated chitosan from shrimp shells requires approximately 6.3 kg of HCl, 1.8 kg of NaOH, nitrogen, 0.5 tons of process water and 0.9 tons of cooling water. The major cost-determining factors include transportation expenses, which vary depending on labor costs and production locations.

Methods for chitin extraction and chitosan synthesis

Countries like the USA, Japan, Norway, Thailand, India, Australia and Poland are major producers of chitosan. The standard process of chitosan production involves demineralization, deproteinization, decoloration and deacetylation, with the sequence of demineralization and deproteinization being interchangeable (Hirano and Nagao, 1989). Alternative methods, such as enzymatic deproteinization using microbial enzymes, have been explored to enhance efficiency and minimize degradation. Enzymatic treatment of shrimp waste using *Aspergillus niger*, followed by fermentation-derived acetic or lactic acid demineralization, yields high-quality chitin (Rinaudo, 2006). Additionally, microbial species such as *Bacillus subtilis, Lactobacillus helveticus* and *Pseudomonas aeruginosa* have been used for demineralization (Choorit *et al*., 2008). Furthermore, enzymatic deacetylation using fungal chitin deacetylase presents a sustainable approach for chitosan synthesis. These advancements ensure a more environmentally friendly and efficient production process, promoting the sustainability of chitosan applications in various industries.

Biological extraction of chitosan

Biological extraction represents an advanced and innovative approach for obtaining chitin. This method employs proteolytic microorganisms, fungi, or purified enzymes, leading to the production of oligomers with an optimal degree of polymerization for various applications. Unlike chemical methods, it preserves the native structure of chitin while yielding a higher molecular weight product through enzymatic processes. In addition to environmental benefits, enzymatic extraction eliminates the hazards associated with reactive chemical reagents. The overall cost of biological chitin extraction can be reduced by optimizing the use of carbon sources such as glucose, dextrose, lactose and sucrose. The application of proteases for deproteinization of crustacean shells eliminates the need for alkali treatment. Moreover, proteolytic bacteria have been utilized for the deproteinization of demineralized shells, leading to valuable by-products (Jung *et al*., 2007).

This method (Fig. 1) produces some liquid fraction rich in proteins, minerals and astaxanthin, along with a solid chitin fraction. The liquid portion can serve as a protein-mineral supplement for human consumption or as animal feed (Einbu *et al*., 2004).

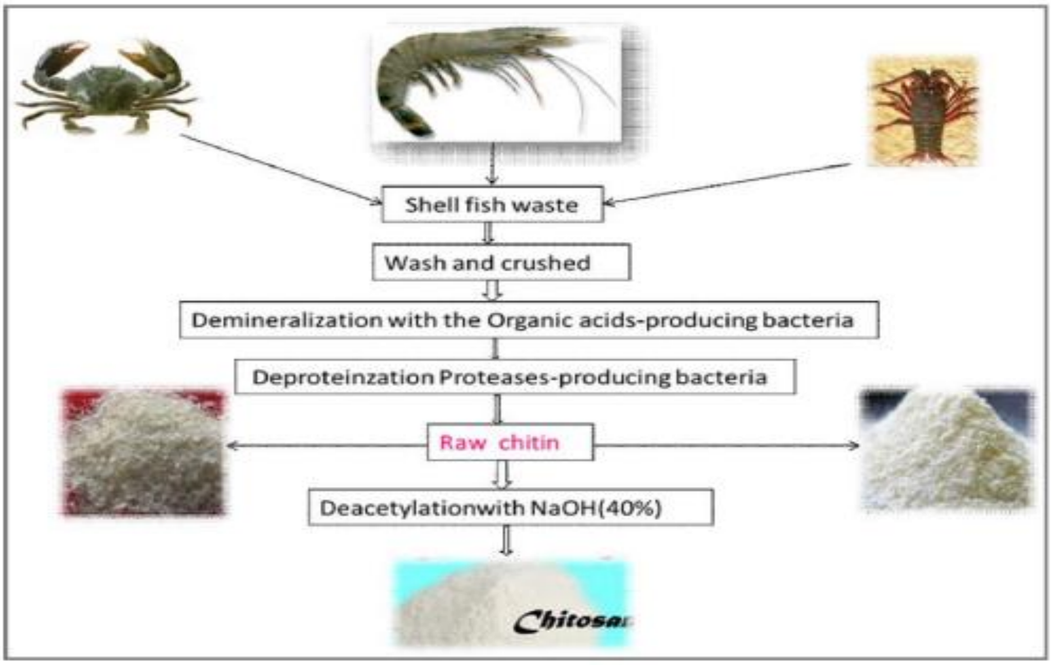

Figure 1: Flow diagram showing biological method of chitosan production (Jag pal *et al*., 2014).

Various deproteinization processes have been employed for chitin extraction, primarily from shrimp waste, using mechanical, enzymatic and microbial techniques. Several bacterial species, including *Lactobacillus* (Rao *et al*., 2000), *Pseudomonas aeruginosa* K-187 and *Bacillus subtilis*, have been reported to facilitate deproteinization. Similarly, biological demineralization has been documented using enzymatic agents such as alcalase (Synowiecki and Al-khateeb, 2000) or microbial strains such as *L. pentosus* 4023 (Bautista *et al*., 2001). Additionally, a natural probiotic (milk curd) has also been explored for its role in biological demineralization (Prameela *et al*., 2010). These processes generally involve simultaneous demineralization and deproteinization, though they may not always be complete (Jung *et al*., 2007).

Fermentation has been a traditional method applied in fish processing for decades due to its affordability and low technological requirements (Fagbenro, 1996). In chitin extraction, this approach involves ensiling crustacean shells and producing lactic acid in situ from by-products such as whey, lignocellulose and starch. The presence of lactic acid bacteria induces liquefaction of semi-solid waste, reducing pH and activating proteases, thereby facilitating protein separation from chitin (Shirai *et al*., 2001). As a result, chitin remains in the sediment while a protein-rich liquid fraction is recovered (Winter *et al*., 2021). This approach offers potential for commercial chitin recovery. Lactic acid production lowers pH, enhancing ensilation and inhibiting spoilage microorganisms. Furthermore, lactic acid interacts with calcium carbonate in crustacean shells to form calcium lactate,

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which can be removed by washing. Organic salts derived from this process have applications as de-icing or anti-icing agents, as well as preservatives (Jung *et al*., 2008). Deproteinization of biowaste and simultaneous liquefaction of shrimp proteins occur primarily through proteolytic enzymes produced by *Lactobacillus* strains, gut bacteria in shrimp, or proteases naturally present in biowaste (Rao *et al*., 2000; Vinusha *et al*., 2022, 2020, 2019, 2017). This results in a liquid fraction containing a high concentration of soluble peptides and free amino acids (Fagbenro, 1996). Khanafari *et al*. (2008) compared chemical and microbial methods for extracting chitin and chitosan from shrimp waste, concluding that microbial methods are significantly more effective, particularly for chitin recovery. This highlights the efficiency and sustainability of biological techniques over traditional chemical approaches.

Chitin and chitosan oligomers exhibit a range of biological activities, including antitumor properties (Pae *et al*., 2001), immune system enhancement (Mac Laughlin *et al*., 1998) and protection against certain pathogenic infections. Additionally, these oligomers possess antifungal and antimicrobial properties (Saifuddin and Dinara, 2011; Vinusha *et al*., 2015). Studies have demonstrated the antitumor potential of N-acetylchitohexaose, a hexamer of chitin oligosaccharide, as well as chitohexaose, a hexamer of chitooligosaccharide. Furthermore, the protective role of N-acetylchitohexaose against Listeria monocytogenes infection in mice has been explored. Despite these promising findings, large-scale production of a specific oligosaccharide homologue remains a significant challenge for practical applications (Liang *et al*., 2007; Vinusha *et al*., 2016).

