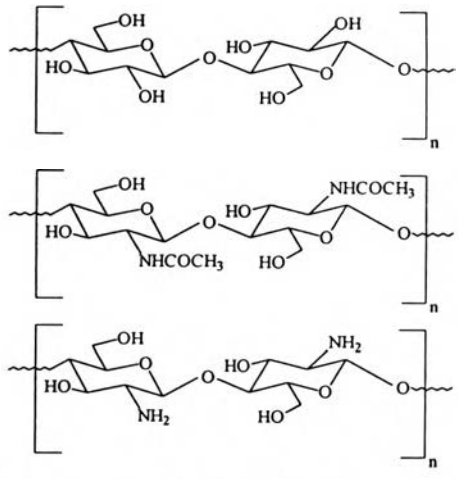
CHAPTER II
LITERATURE SURVEY

2.1.Chemical Structure of Chitin-Chitosan

Chitin-chitosan is the second most abundant natural occurring polymer next to cellulose. Chitin-chitosan can be found in the exoskeleton of insects and crustacean or cell wall of yeast and fungi. The chemical structure of chitin-chitosan is unique for the nitrogen belonging to acetamide or amine group at C-2 position in addition to the primary alcohol at C-6 position. Naturally, chitin-chitosan is a copolymer, which has β–(1–4)–2–acetamido-2-deoxy-β-D-glucose as a main unit and β–(1–4)–2–amino-2-deoxy-β-D-glucose as a sub unit (Figure 2.1). The chemical structure is found to have inter and intramolecular hydrogen bonding. As a result, chitin-chitosan is limited in application owing to the low solubility in common solvents. Practical utilization of chitin–chitosan, thus, is recognized by using physical modification such as powder blending (Sawayanagi *et al.*, 1982), fiber extrusion (Tokura *et al.*, 1979), film (Xu *et al.*, 1996) or membrane casting (Hirano *et al.*, 1978).

Despite the solubility problem, chitin-chitosan has the reactive sites of nitrogen together with primary alcohol for chemical modification. Basically, the nitrogen atom of chitin-chitosan is reported to provide lone pair electrons for metal complexation (Kurita *et al.*, 1988). The hydroxyl and amino groups are known to undergo the oxidation or reduction reaction. Considering the chemical structure, chitin-chitosan can react with other reactive functional groups such as carboxylic acid, acid chloride, alkyl halide.



(a) cellulose

(b) chitin

(c) chitosan

Figure 2.1 Chemical structures of (a) cellulose, (b) chitin, and (c) chitosan.

2.2 Chitin-Chitosan Derivatives

Chitin-chitosan is clarified for its biodegradability, bioactivity and biocompatibility, which are attractive for some advanced application, especially in biochemical, bioclinical, including the medical fields. Recently, various derivatives of chitin-chitosan are proposed in order to achieve a series of value added products.

Conceptually, chitin-chitosan can be modified at C-2 and C-6 positions. It should be noted that the reactions of chitin-chitosan face the problem of dissolvation chitin-chitosan in organic system. In some cases, the heterogeneous reaction has to be operated (Kurita *et al.*, 1992).

2.2.1 Chemically Modification of Chitin-Chitosan at N-position

Basically, acetamide group of chitin is known to change to amino group, by deacetylation with alkali solution (Horton *et al.*, 1965) to produce the reactive chitosan. Owing to the reactive amino group, chitosan has received much more attention in chemical modification study than chitin. The

purification of chitin-chitosan, thus, always includes the treatment of NaOH quantitatively to achieve chitosan as a main unit in the copolymer.

N-acylation (Fujii *et al.*, 1980), (Kurita *et al.*, 1988) is known to achieve the nonpolar structure with a low intra/inter molecular hydrogen bonding by increasing the long chain alkyl group. Hirano *et al.* (1976) investigated the selective N-acylation of chitosan by treatment of a solution in aqueous methanolic acetic acid with carboxylic anhydrides at room temperature. The obtained N-acylations were found to be effective as a selective aggregation for specific cancer cell.

Kurita *et al.* (1988) examined the selective and effective introduction of nonanoyl groups to the amino groups of chitosan by the acylation with the corresponding acyl chloride. The products show a highly swell particle according to the extent of acylation.

