

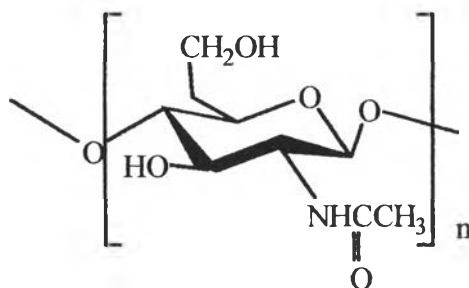
## CHAPTER II

### LITERATURE SURVEY

#### 2.1 Chitin and Chitosan

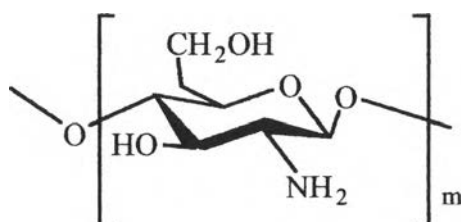
Chitin and chitosan, a cellulose-like biopolymer, is the second most abundant renewable organic resource on earth. Similar to cellulose, chitin and chitosan are long linear polymeric molecule of  $\beta$ -(1,4) linked glycans but the hydroxyl groups at C-2 position has been replaced by acetamide and amino groups for chitin and chitosan unit, respectively (Scheme 2.1). As the result, the characteristics of chitin and chitosan are different from cellulose.

a)



Poly- $\beta$ (1,4)-2-acetamido-2-deoxy-D-glucose

b)



Poly- $\beta$ (1,4)-2-amino-2-deoxy-D-glucose

**Scheme 2.1** Chemical structure of a) chitin, b) chitosan.

Chitin can not be sharply distinguished from chitosan, because fully acetylated and fully deacetylated chitin do not normally occur in nature and are difficult to prepare. Generally, this amino polysaccharide was defined as chitosan when the degree of deacetylation is 70-90%. Most of research work carried out on chitosan concerns the amino groups, which is of course one of the most important functional groups of the macromolecules.

## **2.2 Applications and Properties of Chitosan**

The main driving force in development of new applications for chitosan lies in the reason that this polysaccharide is not only naturally abundant, but it is also nontoxic, biocompatible, biodegradable and easy for membrane casting. One of the most useful properties of chitosan is chelation. Chitosan can selectively bind desired materials such as cholesterol, fats, metal ions, proteins and tumor cells. Chelation has been applied to areas of food preparation, health care, water improvement and pharmaceuticals. Other properties that make chitosan very useful include inhibition of tumor cells, antifungal effects, acceleration of wound healing, stimulation of the immune system and acceleration of plant germination. At the previous time, the major applications of chitosan were centered on sludge dewatering, food processing and metal ion chelation. The present trend, in industrial applications, however, is toward producing high value products, such as cosmetics, drug carriers, feed additives, semipermeable membranes and pharmaceuticals. The difference in value between the products and the low cost starting polymer is one of the main driving forces pushing studies on new applications of chitosan.

## **2.3 Metal Chelating Ability of Chitosan**

Chitosan is a natural polycationic polymer which possesses valuable

properties as a metal binding agent. It was firstly described in terms of a natural chelating polymer by Muzzarelli in 1968. The nitrogen electrons present in the amino and substituted amino groups can establish bonds with transition metal ions, especially in the case of chitosan, where free amino groups are particularly abundant. These polymers are bases and therefore they can also act to form salts of transition metal anions. The only field of applications where complex binding of metals with chitosan currently is in waste-water treatment. More applications are expected to develop in the future where substantial quantities of chitosan will be required.

(1) Waste-water treatment

Higher demand in reduction of harmful metals from waste water to protect the environment and increased scepticism toward the use of synthetic flocculents made chitosan interesting in removal of heavy metals and radio isotopes, and in the recovery of valuable metals.

(2) Water purification

Drinking water supply for single and small numbers of users seldom includes sophisticated purification processes and reduction of pollutants like toxic metals is done by installation of filters with easily replaceable cartridges.

Recycling of larger amounts of process water often requires removal of accumulated metals. Chitosan products exist for removal of iron and manganese from swimming pools and spas.

(3) Agriculture

Chitosan has applications in controlled release of trace metals essential to plant growth.

(4) Food

Storage of precooked meat results in development of bad flavours and odours caused by catalytic oxidation of unsaturated fatty acids. The

catalytic oxidation depends on the available iron in the meat and can be reduced considerably by complex binding of the iron.

#### (5) Biomedical

Chitosan has shown excellent properties as a hypocholesterolemic agent. Coprecipitation in the intestine of trace metals (Fe in particular) has to be limited by careful selection of appropriate chitosan quality.