Properties of chitosan

Physical properties

Chitosan is classified as a weak base, characterized by its insolubility in water but its ability to dissolve in diluted acidic solutions with a pH below its pKa (~6.3). Under such conditions, the glucosamine units ($-NH_2$) undergo protonation, transforming into the soluble form ($-NH_3^+$). The solubility of chitosan is influenced by factors such as its biological origin, molecular weight and degree of acetylation (Sheperd *et al*., 1997). Due to its solubility in acidic media, chitosan can be processed into films through casting or dipping, forming either dense or porous structures (Assis and Pessoa 2004).

Chemical properties

Chitosan contains three reactive functional groups: primary (C-6) and secondary (C-3) hydroxyl groups, along with an amino group at the C-2 position in deacetylated units. These groups allow chemical modifications such as etherification and esterification, which can alter its mechanical properties, solubility and overall functionality. Additionally, chitosan can undergo reactions including N-alkylation, N-acylation and reductive alkylation when interacting with alkyl halides, acid chlorides and aldehydes or ketones, respectively, through its nucleophilic amino group. Selective O-substitution can also be performed by temporarily protecting the amino group during the reaction. Furthermore, modifications like cross-linking and graft copolymerization enhance its properties for specific applications (Dutta *et al*., 2004; Harish Prashant and Tharanathan, 2007).

Biological properties

Chitosan and its derivatives have gained significant interest due to their diverse applications in medical, pharmaceutical and industrial fields. Key properties contributing to their versatility include biodegradability, biocompatibility and low toxicity (Goy *et al.,* 2009; Raafat and Sahl, 2009). Additionally, chitosan has demonstrated various bioactive effects such as analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobial and antioxidant activities (Koide, 1998; Kumar, 2000; Kumar *et al*., 2004).

Biodegradability

Although chitosan is not naturally present in mammals, it can be degraded *in vitro* by nonspecific enzymes, including lysozymes, pepsin, papain, cellulase, pectinase, proteases and lipases (Pantaleone *et al*., 1992; Darmadji and Izumimoto, 1994; Yalpani and Pantaleon, 1994; Kumar *et al*., 2005). Additionally, chitosanases (chitosan N-acetyl-glucosamino-hydrolases) catalyze its degradation, producing non-toxic oligosaccharides of varying lengths that can be integrated into metabolic pathways, glycosaminoglycans, or glycoproteins, or excreted from the body (Pangburn *et al*., 1982).

The biodegradation rate of chitosan is largely dependent on its crystallinity, which is controlled by the degree of deacetylation (DD). The acetyl group distribution also plays a role in determining degradation efficiency, with homogeneous acetylation leading to slower enzymatic degradation (Aiba, 1992; Francis *et al*., 2000). Several studies indicate that a lower DD correlates with a faster degradation rate. For instance, Kofuji *et al*. (2005) observed that chitosan solutions with lower DD exhibited a greater reduction in viscosity due to lysozyme activity. However, other researchers argue that variations in degradation rates result from differences in acetamide group distribution rather than deacetylation conditions alone (Sashiwa *et al*., 1991; Aiba, 1992; Shigemasa *et al*., 1994). These differences influence chitosan solution viscosity by modifying intermolecular and intramolecular repulsion forces (Sashiwa *et al*., 1991).

Biocompatibility

One of the most valuable biological properties of chitosan is its biocompatibility, meaning that it does not trigger adverse local or systemic effects when in contact with living tissues. It is well tolerated by various biological structures, including the skin, ocular membranes and nasal epithelium (Shigemasa *et al.,* 1999). However, biocompatibility is not a fixed characteristic and is influenced by factors such as the natural source of chitosan, its

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method of preparation, molecular weight (Mw) and degree of deacetylation (DD) (Minami *et al.*, 1998).

Low toxicity

Compared to other natural polysaccharides, chitosan is known for its minimal toxicity. Animal studies have demonstrated that chitosan has an LD50 of approximately 16 g/kg, comparable to common substances such as glucose and table salt (Singla and Chawla, 2001). However, individuals with shellfish allergies should avoid chitosan products due to potential allergic reactions. Toxicity levels have been linked to the degree of deacetylation; chitosans with a DD above 35% have shown low toxicity, whereas those with a DD below 35% exhibited dose-dependent toxicity (Aiba, 1992).

Applications of chitosan in various scientific fields

Due to its exceptional physical and chemical properties, chitosan is utilized in a diverse range of industries, from pharmaceuticals and cosmetics to environmental applications such as water treatment and agriculture. The required properties of chitosan differ based on its intended application, with variations in molecular weight and degree of acetylation playing a crucial role in determining its functionality (Pradip Kumar, 2004).

Application of chitin and chitosan nanoparticles in agriculture

The rising global demand for food necessitates the adoption of sustainable and environmentally friendly agricultural practices. Chitin and chitosan nanoparticles have emerged as promising solutions in this regard, offering a range of beneficial properties such as biocompatibility, biodegradability and antimicrobial activity. These characteristics make them highly suitable for improving crop yields and safeguarding plants from pests and diseases. Chitosan nanoparticles, in particular, have shown considerable potential as biopesticides by exhibiting antimicrobial activity against various fungal and bacterial pathogens, thereby restricting the spread of plant diseases (Kumar *et al*., 2019). Additionally, research indicates that chitosan nanoparticles can stimulate the production of plant defense-related enzymes, thereby bolstering plant resistance against pests and diseases (El Hadrami *et al*., 2018). This dual mechanism of action provides an effective and environmentally responsible alternative to synthetic pesticides, minimizing environmental contamination and promoting sustainable farming (Fig. 2).

Moreover, chitin and chitosan nanoparticles serve as effective carriers for delivering nutrients and bioactive compounds to plants. Their porous nature allows for the encapsulation and controlled release of fertilizers and growth stimulants, improving nutrient uptake and reducing wastage (Mahmoud *et al*., 2021). The gradual release mechanism ensures efficient absorption by plants, ultimately enhancing agricultural productivity.

