CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Embellishment of the cloth takes place through various unit operations of wet processing such as desizing, scouring, bleaching, dyeing, printing and finishing. To accomplish these processes, dyes and various chemicals such as acids, alkalies, oxidizing/reducing agents, electrolytes, organic solvents, surfactants, and polymers etc are used. Some of these are retained by the fabric and the excess one are discharged in one or more forms of solid, liquid and gaseous. Many of these dyes and chemicals are toxic and non-biodegradable, and hence deteriorate the ecological balance and the human health.

Ecological considerations, now days, are becoming important factors in the selection of consumer goods, all over the world. The consumers demand not only the right quality product, at right time, at a reasonable price, but also with no harm to ecology during the manufacture as well as in the use. Hence, there has been a constant urge to scientists and industrialists to explore and adopt the substitutes, that are non-hazardous and ecofriendly. The use of natural dyes on textiles has been one of the consequences of increased environmental awareness. Enzymes are commercially available for the processes like desizing, scouring, bleaching, and finishing. Eyes are, today, focused towards biopolymers to minimize the use of hazardous synthetic polymers in textile processing. Natural polymers are becoming more and more attractive as raw material for the manufacture of novelty products with wider applications in textiles, medicine and agriculture. Cellulose the commonest natural polymer is well established source for standard textiles. Others like polyaminosaccharides, alginates, starch, collagen, gelatin etc are promising naturally occurring starting materials for specialty products [1]. One such promising examples among above kind is CHITOSAN (pronounced as kite-o-san), which is derived from naturally occurring polymer CHITIN (pronounced as kite-in). Both chitin and chitosan are biopolymers and are biodegradable, biocompatible with animal and plant tissues, non toxic, and renewable [2]. Chitin, the precursor of chitosan, is a

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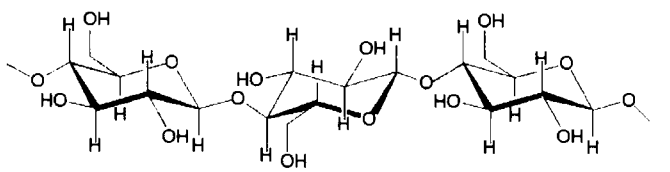
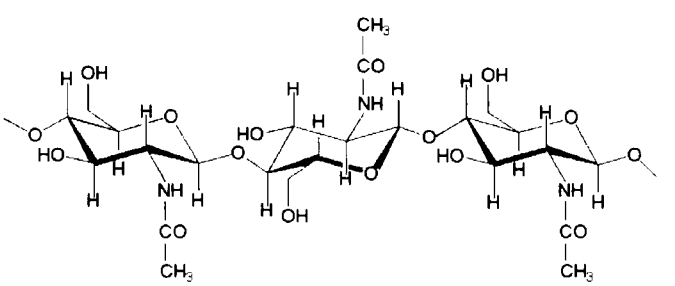
nitrogen containing polysaccharide and is second most abundant biopolymer after cellulose. It is widely distributed in the shells of crustaceans such as crabs, shrimps, lobsters etc as well as in the exoskeleton of marine zoo-plankton, including coral, jellyfish and squid pens. It is believed that at least ten gigatons (10^{13} Kgs) of chitin are synthesized and degraded and it is also estimated that over 1,50,000 tons of chitin is available for commercial use annually [2,3].

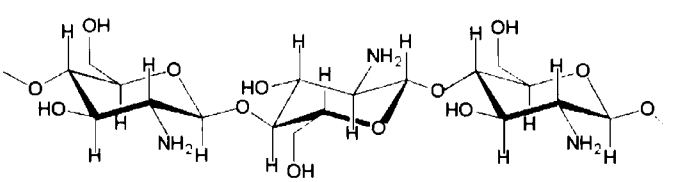
The structure of chitosan is very much close to that of cellulose except the hydroxyl group in C (2) of cellulose is being replaced by amino group in chitosan, figure 1.1. It is composed of a linear (1-4) linked 2- amino-2-deoxy-β- d- glucan (i.e. β- d-glucosamine) in the chair $^4C_1$ conformation.



Cellulose



Chitin



Chitosan

Figure 1.1 Structures of chitin, chitosan and cellulose

Chitosan, due to polycationic nature, is soluble in acidic medium and has gained enormous interest for its unique properties such as biodegradability, biocompatibility with animal and plant tissues, non toxic nature, anti bacterial, anti fungal, anti viral, anti

acid, anti ulcer, non toxic, non allergenic etc as well as film formation, fibre formation, bead formation, hydrogel formation etc. By virtue of these properties, chitosan has prospective applications in many fields such as medical, water treatment, cosmetics, dentifrices, food and textile industries. In textiles it can be in primary production of fibres, textile auxiliary chemicals and finishing agents [2-5].

Keeping in mind many valuable inherent properties and huge application potential of chitosan, the aim of present study was focused on the applications of chitosan and its various derivatives in textile processing. The work was divided into four major areas:

(1) Synthesis of chitosan of different molecular weights and determination of effect of their applications on cotton fabric,

(2) Synthesis of nano chitosan colloids and determination of effect of their applications on various properties of cotton fabric,

(3) Synthesis of *N*-substituted chitosan derivatives and determination of effect of their applications on important properties of cotton fabric, and

(4) Use of chitosan derivatives in the removal of metal ions from feed and drain water of textile processing.

Reporting of the work in this thesis comprises six chapters. Chapter 1 deals with the extensive literature survey and aims of the work. The available literature regarding applications of chitosan on textiles is reviewed in the introductory part of chapter 2 and the literature regarding nano chitosan, chemical modification of chitosan and water treatment is critically reviewed in the initial part of the chapters 3, 4 and 5 respectively.

Chapter 2 deals with the synthesis, stabilization and characterization of chitosans of different molecular weights, synthesized by hydrolytic degradation of high molecular weight chitosan using nitrous acid and subsequent applications of these chitosans on cotton fabrics. The molecular weights of chitosans were determined viscometrically using Ubbelohde capillary viscometer and Mark-Houwink equation. The viscosity behaviour of the synthesized chitosans was studied in presence and absence of electrolyte. The characterization of varying molecular weight chitosans was performed by analysis of FTIR spectra. The degree of deacetylation (DAC) was verified by ^{1}HNMR spectrum and elemental analysis. The effects of applications of chitosan on dyeing and finishing properties of cotton were analyzed. Chitosans of varying molecular weights and

concentrations were applied onto cotton fabric by conventional pad-dry cure method. The surface morphology of treated fabric was examined under scanning electron microscope (SEM). Effect of such treatments on physical properties like looks (in terms of whiteness, yellowness and brightness), stiffness, strength and water absorbency were examined. The chitosan was applied before and after dyeing of cotton, with direct dye and its effect on dyeing properties was examined. The effect of chitosan pre- treatment on the dyeability towards acid dyes was also investigated. The effect of chitosan on crease antibacterial resistance of cotton was also examined.

The very large molecular size and consequently high viscosity of chitosan restricts its penetration into the fibre and fabric structure and leads to only surface deposition. The surface deposition of this high polymer affects the feel and appearance of the treated textiles. This may also leads to maximum accumulation of dye on surface thereby reducing the all round fastness properties especially washing, rubbing and light fastness. In order to sustain the inherent cotton feel with improved performance, the particle size was reduced to nano level for the enhanced surface area, greater penetration in to fibre structure and efficacy of chitosan. In chapter 3, investigations relating to the synthesis, characterization and applications of nano-chitosan on textiles are reported. Nano chitosan dispersions were prepared by gel ionization technique through the reaction between chitosan and pentasodium tripolyphosphate (TPP) in aqueous medium. The samples were characterized by particle size analysis. Effect of various parameters such as molecular weight & concentration of chitosan, concentrations of TPP on particle size were determined. Attempts were made to correlate the viscosity behaviour with particle size of chitosan. The synthesized nano-chitosan was applied to cotton fabric and subsequently various properties of the treated fabric like appearance, absorbency, stiffness, dyeing behaviour, wrinkle recovery, resistance to microbial attack etc were examined. The fabric samples were pretreated with normal and nano chitosan solutions by pad-dry cure technique. The surface morphology of the nano chitosan treated cotton fabric was examined by SEM analysis.