There are many researchers focused on the metal chelation of chitosan and its derivatives.

Maruca and coworkers (1982) reported that the uptake of the metal ion on chitosan was much greater than that on chitin because the free amino groups in chitosan are much better ligands for binding to metal ions than the N-acylated amine groups would be. The uptake of chromium from aqueous solution was determined from changes in concentration as measured by atomic adsorption spectroscopy. The result shown that the uptake of Cr(III) by chitosan increased with increasing solution pH and was enhanced in the presence of phosphate.

Muzzurelli and Tanfani (1982) studied the chelating ability for transition metal ions that was enhanced by reacting the amino groups of chitosan with suitable chemicals. These derivatives are dithiocarbamate chitosan (DTCC), water-insoluble powder, N-(O-carboxybenzyl) chitosan (NCBC) and N-carboxymethyl chitosan (NCCM), water-soluble polyampholytes. The collecting ability of DTCC is mainly due to its high sulfur content while the collecting ability of NCBC and NCCM is due to the combined actions of acetamido, secondary amino and carboxyl groups in the chelating reaction. The results showed that NCBC and NCCM were more favoring the complexation of the metal ions due to the interaction in homogeneous phase.

Onsoyen *et al.* (1990) reported that the mechanism of complex formation of metals with chitosan was manifold and probably dominated by different processes such as adsorption, ion-exchange and chelation under different conditions. The dominant complex in the case of Cu is proposed to have two OH groups and one NH<sub>2</sub> group as ligands and the fourth site either occupied by a water molecule or by the OH group on the C-3 carbon atom.

Bassi *et al.* (2000) studied the effects of various physicochemical parameters, including chitosan amount, reaction time (with and without shaking), pH and initial metal ion concentration, on the simultaneous adsorption of Zn<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup> by chitosan flakes. The results showed that chitosan flakes had a maximum sorption capacity for copper ions and then decrease to lead, cadmium and zinc respectively. The sorption of metal ions was initially rapid up to 3 hours and then remained constant and was optimum at pH 6.0 and 7.0. Shaking of the chitosan-metal ion mixture did not affect to the sorption capacity.

## **2.4 Limitation of Chitosan**

Solubility is a very important criterion for the different uses of chitosan in any fields of applications. Although chitosan combined a number of useful properties, It was known to be insoluble in common solvents. Chitosan is insoluble in water, alkali and organic solvents but soluble in solutions of organic acids when the pH of the solution is less than 6. In this case the free amino groups becomes protonated to form cationic amino groups (-NH<sup>3+</sup>). Acetic and formic acid are two of most widely used solvents for dissolving chitosan. These solvents offer only very limited utility for chemical derivatizations in homogeneous solution, particularly for large-scale applications. Poor solubility of chitosan in common organic solvents was due to its rigid crystalline structure through intra- and intermolecular hydrogen

bonding. It was the main reason that limited chitosan to be utilized widely. In order to solve this problem, chemical modification of chitosan has been studied.