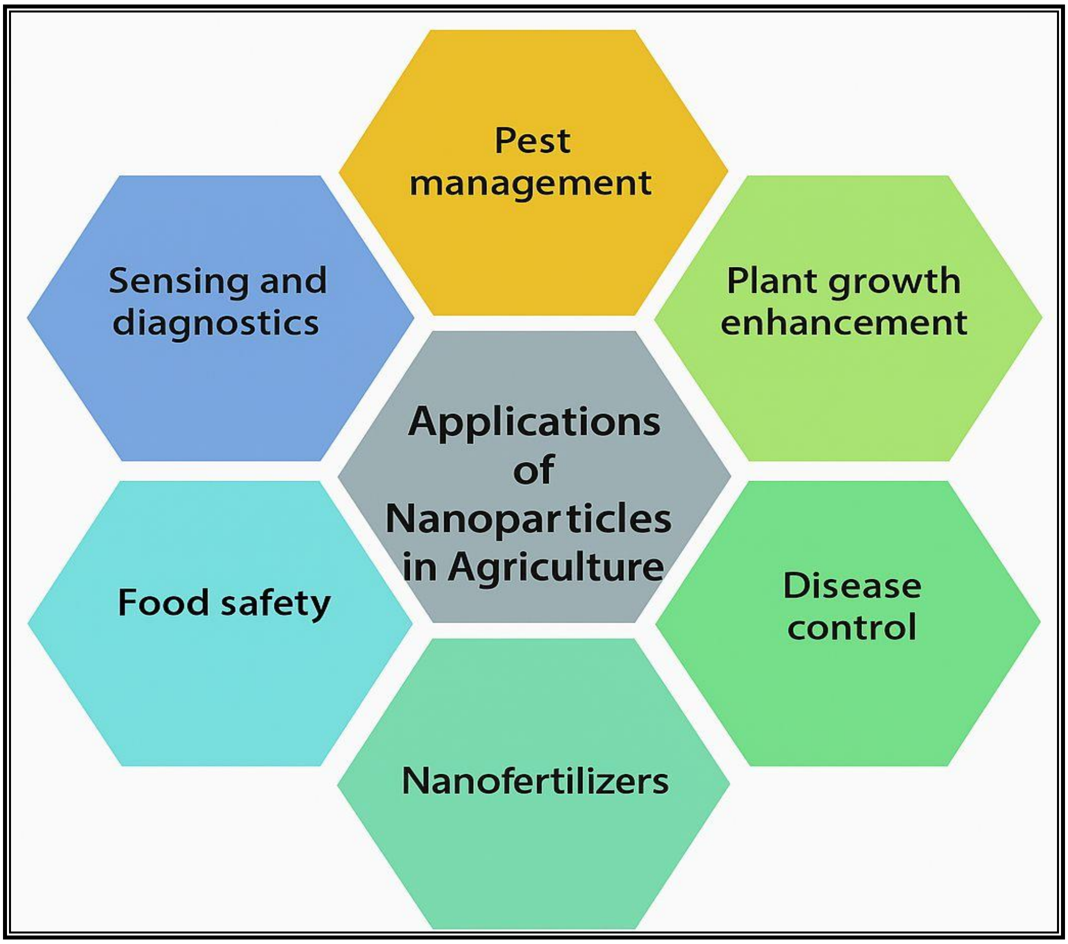

Figure 2: Various steps of chitosan nanoparticles applications in agriculture.

Beyond plant nutrition, these nanoparticles contribute to soil enhancement and fertility. Studies indicate that chitosan nanoparticles support the proliferation of beneficial soil microorganisms, including mycorrhizal fungi and nitrogen-fixing bacteria, which play a crucial role in nutrient cycling and soil health (Ma *et al*., 2017). Furthermore, chitin and chitosan nanoparticles facilitate soil aggregation, thereby improving soil structure and water retention (Li *et al*., 2018). These improvements create optimal conditions for plant growth while mitigating soil degradation. A significant advantage of incorporating chitin and chitosan nanoparticles into agriculture is their minimal environmental footprint compared to conventional agrochemicals. As they are biodegradable and non-toxic, they do not accumulate in the soil or water systems, reducing potential risks to human health and ecosystems (Saharan *et al*., 2019). Their eco-friendly nature aligns with organic farming principles and environmentally sustainable agricultural practices.

Additionally, chitin and chitosan nanoparticles are highly effective in controlling plant diseases. They exhibit activity against soil-borne fungi, viruses, bacteria and other agricultural pests. Their application in the rhizosphere and phyllosphere alters the microbial environment, favoring beneficial

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organisms while suppressing plant pathogens. Moreover, chitin and chitosan fragments have been shown to elicit various plant defense mechanisms, including phytoalexin accumulation, pathogen-related (PR) protein production, proteinase inhibitor synthesis, lignification and callose deposition (EI Hadrami *et al.*, 2010).

Application of chitin and chitosan nanoparticles in aquaculture

The aquaculture industry is integral to global seafood production; however, its sustainability is challenged by factors such as disease outbreaks, environmental pollution and reliance on fishmeal-based feeds. Chitin and chitosan nanoparticles offer an innovative and environmentally friendly approach to addressing these challenges. Disease management is a critical concern in aquaculture, as outbreaks can lead to severe economic losses. Chitosan nanoparticles have demonstrated antimicrobial activity against a range of aquatic pathogens, including bacteria, viruses and fungi, making them valuable tools for disease prevention (Chung *et al*., 2020). These nanoparticles can suppress pathogen growth, reduce virulence and enhance the innate immune response of aquatic organisms (Sukhveer *et al*., 2021). Furthermore, chitosan nanoparticles can serve as delivery systems for vaccines, enhancing immune protection and resistance to diseases in aquaculture species (Raghu *et al*., 2019).

Ensuring optimal water quality is essential for aquaculture productivity and the health of aquatic organisms. Chitin and chitosan nanoparticles have been explored for their potential to improve water quality by effectively removing heavy metals, organic pollutants and excessive nutrients (Khan *et al*., 2018; Hritcu *et al.*, 2012) (Fig. 3). Due to their high adsorption capacity, these nanoparticles can bind to pollutants, preventing their accumulation and mitigating adverse effects on aquatic life. The use of fishmeal in aquaculture feed contributes to overfishing and environmental concerns. Chitosan nanoparticles present an alternative by enhancing feed efficiency and nutrient absorption in aquatic species (Abd El-Ghany *et al*., 2021). By encapsulating essential nutrients within chitosan nanoparticles, their bioavailability and uptake by aquatic organisms are improved, reducing overall feed requirements and supporting sustainable aquaculture practices. Another advantage of chitin and chitosan nanoparticles in aquaculture is their minimal environmental impact. Being biodegradable and non-toxic, they do not pose significant risks to aquatic ecosystems or human health (Mishra *et al*., 2020). Their use aligns with sustainable and responsible aquaculture management practices. Despite their potential, the application of chitin and chitosan nanoparticles in aquaculture presents challenges. Factors such as nanoparticle stability, optimal dosage determination and potential unintended effects on non-target organisms require further investigation.

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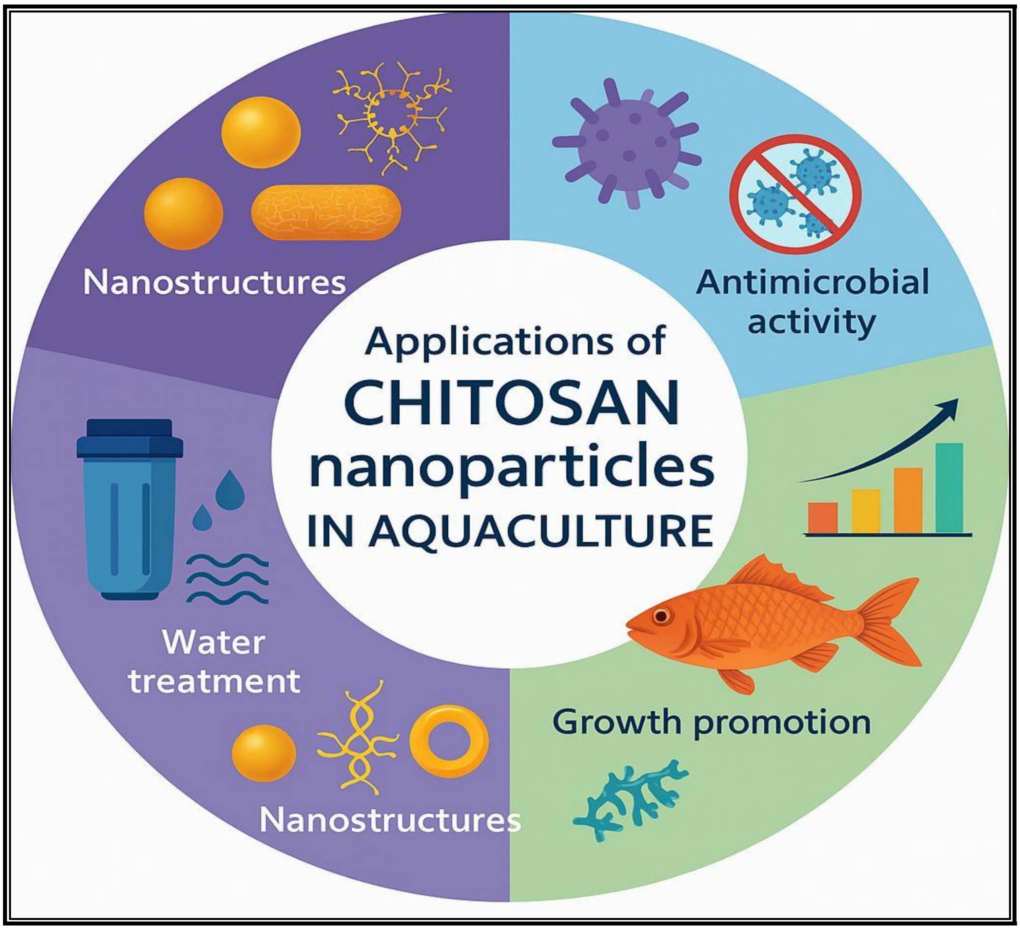

Figure 3: Various branches of applications of chitosan nanoparticles in aquaculture.