The study reported in Chapter 4 is mainly emphasized on synthesizing a series of chitosan derivatives belonging to *N*-substituted quaternary salts having enhanced hydrophilicity and expected freedom from chlorine retention problem. Trimethyl chitosan

chloride (TMCHT) was synthesized by the reaction of chitosan (CHT) with methyl iodide in alkaline medium followed by ion exchange with sodium chloride. Further, *N*- alkyl and *N*-Aryl substituted chitosans of varying molecular size of substituents were synthesized through the reductive amination of Schiff's base obtained by the reaction of chitosan with respective aldehydes. The quaternization of *N*- substituted derivatives were then performed by the reaction of these compounds with methyl iodide as carried out for the synthesis of TMCHT. The chosen *N*-Alkyl chitosan derivates were *N*-ethyl chitosan (N-Et CHT), *N*-butyl chitosan (N-Bu CHT) and *N*-dodecyl chitosan (N-Dod CHT). For *N*-Aryl substituents *N*-benzyl chitosan (N-Bz CHT) and *N*-(1-naphthyl) methylene chitosan (N-Np CHT) derivatives were chosen. These *N*-substituted chitosan derivatives were subsequently quaternized using methyl iodide in alkaline medium. The synthesized chitosan derivatives were characterized by FTIR spectroscopy, ^{1}HNMR spectroscopy, Elemental (CHN) analysis and conductometry. Various reaction parameter variables like methyl iodide concentration, alkali concentration and the role of co-solvent (NMP) on the degree of quaternization of chitosan were studied. The effect of chain length/molecular weight of alkyl groups and molecular size of *N*-aryl substituent on the degree of substitution (DS) of CHT and degree of quaternization (DQ) of *N*- substituted CHT derivatives were investigated. The aqueous behaviour of these CHT derivatives was also studied through viscometry.

Effects of applying varying concentrations of *N*-substituted chitosan derivatives on appearance, feel, dyeing behaviour, chlorine retention, absorbency and dyeing behaviour of cotton were studied. The performance of CHT derivatives on cotton fabric was also examined for various aesthetics and value additions such as wrinkle recovery, soil release and antimicrobial properties.

Chapter 5 deals with the study associated with the application potential of chitosan and its derivatives for the recovery of valuable metals or the treatment of contaminated effluents. This research investigation was focused at understanding the chelation property of chitosan of varying molecular weights and trimethyl chitosan chloride. The study was primarily performed on chelation of calcium ions (Ca^{++}) for its ease of analysis. Study included the effect of molecular weight of chitosan, effect of concentration and kinetic on chelation. The chelation behaviour of nano chitosan

dispersion was also investigated. Similar study was conducted with copper ions. Besides volumetric analysis, gravimetric analysis and flame atomic mass spectroscopy were employed for characterization. Due to presence of hydroxyl and amino functional groups, chitosan has high affinity for different classes of dyes. The efficiency of chitosan in regard to the removal of acid and direct dyes was also determined.

Conclusions and future prospects are summarized in chapter 6.

1.2 HISTORICAL BACKGROUND OF CHITIN AND CHITOSAN

Prof. Henri Braconot, Director of Botanical Garden in Nancy (France) in 1811, first isolated a fibrous substance from the cell walls of mushroom, which he called FUNGINE. He further observed that this substance did not dissolve in aqueous acidic solutions, e.g. sulfuric acid. Later in 1823, Odier discovered that this compound is also one of the major constituent of the exoskeleton of insects and then he renamed as CHITIN (from Greek *khitōn* meaning tunic or envelope). Prof. C. Rouget in 1859, prepared a compound from chitin by treatment with concentrated caustic solution and observed that, unlike chitin, the resulting substance dissolved in acids. This compound was then named as CHITOSAN by Hoppe- Seiler in 1894 [3]. In the mean time, in 1878, Ledderhose proposed chitin to be made of glucosamine and acetic acid. However, the existence of chitosan in nature was discovered in 1954 in the yeast Phycomyces blakesleeanus.

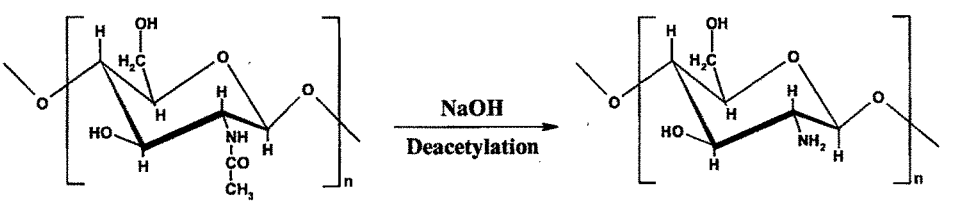
During 1930's and 1940's, researchers in Korea, Japan, Europe and USA have tested chitin and chitosan in biomedical applications. In Japan, chitosan was first used for waste water treatment to absorb grease, oils, heavy metals and other potentially toxic substances. Researchers claim that a tooth paste made from crab's shell could cut dental infections and reduce the number of visits to dentists [3, 6]. During 1970's the interest in these bio-macromolecules resulted in the first ever Chitin-Chitosan conference being held in the United States in 1977. Pioneering work of Muzzarelli during 1980's has greatly advanced our understanding of these materials [2, 3].

Chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively, and thus considerable attention has given to the use of chitosan as a natural preservative to improve the shelf-life of food [7]. In the United States, the food

and drug administration (FDA) has approved chitosan for fruit juice clarification, protein recovery from food process waste, edible coatings, and as an additive for animal feed [8-10].

1.3. CHEMISTRY OF CHITIN AND CHITOSAN

Chitosan $[(C_6H_{11}O_4N)_n]$ is a polysaccharide composed of a linear (1-4) linked 2-amino-2-deoxy-β- d- glucan (i.e. β- d-glucosamine) in the chair $^4C_1$ conformation. It is derived by alkaline deacetylation of chitin i.e. (1-4) linked 2-acetaamido-2-deoxy-β- d-glucan (i.e. *N*-acetyl- β- d-glucosamine), scheme 1.1. Chitin when boiled with highly concentrated sodium hydroxide solution; deacetylation takes place producing chitosan having free amino groups and sodium acetate as byproduct. The mechanism of deacetylation is similar to alkali hydrolysis acid amides [11]. In highly alkaline medium, the hydroxyl ions of sodium hydroxide (OH^-) attack the electron deficient carbonyl carbon of acetamide group on chitin to form an intermediate anion. Chain scission follows and release free amino group on main chain with liberation of acetic acid. The liberated acid then gets neutralized with sodium hydroxide. The overall reaction mechanism is illustrated in scheme 1.2.