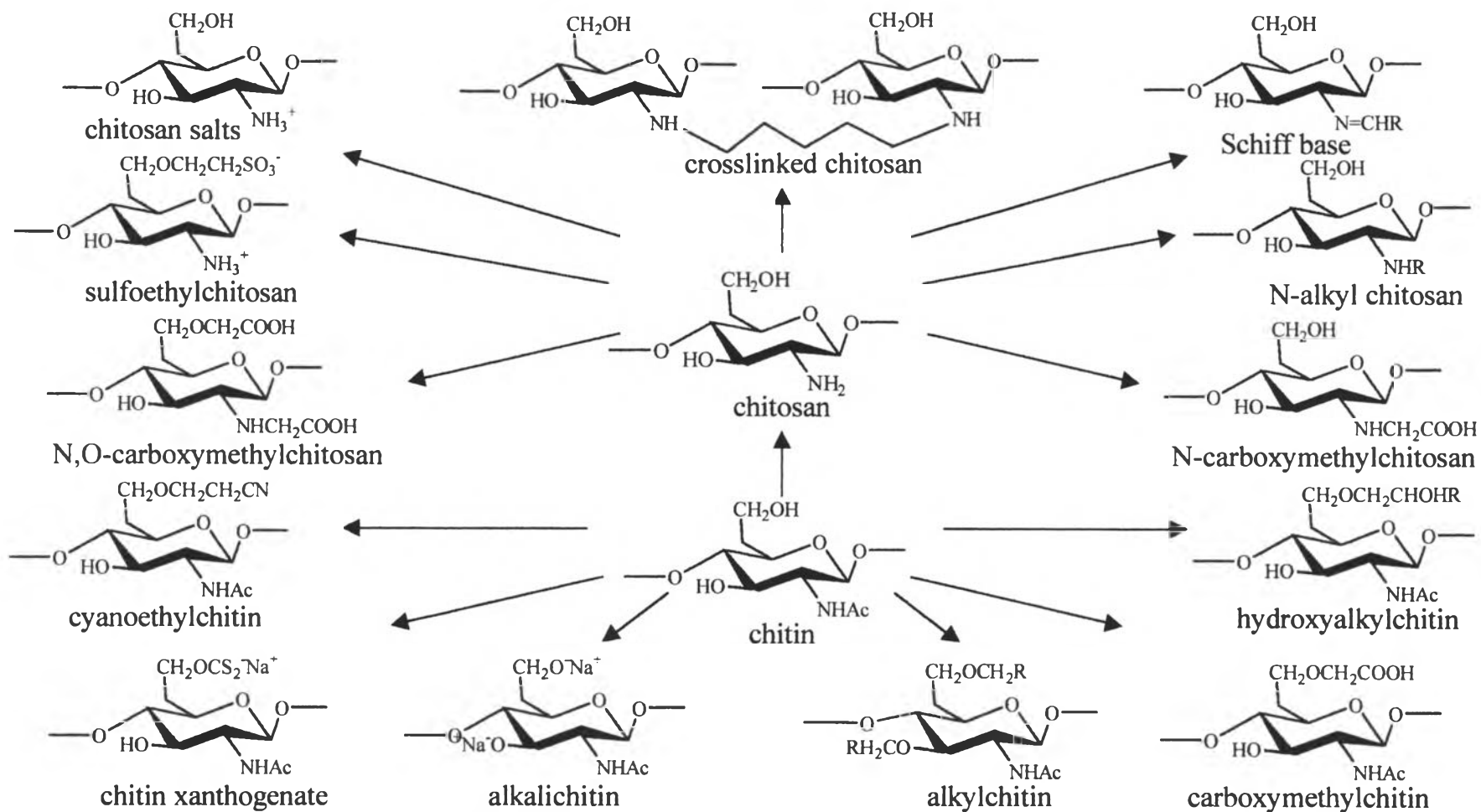
## **2.5 Chemical Modification of Chitosan**

Chitosan, being a high molecular weight biopolymer, is a linear polyelectrolyte whose reactive amino groups and primary and secondary hydroxyl groups are readily available for chemical reactions. The amino groups of chitosan can act as a nucleophile and undergo the reaction as the reactive primary amine while both hydroxyl groups of chitosan can react as the alcohols. Chitosan can undergo etherification, esterification, cross-linking and graft copolymerization reactions. Much emphasis has been paid on the chemical modification of chitin and chitosan in developing new useful materials from the viewpoint of the high potential of this amino polysaccharide. Some of known chitin and chitosan derivatives were shown in Figure 2.1. In many cases, the chemically modified chitosans show greater solubility than the original polymer.

### **2.5.1 Water Soluble Derivatives**

Tokura *et al.* (1983) prepared water soluble chitin derivatives which were carboxymethyl chitin and dihydroxy-propyl chitin. The initial alkylation sites were investigated. The result showed that C-6 hydroxyl group of chitin was attacked predominantly.

Yalpani and Hall (1983) reported on the N-alkylation of chitosan with various types of carbohydrates. Specific attachment of carbohydrates to the amino functional groups of chitosan transformed this water insoluble linear polymer into branched-chain water-soluble derivatives. Facile conversions can be achieved by reductive alkylation using sodium cyanoborohydride and



**Figure 2.1** Chemical derivatization of chitin and chitosan (David, 1996).

any aldehyde or keto sugar, by schiff base formation or amidation reaction using carboxylic acid or lactone derivatives. The synthetic chitosan derivatives exhibited a number of useful and specific properties in terms of their solution characteristics.

Sugimoto *et al.* (1997) reported the modification of chitosan with poly(ethylene glycol)-aldehyde (PEG-aldehyde) to prepare chitosan-PEG hybrid, partially acetylated chitosan-PEG hybrid and chitin-PEG hybrid. The modification with high molecular weight PEG improved the water-solubility of chitosan keeping the main skeleton intact. Additionally, water-soluble chitin-PEG hybrid was obtained by the acetylation of chitosan-PEG hybrid.