Additionally, advancements in cost-effective and scalable production methods are necessary to facilitate their widespread adoption in the aquaculture industry. Continued research and development efforts will be crucial in unlocking the full potential of chitin and chitosan nanoparticles, ensuring their safe and effective integration into aquaculture systems.

Overall, the utilization of chitin and chitosan nanoparticles in aquaculture provides a sustainable and innovative approach to addressing industry challenges. From disease control and water quality improvement to feed supplementation, these nanoparticles offer versatile benefits. However, further research and refinement are essential to maximize their efficacy and sustainability in aquaculture practices.

Application of chitin and chitosan nanoparticles in food processing and storage

The food industry constantly faces challenges in maintaining food safety, ensuring quality and promoting environmental sustainability. As natural and biodegradable materials, chitin and chitosan nanoparticles offer an eco-friendly solution to enhance food processing and packaging. One of the primary concerns in food production is the presence of foodborne pathogens and spoilage microorganisms. Chitosan

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nanoparticles exhibit significant antimicrobial properties, effectively inhibiting the growth of a broad spectrum of bacteria and fungi, including well-known foodborne pathogens (Ribeiro *et al*., 2019). Their integration into food products can help curb microbial proliferation, thus prolonging the shelf life of perishable goods and enhancing food safety. Additionally, chitin and chitosan nanoparticles possess notable antioxidant properties due to their amino and hydroxyl functional groups. These compounds actively scavenge free radicals and mitigate lipid oxidation in food products, thereby preventing oxidative deterioration (Shahidi and Chao, 2018). By minimizing oxidation, chitosan nanoparticles help preserve food quality, ensuring better taste, texture and nutritional value over time.

Beyond direct food applications, these nanoparticles can be incorporated into food packaging materials to improve barrier properties. Studies suggest that chitosan nanoparticles significantly enhance the water vapor and oxygen barrier capacity of packaging films (Kumar *et al*., 2020). This improvement aids in maintaining the freshness of food products, reducing spoilage and ultimately minimizing food waste. Moreover, the application of chitin and chitosan nanoparticles in food storage helps slow down microbial growth and enzymatic degradation, thus extending the shelf life of perishable items (Xu *et al*., 2021). This function contributes to food security by reducing waste and increasing the availability of fresh products. Despite their promising applications, it is essential to address regulatory and safety concerns regarding the use of chitin and chitosan nanoparticles in food (Fig. 4). One key consideration is the potential migration of nanoparticles from packaging materials into food and their subsequent impact on human health. Standardized testing and thorough risk assessments are necessary to ensure their safe incorporation into food processing and packaging systems (Ghormade *et al*., 2019). Proper evaluation will facilitate the responsible utilization of these nanoparticles while ensuring consumer safety.

Role of edible films and coatings in food preservation

Edible films and coatings play a significant role in enhancing food quality and prolonging shelf life. Among these, cellulose- and protein-based films are extensively utilized due to their ability to regulate O_2 and CO_2 partial pressures. However, they are not as effective in controlling moisture exchange between food products and their surrounding environment. In contrast, chitosan forms strong, flexible and semi-permeable films, making it a suitable material for food wraps that help extend the freshness of perishable goods. During postharvest storage, fruits and vegetables undergo various physiological transformations, including tissue softening, increased sugar concentration, chlorophyll breakdown and fluctuations in volatile flavor compounds.

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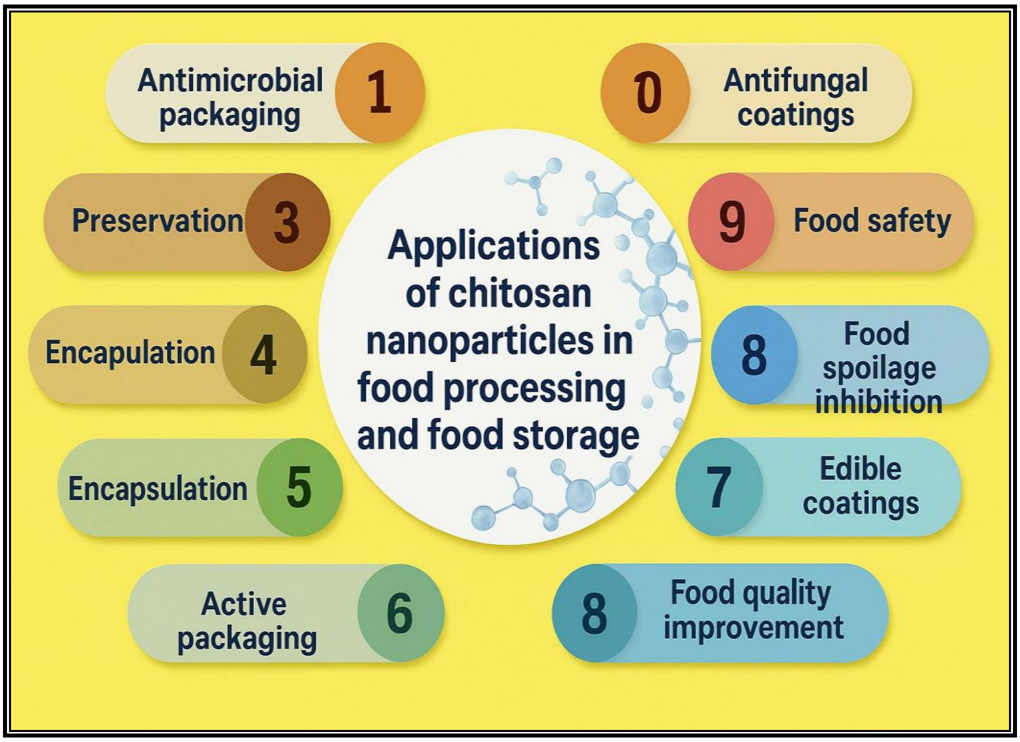

Figure 4: Various branches of applications of chitosan nanoparticles in food processing, food storage and packaging.

Controlling the respiration rate is a key factor in preserving the freshness and longevity of produce, as it delays senescence and prevents spoilage. The appearance, texture, color, flavor, nutritional value and microbial stability of minimally processed food products are essential factors influencing their marketability. Effective management of these attributes is crucial for ensuring consumer satisfaction and maintaining the product's commercial appeal.

Chitosan-based films offer excellent barrier properties that help in reducing moisture loss, preventing drip formation, retaining natural color and flavor and enhancing microbial stability, thereby extending the shelf life of various fresh produce. Instead of solely using chitosan as a packaging material, an alternative approach involves immersing fresh fruits and vegetables in a dilute chitosan solution containing acetic acid. This technique allows for the incorporation of additional protective agents, such as vitamin E, rosemary extract, oleoresin, calcium and potassium, further strengthening the preservation effects of the treatment (Aider, 2010). Studies have highlighted the effectiveness of this method in maintaining the quality of strawberries, bell peppers, cucumbers, peaches, pears and kiwifruit (Bautista-Banosa *et al*., 2006).