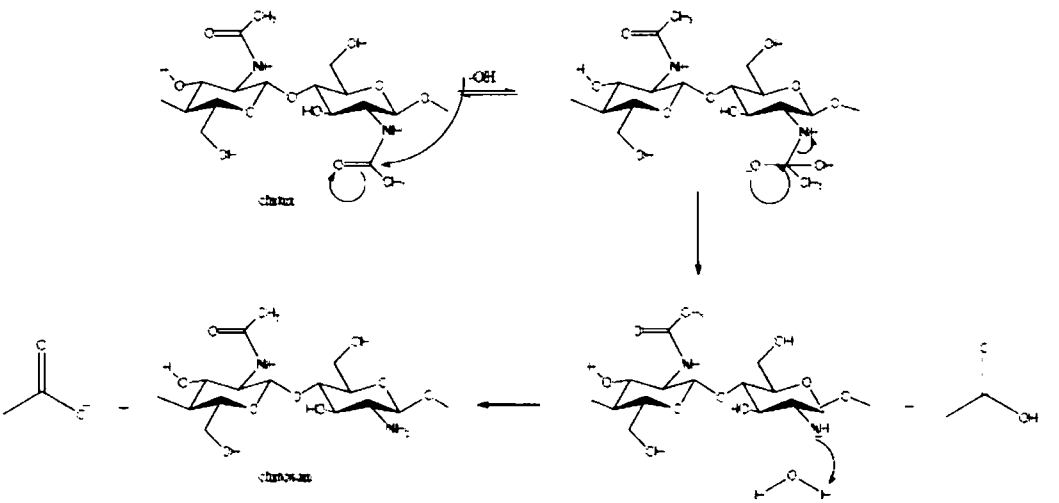


Chitin **Chitosan**

Scheme 1.1 Synthesis of chitosan by deacetylation of chitin

Between chitin and chitosan, a number of derivatives of partially *N*-acetylated chitosans exist, which is characterized by degree of deacetylation (DAC). Degree of deacetylation has a direct impact on the secondary structure of the polymeric chain and can also influence the solubility of the polymer in organic or aqueous solvents. As an evolved nomenclature, chitineous substances that do not dissolve in dilute organic acids, e.g. 1-2% acetic acid, are collectively called 'chitin'. On the other hand, chitineous

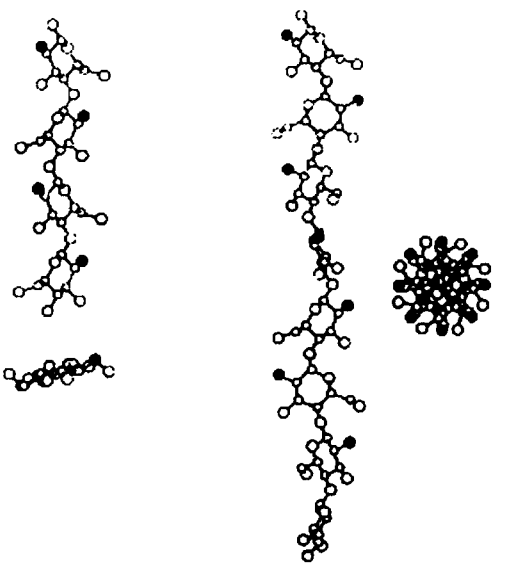
substances that can dissolve in aqueous dilute acids are referred as chitosan, approximately 60 % or above deacetylated products fall in this category. Indeed, it is a copolymer of *N*-acetyl-glucosamine and glucosamine units. The structure of chitosan is very much close to that of cellulose except the hydroxyl group in C (2) of cellulose is being replaced by amino group in chitosan, Figure 1.1 [12-19].



Scheme 1.2 Reaction mechanism of deacetylation of chitin

Chitin occurs naturally as one of three crystalline polymorphic forms, known as α-, β-, and γ-chitin. The chains of α-chitin are antiparallel but the β-chitin has a parallel stack structure. The γ-chitin form has not been fully classified but an arrangement of two parallel chains and one antiparallel chain has been suggested. Although both α- and β-chitins possess C=O$\cdots$H–N intermolecular hydrogen bonds, the β-chitin does not have the intermolecular hydrogen bonds between $-CH_2OH$ groups, which are present in the α-chitin. This fact makes it easy for the β-chitin to swell in water to produce hydrates unlike the α-chitin, which has a strong three-dimensional hydrogen bond network. The α-chitin is the most abundant and found in crustaceans, insects, and fungi, while the occurrence of β-chitin is less common and it is found in squid pens. Chitosan mainly occurs in two molecular conformations, namely (i) as extended two-fold helix and (ii) as extended eight-fold helix (Figure 1.2). The eight fold helix conformation transforms into two-fold helix under high humidity. No ordered conformation, however, is present in the aqueous

acidic solution. The molecular flexibility increases with increase in deacetylation, increase in ionic strength in the solution and increase in temperature. Chitosan is characterized by another feature, namely, the molecular weight which governs the viscosity behaviour. The average molecular weight determined by the viscosity measurement methods is of the order of $7x10^5$. The degree of acetylation of chitosan may be determined by C:N ratio (by elemental analysis) , ^{13}C NMR, ^{15}N NMR, by I.R. Spectroscopy, colloidal titration and gas chromatography. The molecular weights are mainly determined by viscosity measurements, light scattering spectrophotometry and gel permeation chromatography (GPC) [2, 3, 12]. The chitosan molecule is rather stiff, less than DNA and more than polyacrylate; increasing degree of acetylation value lead to a more extended conformation and stiffer chain [20].



(a) Two fold helix (b) Eight fold helix

Figure 1.2 Molecular conformation of chitosan at the solid state: (a) two fold helix conformation with side view(above) and a sectional view (below), (b) eight fold helix conformation with side view(left) and a sectional view (right)

1.4 PRODUCTION OF CHITIN AND CHITOSAN

Chitin, the precursor of chitosan, is a nitrogen containing polysaccharide and is second most abundant biopolymer after cellulose. It is widely distributed in the shells of crustaceans such as crabs, shrimps, lobsters, prawns, squilla etc as well as in the

exoskeleton of marine zoo-plankton, including coral, jellyfish and squid pens. About 20-40% chitin is present the exoskeleton of these animals. It is also present in smaller quantities in insects such as butter flies ladybugs, and the cell walls of yeast, mushrooms and other fungi, Figure 1.3. But since the crustacean shells are waste products (now byproducts) of food industry, these are commercially employed for production of chitin and chitosan [2, 3, 21]. Squid pens are also the potential source of chitin and chitosan. Squid-pens are removed from the squid during processing and are currently regarded as 'waste' so the raw material is cheap. Since, the squid pens are very low in calcium; the acid extraction step is not required. This intern reduces the cost and acid hydrolysis of chitin [22]. Therefore, comparatively cheaper and better quality chitin can be produced. It is believed that at least ten gigatons (10 X 10^9 T) of chitin are synthesized and degraded and it is also estimated that over 150,000 tons of chitin is available for commercial use annually. In India, it is estimated that more than one lakh tons of shrimp processing waste is being disposed annually which would be gainfully utilized for the manufacturing of chitin. Another raw material for chitin is squilla. It is estimated that a potential of around 50,000 tons of squilla is available of which nearly 5000 tons is being thrown back into the sea. This is an important trawl by catch especially in Mangalore (Karnataka) and could be used for chitin /chitosan production. The estimated availability of crab shell is about 40,000 tons in Indian waters. The average distribution of chitin in shells of various living species is listed in Table 1.1 [3].

Chitosan can directly be isolated from some fungi, mainly, Phycomyces blakesleeanus (yeast), Zygomycetes (fungus) etc species. However, the yield is too low [6, 23]. Deacetylation of chitin can also be done enzymatically. Powdered chitin is treated with *N*-deacetylase (EC 3.5.1.41) or with microbes which secrete *N*-deacetylase. The enzymatic method yields chitosan with low degree of *N*-acetylation and low degree of polymerization [24, 25]. To-date, chitosans have been produced commercially by the alkaline deacetylation of crustaceans chitins [22, 26-28].