Sashiwa and Shigemasa *et al.* (1999) prepared the N-acylated chitosan derivatives via ring opening reactions with various cyclic anhydrides in aqueous methanol system. As these derivatives had both amino and carboxy groups. The water solubility of chitosan was changed from only acidic region to all pH region except at pH 3.5-7 which was the isoelectric point of these derivatives. N-alkylation of chitosan were also performed in aqueous methanol with various aldehydes, monosaccharides and disaccharides. Most of these derivatives displayed solubility in water at all pH regions.

### 2.5.2 Organic Solvent Soluble Derivatives

Kaifu *et al.* (1981) studied the hexanoylation, decanoylation and dodecanoylation of chitin by the acyl chloride-methane sulfonic acid method. Perfectly O-hexanoylated chitin (2.0 hexanoyl chitin) was successfully obtained by the standard procedure with 5 equivalent molar of hexanoyl chloride. The reaction seems to proceed more completely when the aliphatic chain of acyl chlorides was shorter because of the smaller steric hindrance. The introduction of a long alkyl chain into a chitin molecule resulted in the remarkable enhancement of the solubility property of chitin. Highly



substituted decanoyl and dodecanoyl and hexanoyl chitins are soluble in benzene, m-cresol, THF, methylene chloride and dichloroacetic acid. Hexanoyl chitin is also soluble in several kinds of solvent such as butanol, dioxane, acetic acid and ethyl acetate.

Grant *et al.* (1989) reported on the O-acylation to prepare benzoylated and butyrylated derivatives of chitin and chitosan. FT-IR results indicated that the reaction proceeded without N-acylation. Butyrylated chitin, benzoylated and butyrylated chitosan can be dissolved in chloroform, dilute acetic acid and DMSO respectively. It has been suggested that substituent groups disrupted the natural packing of the polysaccharide molecules and facilitated solubility in organic solvents.

Tokura *et al.* (1990) reported that the carboxymethyl-chitin (CM-chitin) was converted into a methanol-soluble one through an alkylation reaction by allyl bromide under the basic conditions. The allyl-CM-chitin produced was regenerated into a sparingly soluble film when allyl-CM-chitin-methanol solution was cast on a glass plate and dried under light.

Nishimura *et al.* (1991) reported N-phthaloylation of chitosan by reacting with phthalic anhydride in N,N-dimethylformamide (DMF) at 130°C. The resulting phthalimido chitosan exhibited much improved solubility in common organic solvents. The 6-O-substituted derivatives were then carried out by the reaction with bulky substituents such as triphenylmethyl (trityl) and (p-tolylsulfonyl)oxy (tosyloxy) groups under mild conditions in homogeneous solution. Subsequent 3-O-acetylation of the secondary hydroxyl groups of 6-O substituted materials gave rise to regioselectively modified chitosan derivatives showing much better solubility. The preparative procedures based on N-phthaloyl-chitosan were useful for regioselective and quantitative introduction of substituents. After desired modification reactions, the phthaloyl groups were easily removed with hydrazine to regenerate the free amino groups.

Kurita *et al.* (1992) reported on the tosylation on C-6 hydroxyl groups of chitin prepared by the interfacial reaction between an aqueous alkali chitin solution and a chloroform solution of tosyl chlorides at 0°C. The resulting tosylchitin was easily converted to iodo-chitin when treated with sodium iodide in DMSO solutions at 85°C. The tosyl-chitin with degree of substitution above 0.5 were soluble in organic solvents such as DMSO and DMAc. The derived iodo-chitin showed even better solubility in organic solvents.

Ren *et al.* (1993) studied on graft copolymerization of methyl methacrylate (MMA) onto chitosan initiated with cerium(IV) ammonium nitrate carried out in a suspension system. Solubility of the highly grafted chitin derivatives in various solvent systems changed significantly, and a gel-like mass swelling in organic solvents was obtained.

Xu *et al.* (1996) reported the acylation of chitosan films under heterogeneous conditions in methanol with acetic and hexanoic anhydrides. FT-IR results indicated that acylation occurred at the surface was site selective for amino (N) functionalities. Acetylation beyond the surface proceeded more rapidly and to higher extents than hexanoylation. Film acetylation resulted in an unreacted inner core sandwiched between outer layers of chitin. By regenerating chitin at film surfaces via N-acetylation, the rate of film biodegradation was dramatically enhanced.

Zong *et al.* (2000) prepared a series of acylated chitosans by reacting chitosan with hexanoyl, decanoyl and lauroyl chlorides in pyridine and chloroform solvent mixture. The fully acylated chitosans exhibited excellent solubility in common organic solvents and good film formability. The thermal stability of these polymers was lower than that of chitosan with losing hydrogen bonding that might imparted by the disordered packing of side chains prevented the occurrence of well-defined structure of chitosan main chains.