Chitin and chitosan nanoparticles: Advancements in wastewater treatment and environmental pollution control

Chitin and its derivative, chitosan, have garnered significant interest for their potential in wastewater treatment and environmental remediation. Their biocompatibility, non-toxicity and remarkable adsorption capacities make

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them suitable candidates for addressing pollutants in aquatic ecosystems. This review explores the role of chitin and chitosan nanoparticles (CNPs) in mitigating environmental contamination, particularly focusing on their efficiency in heavy metal adsorption, dye removal and microbial decontamination. Additionally, it discusses recent advancements in CNP synthesis and their modifications for enhanced pollutant removal. Water pollution remains a pressing global concern, necessitating innovative and sustainable treatment approaches. Conventional wastewater treatment methods, such as chemical precipitation, ion exchange and activated carbon adsorption, have certain limitations, including high costs and secondary waste production (Kyzas and Bikiaris, 2019; Gan *et al*., 2020). In contrast, biopolymer-based materials like chitin and chitosan offer eco-friendly and effective alternatives (Fig. 5). Chitin, a naturally occurring polysaccharide found in the exoskeleton of crustaceans and insects, can be transformed into chitosan through deacetylation (Kumar *et al*., 2020; Vinusha *et al*., 2023). The nano-scale derivatives of these biopolymers have demonstrated enhanced physicochemical properties, making them highly efficient in pollutant adsorption and removal.

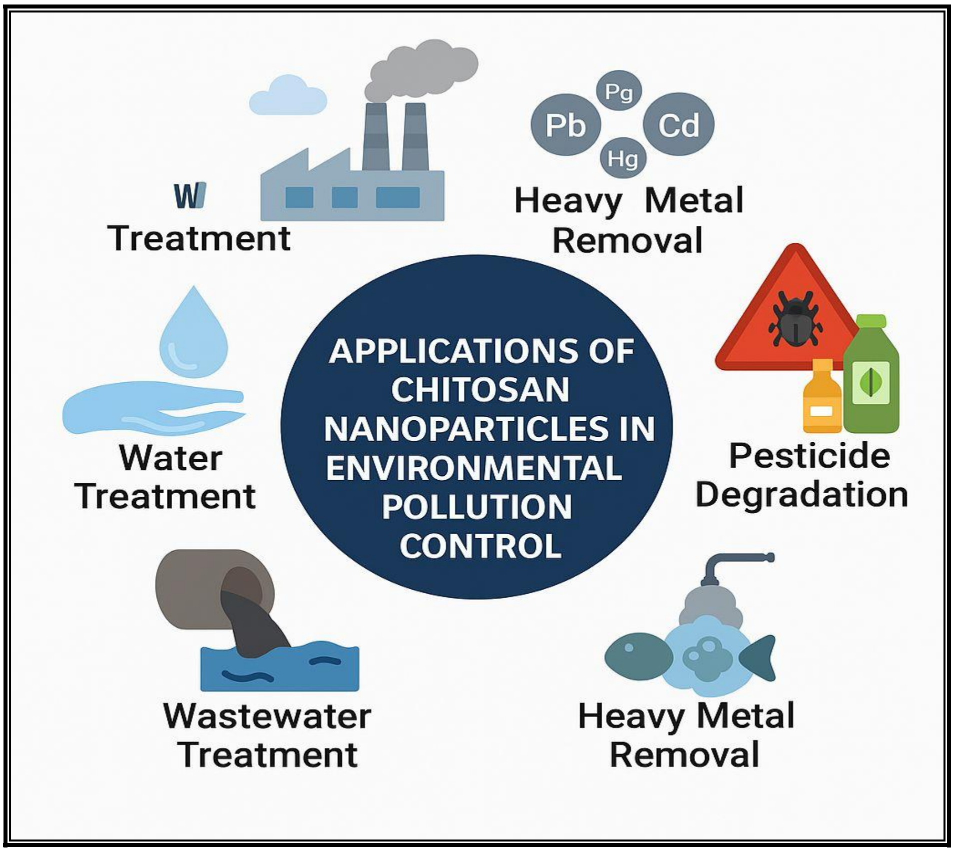

Figure 5: Various applications of chitosan nanoparticles in environmental pollution control.

Chitin and chitosan nanoparticles exhibit multiple mechanisms for pollutant remediation, including chelation, electrostatic interactions and hydrogen

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bonding. These mechanisms play a crucial role in the adsorption of heavy metals such as lead (Pb), cadmium (Cd) and arsenic (As) from contaminated water sources (Ali and Alharbi, 2021; Wang and Chan, 2014). Furthermore, the presence of functional groups like hydroxyl (-OH) and amine (-NH2) enhances their affinity toward metal ions, facilitating their sequestration from aqueous solutions (Nguyen *et al*., 2021). In addition to heavy metal removal, chitosan nanoparticles are highly effective in eliminating dyes from industrial effluents. The adsorption of anionic and cationic dyes onto CNP surfaces occurs through van der Waals forces and π-π interactions, resulting in significant color removal from wastewater (Crini and Badot, 2008; Zainal *et al.,* 2009; Rhazi *et al.,* 2002). Moreover, CNPs have been explored for their antimicrobial properties, which aid in the disinfection of water bodies contaminated with pathogenic bacteria and fungi (Wang *et al*., 2002; Shahram *et al.,* 2014).

Recent studies have emphasized the functionalization of chitin and chitosan nanoparticles to improve their adsorption capacity and selectivity. Surface modifications using metal oxides, graphene-based materials and polymeric composites have been reported to enhance pollutant removal efficiency (Abdelhakin *et al.,* 2014; Bina *et al.,* 2009; Barba *et al.,* 2001). Additionally, green synthesis approaches utilizing plant extracts and microbial sources have been investigated to reduce the environmental impact of nanoparticle production (Sundara Rajan and Howard, 1999). Despite their promising applications, certain challenges hinder the large-scale implementation of chitin and chitosan nanoparticles in wastewater treatment. Issues related to nanoparticle stability, regeneration and cost-effective synthesis need to be addressed (Suresh *et al.*, 2016; Wibowo *et al.,* 2005). Future research should focus on developing scalable production techniques and exploring the recyclability of CNPs to ensure sustainable wastewater management. Additionally, interdisciplinary collaboration between environmental scientists, chemists and engineers is crucial for advancing the practical applications of these biopolymeric nanomaterials. Chitin and chitosan nanoparticles have emerged as sustainable and highly efficient materials for wastewater treatment and environmental pollution control (Bhatnagar, 2009; Thakre *et al.,* 2010). Their unique physicochemical properties and functional versatility make them promising candidates for addressing various contaminants in water systems (Fig. 6). Further advancements in synthesis techniques and modification strategies will enhance their applicability, paving the way for their widespread use in environmental remediation.