The crustacean shells mainly consist of chitin (20-30%), proteins (30-40%), Calcium Carbonate (30-50%), lipids and traces of pigment. The dried/wet shells washed thoroughly to make it free from sand extraneous matter so as to reduce the ash content of

final product to less than 2%. The material is then crushed into a pulverous powder to help make a greater surface area available for the heterogeneous processes to follow.

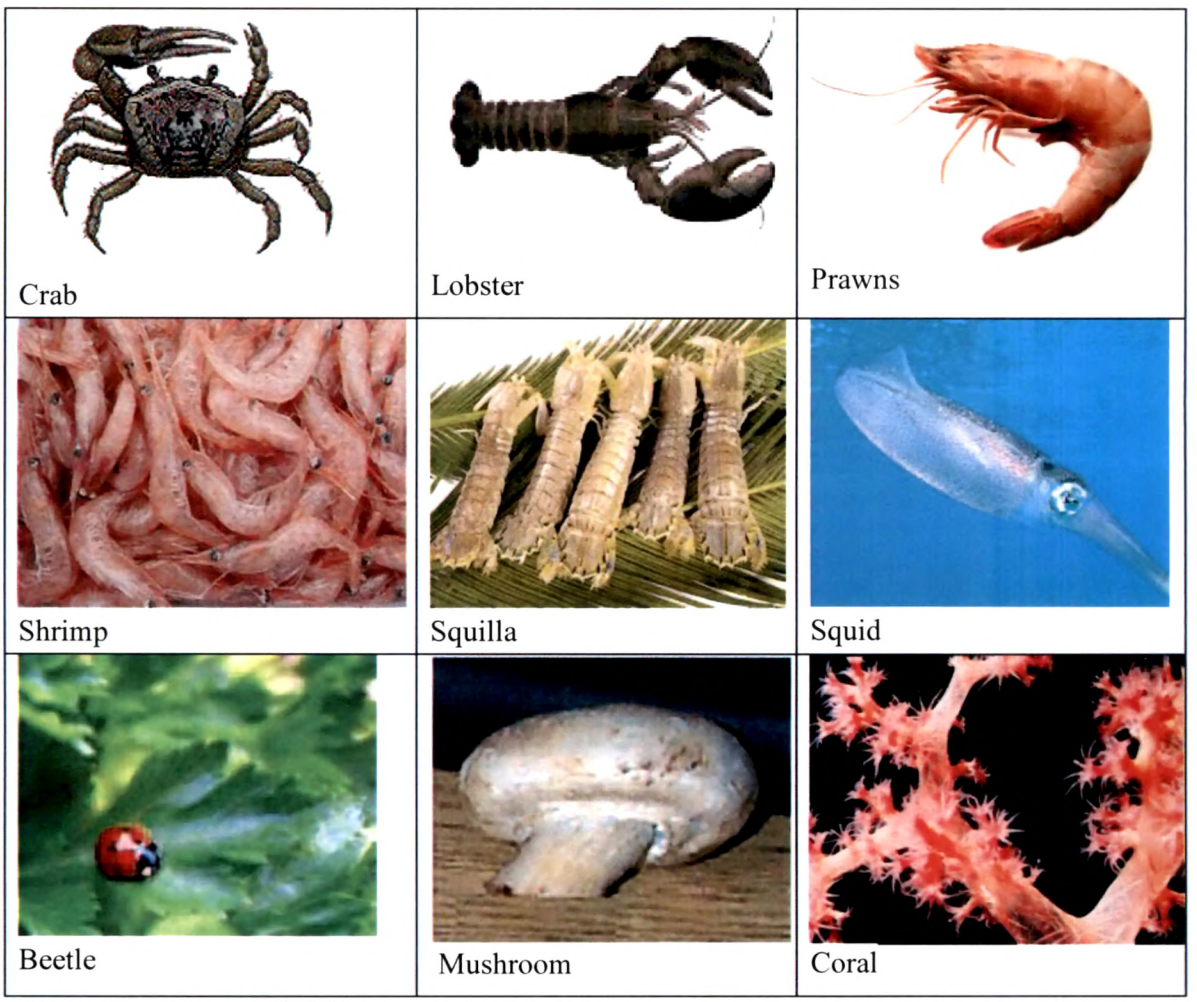


Figure 1.3 Sources of chitin

The proteins are removed by treatment with sodium hydroxide solution (5%) at about 85° C to 100° C for 30 minutes in a stainless steel vessal or digested enzymatically by proteases or micro-organism. The boiled raw material is allowed to cool and is then washed to with water to remove all the traces of alkali. The deproteinised shells are transferred to a mild steel vessel lined with fiber glass and is treated with hydrochloric acid (3 %) at room temperature to remove calcium carbonate. The excess acid is decanted and the residue is washed till the pH is neutral. Excess water is removed using a screw press and the dried. Lipids are extracted by soaking in organic solvents such as acetone or

ethanol. An oxidative bleaching treatment with hydrogen peroxide or sodium hypochlorite is also given to obtain a white chitin powder.

Table 1.1 Chitin content in shells of living species

Species	*** Chitin content, %**
Crustaceans	
Crab	15-35
Shrimp	14-27
Squilla	15
Insects	
Cockroach	10
Beetle	5-15
Mollusks	
Krill	40
Oyster shell	4
Squid pen	41
Microorganisms	
Aspergillus niger	42
Lactarius vellereus (Mashroom)	19
Saccharomyces cerevisiae (baker's yeast)	2.9

**On dry body weight*

Conversion of chitin into chitosan involves the deacetylation process, which is a harsh treatment usually performed with concentrated sodium hydroxide solution. Chitin flakes are treated in suspension with aqueous 40- 50 % caustic solution at 80 – 120 ^{0}C with constant stirring for 4 - 6 hours and this treatment is repeated for once or more times for obtaining high amino content product. To avoid depolymerization due to oxidation, sodium borohydrate is added. Excess alkali is drained off and the mixture is washed with water several times till it is free from alkali. Most of the alkali is then used either in deproteinisation or in deacetylation. Excess water is removed in screw press and the wet chitosan cake is either sun dried or in drier at 60 ^{0}C. Chitosan thus obtained is in the form of flakes and can be pulverised to powder. The flow chart for the manufacturing of chitosan from the starting material (crustacean shells) is shown in Figure 1.4.