Antimicrobial activity of chitosan nanoparticles: Applications in microbiology

Chitosan exhibits extensive antimicrobial properties against a wide range of bacteria, molds and yeasts. Its efficacy extends to both Gram-positive and Gram-

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negative foodborne microorganisms, including *Aeromonas hydrophila*, *Bacillus cereus*, *B. licheniformis*, *B. subtilis*, *Clostridium perfringens*, *Brochothrix* spp., *Enterobacter sakazakii*, *Lactobacillus* spp., *Listeria monocytogenes*, *Pseudomonas* spp., *Salmonella typhimurium*, *S. enteritidis*, *Serratia liquefaciens*, *Staphylococcus aureus* and *Escherichia coli* O157H7. Additionally, it demonstrates antifungal activity against various yeast species such as *Candida*, *Saccharomyces* and *Rhodotorula*, as well as molds belonging to the genera *Aspergillus, Penicillium* and *Rhizopus*. Chitosan and its derivatives have also been reported to inhibit plant pathogenic bacteria, including *Agrobacterium tumefaciens, Clavibacter fascians*, *Erwinia amylovora*, *E. carotovora*, *Pseudomonas solanacearum* and *Serratia lutea*. Likewise, it is effective against plant pathogenic fungi such as *Alternaria alternata*, *Botrytis fabae, Fusarium oxysporum, Penicillium digitatum*, *P. debaryanum* and *Rhizoctonia solani* (Vishnukumar *et al*., 2005). Numerous studies have confirmed the antimicrobial effects of chitosan nanoparticles against both Gram-negative and Gram-positive bacteria, as well as fungi and yeasts (Kong *et al*., 2008ab; Sadeghi *et al*., 2008). The antimicrobial mechanism is likely due to chitosan's ability to disrupt microbial metabolism by interacting with the cell surface or interfering with transcriptional processes by binding to DNA and RNA after cellular penetration.

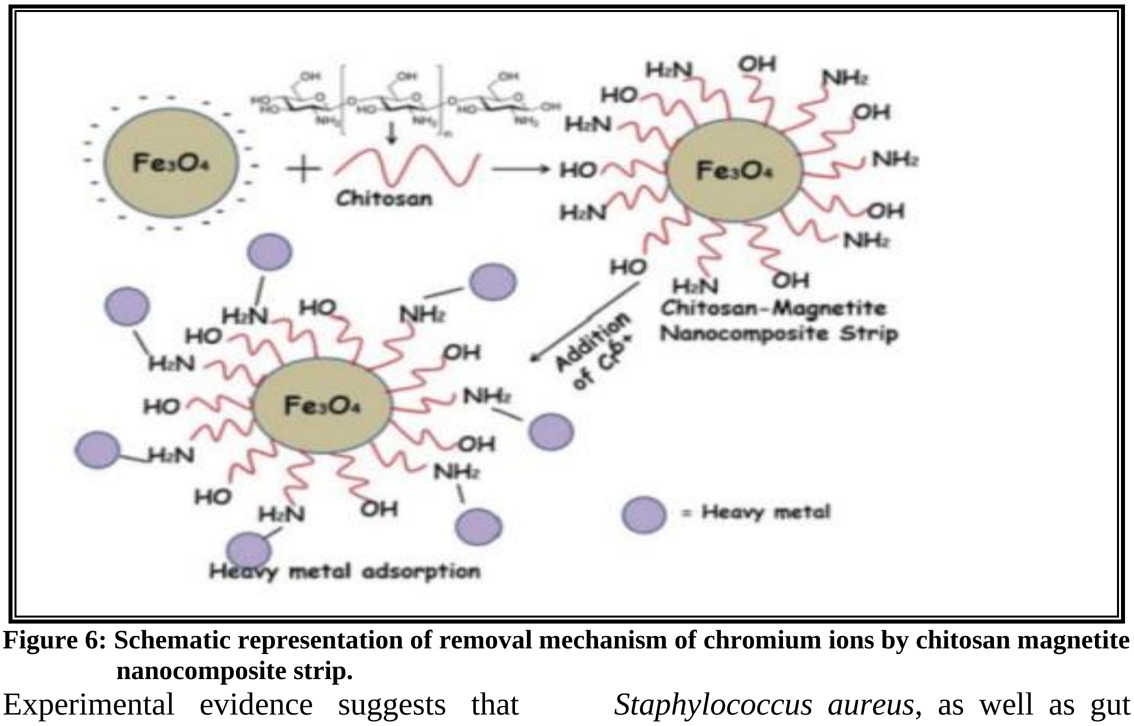

Figure 6: Schematic representation of removal mechanism of chromium ions by chitosan magnetite nanocomposite strip.

Experimental evidence suggests that chitosan nanoparticles significantly inhibit the growth of pathogenic bacteria such as *Enterococcus faecalis, Enterobacter aerogenes* and *Staphylococcus aureus*, as well as gut pathogens like *E. coli*. Moreover, research conducted by Benhabiles *et al*., (2012) highlighted the strong antimicrobial effects of chitosan nanoparticles against *S. aureus, Salmonella typhimurium, Bacillus subtilis, B. cereus* and *Vibrio cholerae*. Kaya *et al*. (2015) reported that chitosan generally exhibits a stronger inhibitory effect on Gram-positive bacteria, including *Listeria monocytogenes, Bacillus megaterium, B. cereus, Staphylococcus aureus, Lactobacillus plantarum, L. brevis* and *L. bulgaris*, compared to Gram-negative bacteria such as *E. coli, Pseudomonas fluorescens, Salmonella typhimurium* and *Vibrio parahaemolyticus*. Furthermore, chitosan nanoparticles synthesized through ionic gelation have demonstrated significant antibacterial activity against Gram-positive microorganisms. Notably, *E. coli* displayed the highest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), recorded at 0.25 µg/mL and 1 µg/mL, respectively (Qi *et al*., 2004). This growing body of research underscores the potential of chitosan nanoparticles as an effective antimicrobial agent in various microbiological applications.

Application in the cosmetics industry

Chitosan is unique among natural hydrocolloids due to its cationic nature, which enables it to become viscous when neutralized with acid. This property enhances its interaction with biological surfaces such as skin and hair (Venugopal, 2011). Chitin, chitosan and their derivatives have significant applications in cosmetics, particularly in hair care, skin care and oral care. Chitosan's positive charge allows it to interact effectively with negatively charged hair, forming a transparent and elastic film that enhances hair texture by improving softness, smoothness and mechanical strength. It can also create a gel when combined with alcohol and water, making it useful in hair care products such as shampoos, rinses, hair dyes, styling lotions, hair sprays and tonics. Several chitosan derivatives, including glyceryl chitosan, hydrolyzed chitosan oligomers, N-hydroxypropyl chitosan, quaternary hydroxypropyl-substituted chitosan, polyoxyalkylene chitosan, chitosan oligosaccharides, chitin sulfate and carboxymethyl chitin, have shown potential in hair care formulations. In skincare, chitosan derivatives are valuable due to their cationic charge and high molecular weight, which prevents penetration into the skin, allowing them to form a protective film on the surface. These properties make chitosan an excellent candidate for various dermatological applications (Pradip *et al.,* 2004).

Application in water resource and engineering

Chitosan's polycationic nature allows it to function as a flocculant, chelating agent and heavy metal absorber. It has been used in wastewater treatment to remove metal ions in acidic conditions through sorbents like chitosan N-benzyl sulfonate derivatives (Weltrowski *et al.*, 1996). Studies have shown its effectiveness in removing color from dye house effluents when used as an adsorbent (Bhavani and Dutta, 1999; Sridhari and Dutta, 2000). Chitosan promotes the aggregation of anionic

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waste particles in solution, forming precipitates and flocs, making it useful for recycling food processing waste (Sridhari and Dutta, 2001). Compared to synthetic resins, chitosan is highly effective in capturing heavy metals from industrial wastewater. Chitin has also been used for decontaminating wastewater containing plutonium and methyl-mercury acetate, a toxic byproduct from acetaldehyde production (Jeon and Holl, 2003). Additionally, chitosan-chitin mixtures have demonstrated efficiency in arsenic removal from drinking water. Another significant application includes the removal of petroleum contaminants from wastewater. Regenerated forms of chitin and chitosan, as well as their membranes, are widely used in processes such as osmosis, reverse osmosis, microfiltration, desalination, dialysis and hemodialysis.

Application in the paper and textile industry

Chitosan plays a key role in paper manufacturing due to its structural similarity to cellulose, the primary component of plant cell walls. Incorporating chitosan in paper production reduces the need for chemical additives, increases output and enhances the final product's surface smoothness and moisture resistance. This makes it particularly valuable in manufacturing toilet paper, wrapping paper and cardboard (Pradip Kumar *et al*., 2004). Additionally, water-soluble derivatives such as hydroxymethyl chitin are beneficial in papermaking. Chitosan also serves as a biodegradable alternative in food packaging materials. Moreover, its adsorption properties facilitate dye removal from textile effluents, making it an environmentally friendly solution for wastewater treatment. Beyond its role in paper and textile industries, chitosan and chitin have applications in medical textiles, contributing to the production of sutures, threads and fibers for healthcare purposes (Fig. 7).

Application in photography

Chitosan has been utilized as a fixing agent for acid dyes in gelatin, aiding in improving diffusion, a crucial step in developing photographic images. This enhances the quality and efficiency of photographic processes (Dutta and Ravishankar, 2002).

Application in chromatographic separations

Due to the presence of free amino (-NH_2), primary hydroxyl (-OH) and secondary hydroxyl (-OH) functional groups, chitosan and chitin have extensive applications in chromatographic techniques. They have been used as stationary phases in thin-layer chromatography for nucleic acid separation (Ottoy *et al*., 1996). Additionally, chitosan has been employed as a sorbent material in solid-phase extraction methods for detecting phenol and chlorophenols through High-Performance Liquid Chromatography (HPLC) (Rhee *et al*., 1998).

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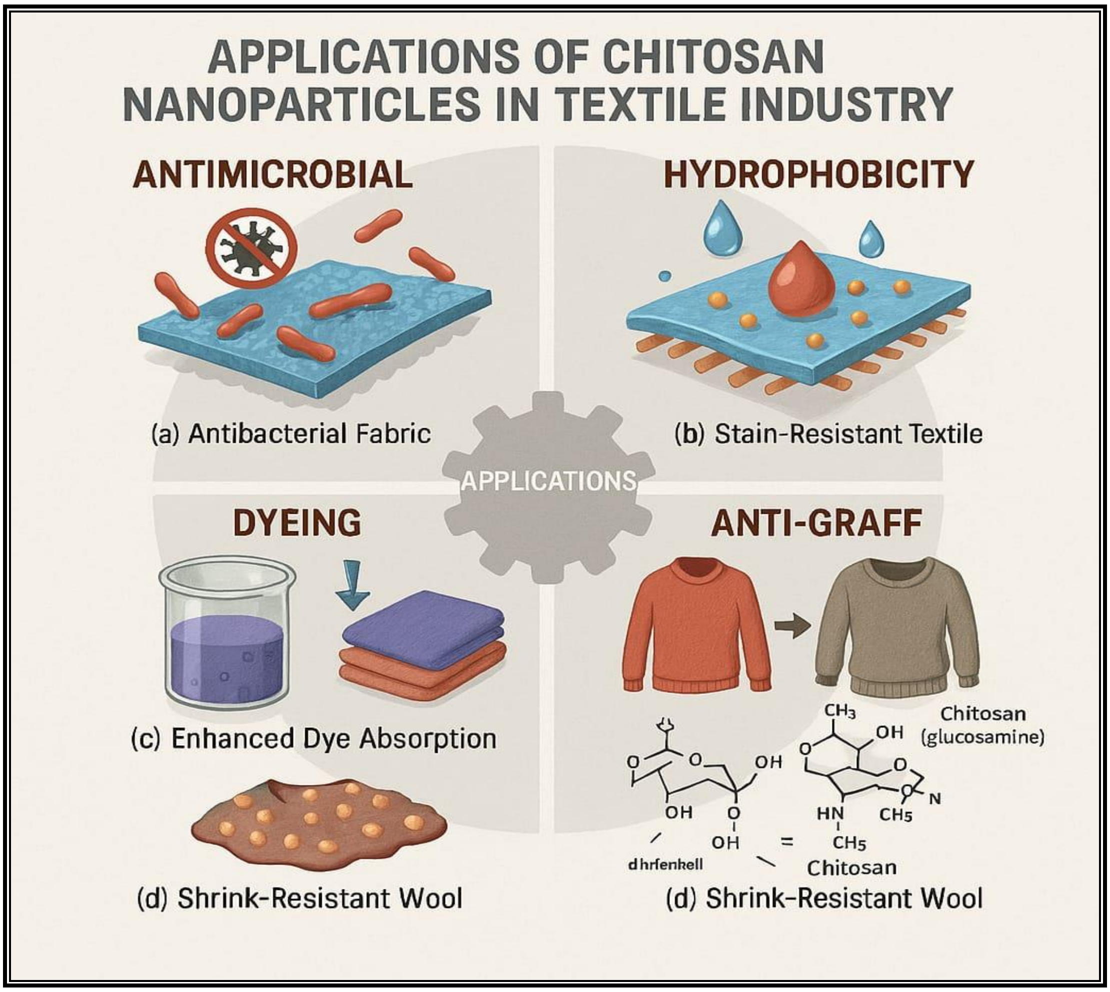

Figure 7: Application of Chitin/Chitosan Nanoparticles in various steps of textile industry.

Application in solid-state batteries manufacturing

Chitosan's inherent water insolubility prevents it from being used independently in the fabrication of solid-state proton-conducting polymer batteries. To overcome this limitation, it is dissolved in acetic acid, which enhances its ionic conductivity by facilitating proton transport within the polymer matrix. The presence of numerous microvoids in the polymer enables this proton movement, as confirmed by piezoelectric studies that associate small dielectric constants with the structural voids. Selecting an optimal electrode material could further enhance battery performance (Ravi Kumar and Dutta, 1998).

Chitosan gel for LED and nonlinear optical (NLO) applications

Recent studies have explored chitosan-based gels doped with specific dyes for potential use in light-emitting devices (LEDs) and lasers. This doping process utilizes porphyrin compounds, structurally resembling heme groups in blood, to improve the optical properties of chitosan-based materials. Researchers have also investigated fluorinated coumarin and rhodamine B for transparent thin films, as well as nickel

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porphyrins to uncover novel film properties (Pradip Kumar, 2004).

Biomedical applications of chitosan

Chitosan exhibits remarkable biomedical potential due to its biodegradability, biocompatibility, polyelectrolyte nature, antimicrobial properties and ability to form gels in mildly acidic environments. These characteristics make it suitable for diverse medical applications, including hemodialysis membranes, artificial skin, hemostatic agents and drug delivery systems. Its ability to form gels under slightly acidic conditions contributes to its antacid and antiulcer properties (Fig. 8).

Figure 8: Various applications of chitosan nanoparticles in biomedical and pharmaceutical industry.

Additionally, intravenous administration of chitin and chitosan oligosaccharides has demonstrated enhanced antitumor activity through macrophage activation (Dutta and Ravi shankar, 2002). Chitosan membranes, owing to their permeability and high tensile strength, have been proposed as artificial kidney membranes. The semipermeable membranes currently used in artificial kidneys are typically made from regenerated cellulose or cuprophane, but research suggests that chitosan-derived membranes could enhance dialysis properties. Due to its

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polycationic nature, chitosan also facilitates cellular attachment, a crucial factor in tissue engineering. Studies indicate that cell adhesion efficiency depends on the degree of chitosan deacetylation. Prasitsilp *et al*. (2000) analyzed the effect of deacetylation levels on cellular responses to chitosan sourced from shrimp and cuttlefish, demonstrating its impact on tissue interaction.