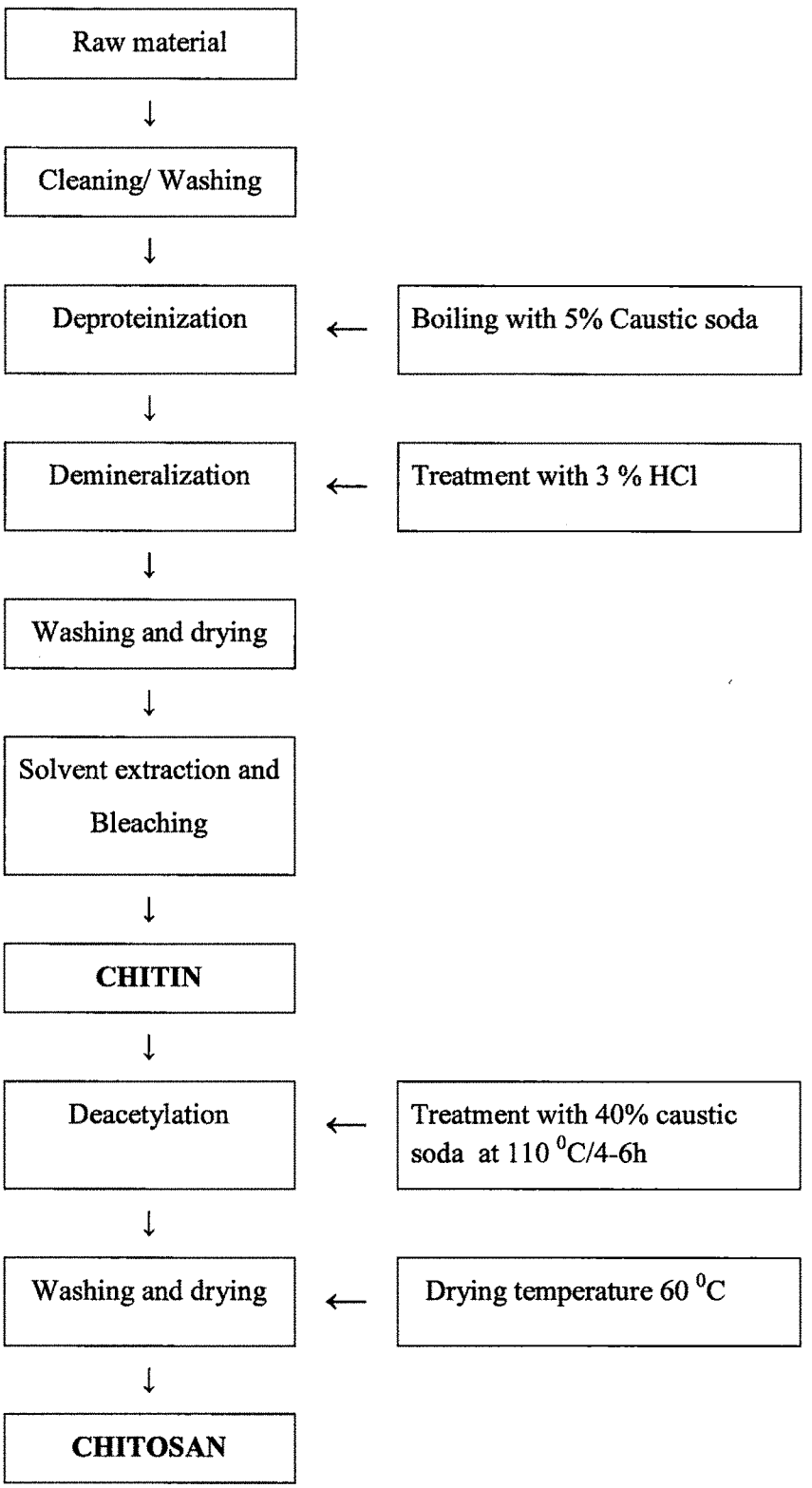


Figure 1.4 Flow chart for the manufacturing of chitin and chitosan

Alternatively, if isolation of chitin is not desired, the acid-base sequence may be reversed to directly produce chitosan.

1.5 PHYSICAL PROPERTIES OF CHITOSAN

Chitosan is a white fibrous material produced in different grades such as fibres, beads and membranes. The physical properties of chitosan are governed, at large, by two factors; the degree of deacetylation (DAC) and the molecular weight.Various factors such as source of chitin and processing parameters deacetylation determine the grades of chitosan. Chitosan being a primary aliphatic amine, it can be protonated by selected acids (p^{ka} of chitosan= 6.3). It is insoluble in water, organic solvents and alkalies, but is soluble in organic acid solutions. Structural characterization of chitosan concerning to various spectral analysis and viscometry is discussed in chapter 2 and physical properties are discussed below.

1.5.1 Colour of chitin and chitosan

The typical yellowish to brownish colour of chitin and chitosan (if not bleached) is mainly associated with the carotenoid pigment composed of astaxanthin. The carotenoids are strongly bound with proteins in the epithelial layer of the exoskeleton of chitin. The carotenoid level in crustacean is very low and varies depending on dietary pigment availability, crustacean size, its maturation, and genetic differences. The average values of pigment concentration found in the shell waste from crab, shrimp and Louisiana crawfish were estimated as 139, 147 and 108 ppm, respectively. This undesired colour can be destroyed by bleaching with hydrogen peroxide or sodium hypochlorite or by chemicking followed by peroxide bleach [28-30].

1.5.2 Degree of deacetylation

The deacetylation process involves the removal of acetyl groups from chitin molecules. The degree of deacetylation (DAC) is defined as the average number of D-glucosamine units per 100 monomers expressed as a percentage. It determines the content of free amino groups ($-NH_2$) in the chitosan and is one of the most important chemical characteristics that influence the physicochemical properties, biological properties,

antibacterial activity and applications of chitosan. In other words DAC value determines the functionality, reactivity, polarity and water solubility of the polymer. Chitin does not dissolve in dilute acetic acid. When chitin is deacetylated to a certain degree (~ 60% deacetylation) where it becomes soluble in the acid, it is referred to as chitosan [21, 31].

The DAC value can be obtained directly by determining amino group content of a chitosan sample or indirectly by determining acetyl content (degree of *N*-acetylation). Acid-base titration is one of the simplest methods to determine DD which involves dissolving a known amount of chitosan in an excess of dilute acid (e.g. hydrochloric acid), titrating it with a standard sodium hydroxide solution and measuring the pH to determine the stoichiometry. Conductometric titrations can be the convenient way [32]. Other methods used to determine the DD are IR spectroscopy [33], UV spectroscopy [34], ^{1}HNMR spectroscopy [35, 36], 13C solid-state NMR spectroscopy [37], gel permeation chromatography [34], elemental analysis [38] etc

1.5.3 Molecular weight

The molecular weight of chitosan is another important property that determines its suitability for a particular application. It determines the viscosity of its solution and the strength of chitosan fibre and film. The molecular weight of chitin and chitosan depend on its source and deacetylation conditions (time, temperature, and concentration of NaOH), respectively [2,3]. Molecular weight of chitosan mostly determined by Viscometry [39], gel permeation chromatography [40] and light scattering spectrophotometry [32]. Chitosan obtained from deacetylation of crustacean chitin may have a molecular weight over 10^5~10^6. Consequently, it is necessary to reduce the molecular weight by chemical methods to much lower molecular weight for easy application as a textile finish.

Depolymerisation is a process of preparation of low molecular weight chitosan from high molecular weight chitosan. It can be achieved by chemical, physical and enzymatic methods. Chemical depolymerisation using hydrochloric acid, nitrous acid and phosphoric acid have been attempted [41-43]. The enzymatic depolymerisation with chitosanase [42, 44] is milder and easy to control but is expensive. Oxidative depolymerisation using ozone [45] and hydrogen peroxide [46] has also been reported.

Physical methods such as ultrasonification [47], irradiation [48, 49] have been attempted by researchers.

1.5.4 Solubility

The solubility of chitosan is very important for its utilization, such as for chemical modification and film or fiber formation. Neither chitin nor chitosan are soluble in neutral water. Chitin is a semicrystalline polymer with extensive inter- and intramolecular hydrogen bonds, which make it difficult to dissolve in dilute acids or organic solvents under mild conditions. Although the polymer backbone consists of hydrophilic functional groups chitosan is normally insoluble in water and most common organic solvents (e.g. DMSO, DMF, NMP, organic alcohols, pyridine). The insolubility of chitosan in aqueous and organic solvents is a result of its crystalline structure, which is attributed to extensive intramolecular and intermolecular hydrogen bonding between the chains and sheets, respectively (Figure 1.5) [50, 51].