Tissue engineering research has increasingly focused on chitosan's role as a scaffolding material. Jarry *et al*. (2001) successfully developed porous scaffolds, films and beads from chitosan, while Kast *et al*. (2000) introduced chitosan-thioglycolic acid (chitosan-TGA) as a promising scaffold candidate. Gan *et al.,* (2020); and Thakre *et al.,* (2010) proposed methods to fabricate porous chitosan scaffolds for various engineered tissues. Microporous chitosan/calcium phosphate composite scaffolds have also been synthesized and studied for tissue engineering applications. Zhang and Zhang (2001) demonstrated that chitosan provides structural support, while calcium phosphate enhances bioactivity, fostering osteoblast attachment and scaffold strength. The attachment efficiency depends on the chitosan-to-calcium phosphate ratio, particularly in formulations incorporating β-tricalcium phosphate and calcium phosphate inverted glass. Research has also examined chitosan's potential in repairing articular cartilage. Studies on chitosan-glycosaminoglycan (GAG) composites indicate promising interactions with chondrocytes (Francis *et al.,* 2000). Additionally, chitosan and its derivatives have been explored for applications in bone tissue engineering and treatments for central nervous system damage.

Chitosan for wound healing and burn treatment

Chitosan-based materials such as regenerated fibers, non-woven mats, sponges and films have been shown to accelerate wound healing by over 30%. Chitin-coated silk and catgut sutures exhibit nearly equivalent healing efficiency compared to pure chitin fibers. Given its bacteriostatic and fungistatic properties, chitosan is a key component in wound-healing skin ointments. When implanted in animal tissues, chitosan enhances wound healing and hemostatic activity. Biocompatible chitin-based wound dressings are available in the form of hydrogels, xerogels, powders, composites and films (Gavhane *et al*., 2013). In burn treatment, chitosan's ability to form strong, water-absorbent and biocompatible films is particularly beneficial. These films can be directly applied to burn sites using aqueous chitosan acetate solutions. They facilitate oxygen permeability, preventing tissue hypoxia while ensuring that the dressing does not require removal, thus minimizing additional damage to the burn site. Chitosan's natural biodegradability allows it to degrade without manual removal, unlike conventional wound dressings (Pradip *et al.,* 2004).

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Chitosan in artificial skin and regenerative medicine

In designing artificial skin, a key focus is creating a non-antigenic, biodegradable membrane that acts as a template for new dermal tissue formation. Given its structural similarity to glycosaminoglycans, chitosan polysaccharides are being considered for artificial skin applications (Dutta *et al.*, 2003). Studies suggest that chitosan-based materials can support plastic skin surgery and brain skull damage repair (Calvo *et al*., 1997; Mucha, 1997).

Ophthalmological applications of chitosan

Chitosan has emerged as a viable alternative to synthetic polymers in ophthalmology, particularly in contact lens manufacturing. It possesses the essential properties of an ideal contact lens material, including optical clarity, mechanical stability, gas permeability (especially oxygen), wettability and immunological compatibility. Contact lenses manufactured from depolymerized and purified squid pen chitosan via spin-casting technology exhibit excellent transparency, durability and mechanical strength. The antimicrobial and wound-healing properties of chitosan make it a strong candidate for ocular bandage lenses (Jing *et al*., 1996). Chitosan's extensive biomedical applications demonstrate its potential to replace synthetic polymers in multiple fields, from wound healing and tissue engineering to ophthalmology and artificial skin development.

Chitosan in drug delivery systems

Controlled-release drug technology has been widely recognized since the 1980s as an effective and commercially viable method for drug administration. This technology facilitates a predictable and sustained release of therapeutic agents, ensuring an optimal response with minimal side effects and prolonged efficacy. Since it involves an interdisciplinary scientific approach, it remains an evolving field. Among the many materials explored for controlled drug delivery, chitin and chitosan have gained considerable attention due to their biocompatibility, cost-effectiveness and non-toxic nature, making them suitable candidates for pharmaceutical applications (Andrady and Xu, 1997).

Chitosan nanoparticles have emerged as promising carriers for delivering a wide range of drugs, including gene-based therapeutics, protein-based drugs, antibiotics and anticancer agents. These nanoparticles offer advantages such as low toxicity, enhanced stability, ease of preparation and multiple administration routes, including oral, nasal, intravenous and ocular pathways. Coating drugs with chitosan nanoparticles has been shown to improve drug payload capacity, enhance bio-adhesion and extend drug release duration compared to uncoated drugs (Agnihorti *et al*., 2004). For instance, Gnanadhas *et al*. (2013) successfully utilized chitosan–dextran sulfate nanocapsules loaded with ciprofloxacin for targeted delivery, enabling efficient intracellular pathogen eradication at reduced dosages compared to free antibiotics. Chitosan nanoparticles also exhibit antitumor activity due to their ability to interact with negatively charged tumor cell surfaces, facilitating selective drug absorption at tumor sites and enhancing therapeutic efficacy. Studies have demonstrated that doxorubicin/methoxy PEG grafted carboxymethyl chitosan nanoparticles significantly improve cellular uptake and inhibit tumor cell proliferation (Katas and Alpar 2006).

Furthermore, chitosan nanoparticles are increasingly being explored for gene delivery applications due to their excellent biocompatibility and biodegradability. Recent research indicates that folic acid-modified chitosan nanoparticles exhibit low cytotoxicity while effectively condensing DNA, forming an optimal size and charge balance for gene delivery. The results suggest that these nanoparticles serve as a viable non-viral gene carrier with strong potential for therapeutic applications (Liu, 2010). Beyond drug delivery, chitosan nanoparticles provide effective protein protection and facilitate enhanced drug-biomembrane interactions, thereby improving bioavailability. Additionally, these nanoparticles have been utilized to deliver a range of therapeutic agents, including antiviral, antiallergic and hormonal drugs. Their potential extends to vaccine adjuvants, with applications in influenza, hepatitis B and piglet paratyphoid vaccines. Moreover, chitosan nanoparticles are used in food packaging for preservative purposes and in dentistry to prevent caries. They have also been incorporated into antimicrobial textiles for protective clothing in healthcare settings. Furthermore, research indicates their potential for skin regeneration, as they have shown positive effects on fibroblasts and keratinocytes in laboratory settings, paving the way for their inclusion in anti-aging skincare products.

Chitosan as a lipid-lowering agent

Chitosan has gained attention as a potential dietary supplement due to its ability to reduce serum cholesterol levels. Its lipid-lowering effect is attributed to its capacity to bind with neutral lipids, including cholesterol and sterols, through hydrophobic interactions. This property enables chitosan to act as a fat scavenger within the digestive system, facilitating the excretion of fats and cholesterol (Luo and Wang, 2013). As a dietary fiber, chitosan possesses key characteristics such as non-digestibility in the upper gastrointestinal tract, high viscosity and strong water-binding ability in the lower digestive system. One of its primary physiological functions is to support cholesterol reduction and weight management by limiting intestinal lipid absorption. Unlike traditional dietary fibers, chitosan carries a positive ionic charge, allowing it to interact with negatively charged fats, lipids and bile acids. However, prolonged consumption should be carefully monitored, as excessive use may disrupt gut microbiota balance or interfere with the absorption of fat-soluble vitamins and essential minerals. Despite these considerations, chitosan is widely regarded as safe for consumption. Its median lethal dose

(LD_{50}) is estimated to be around 16 g/kg, which is comparable to commonly consumed substances such as salt and glucose, indicating its potential suitability for long-term dietary use (Singla and Chawla, 2001).

Chitosan as a clarifying agent in wines and vinegars

Oxidative browning is a major quality concern in white wines, primarily caused by the presence of phenolic compounds. The use of adsorbents to reduce these compounds is a well-established method for improving wine stability. Chitosan has demonstrated significant potential as a clarifying agent in both wines and vinegars due to its strong affinity for various phenolic compounds, particularly cinnamic acid. This property enables chitosan to effectively prevent browning, preserving the visual and sensory quality of white wines (Spagna *et al*., 1996).

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