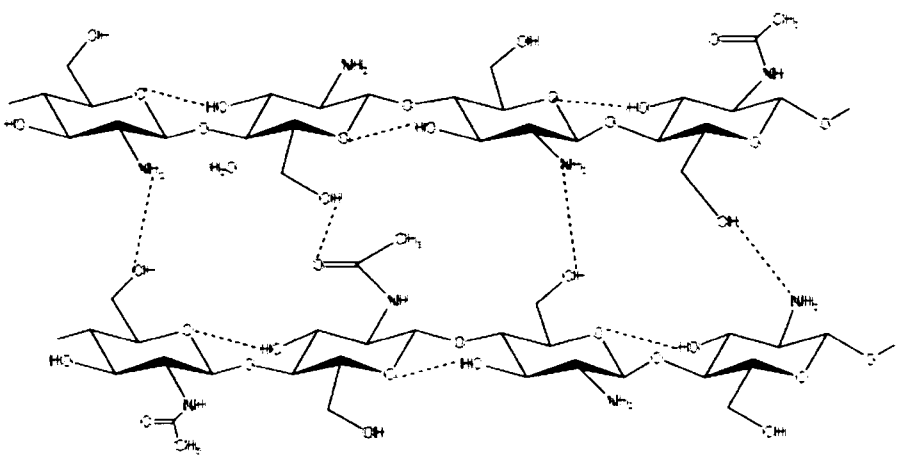


Figure 1.5 Crystalline structure of chitosan

The pKa value of chitosan is 6.3 and is therefore soluble in dilute mineral or organic acids by protonation of free amino groups at pH below about 6.5. This cationic nature is the basis of a number of applications of chitosan. Acetic and formic acids are most widely used for research and applications of chitosan [52]. The solubility of chitosan is determined by molecular weight, DAC value and pH and ionic valency of acid used. Generally, the solubility of chitin and chitosan decreases with an increase in

molecular weight. Oligomers of chitin and chitosan with a degree of polymerization (DP) of 8 or less are water-soluble regardless of pH. When chitin is deacetylated to a certain degree (~ 60% deacetylation) where it becomes soluble in the acid, it is referred to as chitosan [21]. Polyions such as pentasodium tri polyphosphate (TPP), EDTA etc lead to gel ionization at certain concentration and then precipitation at higher concentrations of acid [53-56]. Chitosan also precipitates in sulphuric acid solution [57, 58]. Such polyionic acids can be employed in the synthesis of nano- chitosan particles, which is discussed elaborately in chapter 3.

1.5.5 Viscosity

Viscosity of chitosan solution is another property that determines its commercial applications and is determined by the degree of deacetylation, molecular weight, concentration, ionic strength, pH, and temperature [59]. The viscosity of chitosan increases with an increase in molecular weight and concentration of chitosan, while it increases with decrease in pH in acetic acid and decreases with decreasing pH in HCl. Moreover, the antimicrobial activity of chitosan is also affected by its viscosity [60]. Several studies have shown that physical and chemical treatments affect its viscosity. Viscosity of chitosan decreased with increasing treatment time of grinding, heating, autoclaving, ultrasonication and ozonation [61], and decreased from 248 to 32 cP with increasing deproteinization time from 0 to 30 min [62].

In aqueous solutions, above certain polymer concentration, intermolecular interactions lead to the formation of associations thus exhibiting thickening properties. The viscous solution shows Newtonian flow. Viscosity can be used to determine the molecular weight of chitosan. Viscosity behaviour of chitosan and its different derivatives is discussed elaborately forthcoming chapters.

1.5.6 Chitosan hydrogel

Hydrogels are the crosslinked polymer networks that hold a large amount of water. The polymers used to prepare hydrogels normally consist of a large portion of hydrophilic groups and the formed networks are prevented from dissolving due to the chemical or physical bonds between the polymer chains. Water can penetrate into the

networks, resulting in the swelling of the hydrogels. Depending on the methods fabrication, the dimensions of hydrogels can vary from nanometer to centimeters in width and in different shapes such as films, capsules, sponges, microparticles, composites, beads, etc.

Chitosan based gels may be broadly divided into thermally reversible gels and non reversible gels. Reversible gels are normally obtained by physical cross linking via ionic gelation with anionic molecules while non reversible gels are produced by *N*-acylation and Schiff's base (aldemide) formation. One of the simplest ways to prepare chitosan gel is to treat chitosan acetate solution with carbodiimide. Chitosan hydrogel beads by physically crosslinked with TPP was developed as a pH-sensitive drug release system to encapsulate glipizide, an insulin stimulating drug. The hydrogel beads were proposed to release the encapsulated drug slowly uponsubcutaneous injection, with good tolerability and prolonged half life [63]. In chemical crosslinking, the Schiff's base formation system with dialdehydes such as glyoxal, glutarldehyde etc is the most widely accepted one. This involves the covalent cross linkages formation between chains [2].

Chitosan hydrogel found several applications due their unique features depending on the polymer used, including slow release profile due to physical or chemical crosslinking, enhanced drug residence time and tissue permeability, mucoadhesive characteristics, survival against gastrointestinal tract and colon delivery, etc. The chitosan–polyphosphoric acid gel bead is used as a carrier for the sustained release of anticancer drugs in simulated intestinal and gastric juice. Further, for food industry, hydrogel beads may be more feasible to achieve and industrialize, due to the low cost and controllable preparation parameters, and scalable procedures, compared with nano/microparticles delivery systems in food industry. Hydrogels are also used as a media for affinity chromatography for enzymes, media for gel permeation chromatography, for the isolation of bovine serum albumin, and a wound dressing materials [2, 3, 64-66].

1.5.7 Chitosan membranes

Chitosan, particularly high molecular weight, exhibits good film-forming properties as a result of intra- and intermolecular hydrogen bonding. The films are mostly

flexible, tough, transparent, and colourless with smooth and shiny surface. The quality of film depends on the source from which the precursor 'chitin' is obtained. The films of squid-pen chitosan are clearer and rigid than that of crab and cray fish chitosan [22]. Chitosan films, in general, are produced from acidic solutions containing volatile acid such as formic or acetic acid. The viscous solution is spread on glass plate and dried on water bath to evaporate the acid and moisture. The film i.e. chitosan formate or acetate, which is soluble in water, is detached from glass sheet and the treated with 1M NaOH solution and washed thoroughly to neutral pH and then dried at 60 ^{0}C [67].

The film of chitosan takes place through a series of phases. When the polymer solution is cast on a surface, cohesion forces form a bond between the polymer molecules. When the cohesive strength of the polymer molecules is relatively high, continuous surfaces of the polymer material coalesce. Coalescence of an adjacent polymer molecule layer occurs through diffusion. Upon evaporation of water, gelation progresses and allows the polymer chains to align in close proximity to each other and to get deposited over a previous polymer layer. When there is adequate cohesive attraction between the molecules, sufficient diffusion, and complete evaporation of water, polymer chains align themselves to form films [68].

Chitosan membranes are useful in selective isolation of heavy metals ions such as copper, cadmium, cobalt, molybdenum, zinc, mercury, chromium etc. Other applications are in edible films for coated food and tablets, tissue engineering etc. These are used as active transport membrane for halogen and organic ions in protein purification, as affinity membrane for purification lysozyme, in dialysis, in reverse osmosis and ultrafiltration [3, 5, 69].

1.5.8 Chitosan beads

Chitosan is known to chelate heavy metal ions by residual amino groups, it was expected that the chelating capacity and adsorption for heavy metal ions or organic compounds could be modified by reforming standard chitosan into other forms with increased relative surface. Chitosan formed in porous beads seems to be the most suitable shape for industrial applications in waste water treatment. Chitosan beads find several miscellaneous applications such as media for anion exchange and affinity

chromatography, as controlled release carriers for drug and agrochemicals, as encapsulating materials for mammalian cells, microbes, and drugs and for immobilization of enzymes [1, 3]. The fabrication of chitosan beads, chitosan of suitable molecular weight and desired concentration is first prepared in acidic aqueous solution. A viscosity regulator such as sodium acetate or urea may also be added to chitosan solution. The chitosan solution is poured into a coagulating bath through a discharge hole. The coagulating bath is normally alkaline containing any one of the following: sodium hydroxide, potassium hydroxide, ammonia, ethylene diamine etc preferably in presence of alcohol (e.g. methanol). Alcohol reduces the surface tension of bath and moderates the shocks during pouring and therefore control the specific surface area[70].

1.5.9 Chitosan fibres

The linear structure of chitosan is mainly responsible for the fibre forming property. Chitosan fibres are produced by wet spinning method. Chitosan is dissolved in acetic acid to give a solution of 3% w/v and then extruded through the spinneret into a caustic coagulation bath (0.5%) to obtain a regenerated fibre. Chitosan fibres find use in the production of textiles having antimicrobial, antithrombogenic, haemostatic, deodorizing, moisture controlling, and non allergenic properties which are intern used as bandages for wound- dressing, as sutures, as perfume releasing fabrics, carriers to for active drugs and artificial limbs [1, 3, 71].

1.6 BIOLOGICAL PROPERTIES OF CHITOSAN

1.6.1 Biodegradability

Chitin and chitosan are biodegradable in the biosphere, in the agriculture soil, and in the hydrosphere to produce oligosaccharides [3]. These are mostly attacked, *in vivo*, by several non specific proteases such as lysozyme, papain, pepsin etc. present in all mammalian tissues. Their biodegradation leads to the release of non toxic oligosaccharides of variable length which can be subsequently incorporated to glycosaminoglycans and glycoproteins, to metabolic pathways or be excreted. The rate of degradation is governed by molecular weight, degree of deacetylation (DAC) and the distribution of acetyl groups. The absence of acetyl groups or their homogeneous

distribution (random rather than block) are reported in very low rates of enzymatic degradation. This occurs due to differences in deacetylation conditions which influences viscosity of the chitosan solution by changing the inter- or intra-molecular repulsion forces. The degradation rate influnces the biocompatibility since very fast rates of degradation will produce an accumulation of the amino sugars and produce an inflammatory response. Chitosan samples with low DAC induce an acute inflammatory response while chitosan samples with high DAC induce a minimal response due to the low degradation rate. Degradation has been shown to increase as DAC decreases [10, 72, 73].

1.6.2 Non toxicity and biocompatibility

Chitosan is totally non toxic and its degradation products namely glucosamines are biocompatible. Although the gastrointestinal enzymes can partially degrade both chitin and chitosan, when both polymers are orally administered they are not absorbed. For this reason, they are considered as not bioavailable. Chitosan shows a LD_{50} of around 16g/kg, very similar to the salt and glucose values in assays carried out on mice. LD_{50} is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. No abnormal symptoms are observed with several animals after the oral administration of chitosan for 8 months at a daily dose of 0.7-0.8 g per kg body weight, and after the intravenous injection of low molecular weight chitosan for 11 days at a daily dose of 4.5 mg per kg body weight [3, 74].

Toxicity of chitosan is reported to depend on DAC, which decreases with increase in DAC. On the other hand, Mw of chitosan did not influence toxicity [74]. Chitosan presents higher cytocompatibility *in vitro* than chitin. The cytocompatibility of chitosan has been proved *in vitro* with myocardial, endothelial and epithellial cells, fibroblast, hepatocytes, condrocytes and keratinocytes [75]. This property seems to be related to the DD of the samples. When the positive charge of the polymer increases, the interactions between chitosan and the cells increase too, due to the presence of free amino groups [10].

1.6.3 Antimicrobial activity

One of the most unique biological properties of chitosan is its antibacterial activity against different groups of microorganisms, such as bacteria, yeast, and fungi. This unique property, due to the polycationic nature of chitosan, facilitated its application in a variety of fields, including food science, agriculture, medicine, pharmaceutics, and textiles. Different mechanisms are proposed to explain the inhibition of microbial cells by chitosan. A positive charge on the amine group of the glucosamine monomer at pH<6.3 allows interactions with negatively charged microbial cell membranes that lead to the leakage of intracellular constituents. Also, the interaction with anionic groups on the cell surface causes the formation of an impermeable layer around the cell, which prevents the transport of essential solutes [76]. The second mechanism involves the inhibition of the RNA and protein synthesis by permeation into the cell nucleus [77]. Other mechanisms have also been proposed. Chitosan may inhibit microbial growth by acting as a chelating agent rendering metals, trace elements or essential nutrients unavailable for the organism to grow at the normal rate [78]. It has also been proposed that the antimicrobial action of chitosan against filamentous fungi could be explained by a more direct disturbance of membrane function [79]. Thus, any modifications that increase the cationic charge on the polymer surface, increase the fluidity and impart the chelation property are key factors determining the antimicrobial efficiency of chitosan. Cationic charge is increased by lowering the pH, increased DAC and increased degree of quaternization. Permeability is associated with molecular weight/viscosity and the particle size (hydrodynamic volume) of chitosan, which should be lower [10, 77, 80]. The antibacterial property of chitosan is also variable on the type of the bacteria [7, 81]. Chitosan has shown stronger bactericidal effect on gram-positive bacteria (*Listeria monocytogenes, Bacillus megaterium, B. cereus, Staphylococcus aureus, Lactobacillus plantarum, L. brevis, and L. bulgaris*) than on gramnegative bacteria (*E.coli, Pseudomonas fluorescens, Salmonella typhymurium, and Vibrio parahaemolyticus*).

Besides above properties, chitosan exhibits several other biological properties which are of interest to medical and other relevant areas, such as: antiviral, antacid, haemostatic, analgesic, antitumor, mucoadhesive, fat binding, permeation enhancer, anticholesterolemic, antioxidant, wound healing, sequestering etc [10, 82, 83].

1.7 CHEMICAL PROPERTIES OF CHITOSAN

Chitosan possesses three reactive groups, viz., primary (C-6) and secondary (C-3) hydroxyl groups on each repeat unit, and the amino group at the C-2 position on each deacetylated unit. These reactive groups can be chemically modified to alter the mechanical and physical properties, and solubility of chitosan. The typical reactions involving the hydroxyl groups of chitosan are etherification and esterification. The nucleophilic amino group in chitosan allows reactions such as *N*-alkylation, *N*-acylation and reductive alkylation by reacting chitosan with alkyl halides, acid chlorides and aldehydes or ketones, respectively. Selective *O*-substitution can be carried out by protecting the amino group during the reaction. Cross-linking or graft copolymerization can also be carried out to modify chitosan [3, 82, 83].

1.8 APPLICATIONS OF CHITOSAN

Chitosan is versatile biopolymer derived from chitin with abundance ranking next to cellulose and is renewable. The major driving force in the development of new applications for chitosan lies in the fact that the polymer is biodegradable, biocompatible, and possesses selective adsorption properties. Attributing to the polycationic nature and several inherent properties, chitosan is currently receiving a great deal of interest as regards to its applications in several diversified areas such as medical, food, textiles, water processing, cosmetics, agriculture, paper etc. An overview on applications of chitosan and its derivatives is presented below.

1.8.1 Agriculture

Chitosan has many potential applications in agriculture for its essentially biodegradable characteristics. It is used primarily as a plant growth enhancer, and as an ecofriendly biopesticide substance that boosts the ability of plants to defend against fungal infections such as *Fusarium solani*. Chitosan triggers the defensive mechanisms in plants, acting much like a vaccine in humans. The biocontrol mode of action of chitosan elicits natural innate defense responses within plant to resist insects, pathogens, and soil-borne diseases when applied to foliage or the soil [84, 85].

In addition to growth stimulation properties, chitosan is used for seed-coating on cotton, corn, seed potatoes, soybeans, sugar beets, tomatoes, wheat etc, frost protection, protective coating for fruits and vegetables, controlled release of fertilizers, nutrients etc into the soil. Chitosan increases photosynthesis, promotes and enhances plant growth, stimulates nutrient uptake, increases germination and sprouting, and boosts plant vigor. [5, 86].

1.8.2 Applications in food technology

Chitosan has been already been used as a food ingredient in Japan, Europe and in United States as a lipid trap, an important dietetic breakthrough. Since, chitosan is not digested by the human body; it acts as a fibre, a crucial diet component. It binds the lipids arriving in the intestine, thereby reducing the cholesterol absorption by about 20 to 30% by the human body [10, 87].

Chitosan and its derivatives has a significant role in food application area in view of recent outbreaks of contaminations associated with food products as well as growing concerns regarding the negative environmental impact of packaging materials currently in use. Chitosan can be used in meat preservation by inhibiting some meat spoilage bacteria such as *Pseudomonas fragi, Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus* and some meat starter cultures namely *Lactobacillus plantarium, Pediococcus pentosaceus,* and *Micrococcus varians*. The addition of sunflower oil to chitosan-based films improves gloss of films and the water vapour barrier properties when acetic acid was used as a solvent to prepare the films. Besides, chitosan films can be used as a packaging material for the quality preservation of a variety of food [10, 88, 89].

1.8.3 Applications of chitosan in cosmetics

A cosmetic is defined as any substance to be placed in contact with various surface parts of the human body (e.g. epidermis, hair systems, nails, lips, and external genital organs), or with teeth and the mucous membranes of the oral cavity with a view exclusively or principally to perfume them, protect them, and keep them in good condition, to change their appearance, or to correct body odour. Chitosan is a natural

cationic gum that has been used for various cosmetic applications, it is used to maintain skin moisture, treat acne, tone skin, protect epidermis, reduce static electricity in hair, fight dandruff etc. Incorporation of chitosan salt in shampoos confer shine and strength to hair due to the ionic interactions between chitosan and hair proteins. When applied to the surface of the skin, chitosan forms a protective and moisturizing elastic film[5, 90].

1.8.4 Applications of chitosan in biomedicine

It is in the field of health that the many properties of chitosan (bacteriostatic, immunologic, antitumoral, cicatrizant, hemostatic and anticoagulant) are of interest. For example, because of its biocompatibility with human tissue, chitosan's cicatrizant properties have proven its effectiveness as a component, notably, in all types of dressings (artificial skin, corneal dressings, etc.), surgical sutures, dental implants, in rebuilding bones and gums, and in opthalmological applications. Due to the high *N*-amino content, chitosan acts as a powerful natural magnetic attraction for lipids, fats, and bile in the digestive tract, and actually binds with them to prevent their absorption into the bloodstream. The attracting ability of chitosan can possibly reduce cholesterol and triglycerides blood plasma levels, which contribute to obesity and cardiovascular disease. Various hypolipemic formulations including particles, powders, solutions, and injections containing chitosan, were prepared for oral administration [5, 10, 82,91,92].

It is known that compounds having molecular weights less than 2900 pass through membranes derived from chitosan. Attributing to non hazardous nature and inexpensive, chitosan may be suitable for use in the preparation of dosage form commercial drugs. With reference to pharmaceutical excipient for directly compressed tablets, chitosan-alginate combination showed an extended drug release property. Dry coated tablets having a long induction period in drug release have been prepared by an ion-complex of alginate-chitosan. Sustained intestinal delivery of drugs such as 5-fluorouracil (choice drug for colon carcinomas) and insulin (for diabetes mellitus) seems to be a feasible alternative to injection therapy. For the latter, the drug should be delivered at proper sites (intestine) for long duration for producing maximum pharmacological activity. Sustained release of oxytetracycline, an antibiotic agent, from chitosan microspheres (5-30 μ) for

both oral administration and injection has been reported. This can be prepared by spray hardening and interfacial acylation methods [10, 82, 83, 93].

Tissue engineering is the development is the development and manipulation of laboratory grown cells, tissues or organs that would replace or support the function of defective or injured parts of the body. Chitosan is a promising polymer as a supporting material for tissue engineering for its non-toxicity, biocompatibility, biodegradability, porous structure, gel forming properties, ease of chemical modification, high affinity to in vivo macromolecules, and so on. [5, 82, 108, 94].

1.8.5 Paper industry

Biodegradable chitin and chitosan can strengthen recycled paper and increase the environmental friendliness of packaging and other product. Chitosan is already involved in the manufacture of paper because chitosan molecules greatly resemble those of cellulose the fain constituent of plant walls [5, 82]. Chitosan can be a useful component in paper printing due its film forming property. Coating of paper with chitosan/gelatine coatings improves dye fixation on the paper and kept the light fastness of the printed image. In ink jet printing, pigment based ink jet inks containing a polystyrene maleic anhydride polymer system that reacts with a fixer fluid containing chitosan salt such as chitosan acetate. A gel precipitate is formed on the substrate by the interaction of the chitosan in the fixer fluid and the reactive polymer system in the ink. The gel forms a protective film on the substrate providing benefits such as increased drying time, smear fastness, smudge fastness and water fastness [95].

1.8.6 Chromatography

Chitosans find wide variety of applications in chromatographic separations. The presence of free amino and hydroxyl groups in chitosan makes it an useful chromatographic support. Chitosan can be used in thin layer chromatography for separation of nucleic acid and solid phase extraction of phenols and chlorophenols [82].

1.8.7 Solid state batteries

Chitosan dissolved acetic acid solution can be employed for the fabrication of solid state proton conducting batteries. The conductivity is due to the transport of protons through microvoids in polymer. Small dielectric constants from piezoelectric studies attributed the presence of many microvoids in polymer structure. The choice of a more stable electrode material may produce better battery system [82].

1.8.8 Biocatalysis

Chitosan is widely used as supports for enzyme and cell immobilization due to its appropriate characteristics. Immobilization is the process in which the enzyme, cells or organelles is confined in a definite position thus rendering an insoluble form which retains the catalytic activity and can be reused several times[10, 83].

1.8.9 Molecularly imprinted materials

Chitosan, due to the presence of reactive sites, can be used in making molecularly imprinted polymeric matrices. Molecularly imprinted polymer represents a new class of materials that have artificially created receptor structures. This potential technology is a method for making selective binding sites in polymers by using a molecular template. These have steric and chemical memory toward the template and hence could be used to rebind it. Choice template for bio fabrication is mainly rendered on the end use such as dibenzothiophene sulfone for fuel desulfurization, hemoglobin for protein binding, nikel or copper for metal recovery and photodegradation etc [10].

1.8.10 Water processing

Chitosan can also be used in water processing engineering as a part of a filtration process. Chitosan causes the fine sediment particles to bind together and is subsequently removed with the sediment during sand filtration. Chitosan also removes phosphorus, heavy minerals, and oils from the water. Chitosan is an important additive in the filtration process. Sand filtration apparently can remove up to 50% of the turbidity alone while the chitosan with sand filtration removes up to 99% turbidity [5, 82, 96, 97]. Chapter 5 covers various aspects regarding applications chitosan in context to water processing.

1.8.11 Textiles

In textiles, it finds applications in the primary production of fibers (useful for sutures, wound dressings etc), in the manufacture of textile auxiliary chemicals and finishing agents. Investigations have shown that it can be used as a dye fixing agent, for shade and neps coverage, to improve the fastness of dyed fabrics, as a binder in pigment printing, as a thickener in printing. Due to its unique ability to dissolve and bind fats, it can be used as soil repellant agent. By virtue of its bacteria impeding property, chitosan can prevent garments to develop bad odour. Detailed studies on the applications chitosan and its derivetives in textiles are discussed in subsequent chapters.

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