N-alkylation of chitosan is also attractive to give the product soluble in aqueous system. Nikalaev *et al.* (1985) reported hydroxyalkyl chitosan by using Eschweiler-Clarke reaction (Figure 2.2). Amino group is also occasionally used in terms of forming quaternary salt to purify the product or to attach the protecting group on the N-position (Nud'ga *et al.*, 1973).

Recently, Nishimura *et al.* (1991) reported N-phthaloylation of chitosan by using phthalic anhydride in N, N-dimethylformamide (Figure 2.3). The phthaloylchitosan exhibits the high solubility in common organic solvents and is expected for the biomedical materials including the artificial organs.

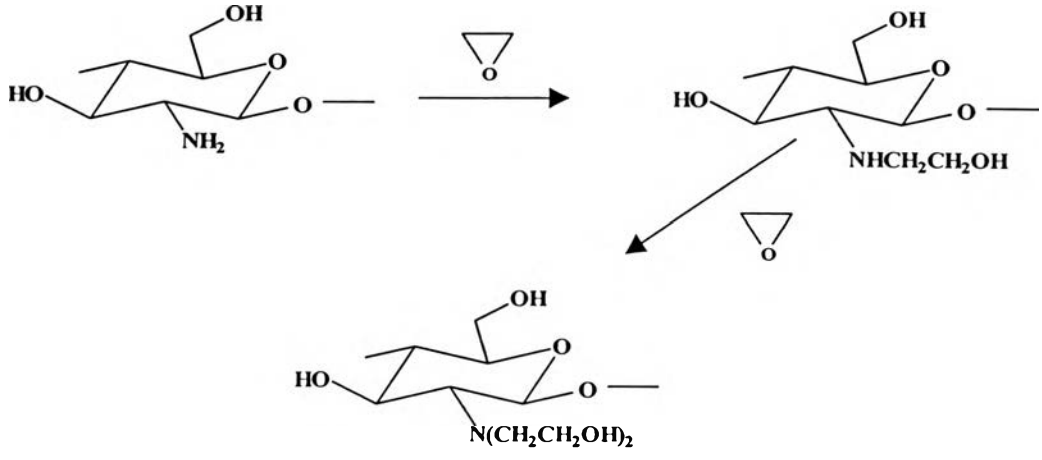


Figure 2.2 Alkylation of chitosan (Nikalaev *et al.*, 1985).

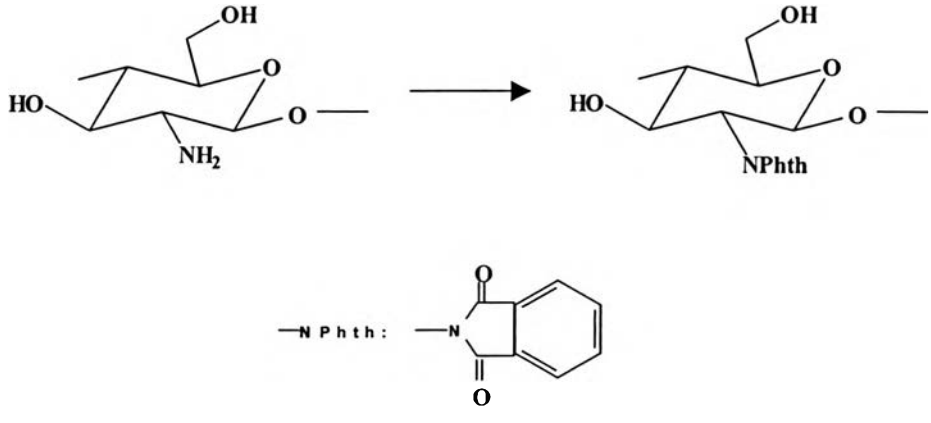


Figure 2.3 N-Phthaloylation of chitosan (Nishimura *et al.*, 1991).

2.2.2 Chemically Modification of Chitin-Chitosan at O-position

Similar to the N-position, the reaction of chitin with ethylene oxide at O-position gives hydroxyethyl chitin and shows the water solubility. The products receive much interest for practical *in vivo* applications in biomedical field. Ohya *et al.* (1995) proposed the preparation of CM-chitin conjugated with doxorubicins via tetrapeptide spacer groups. The *in vivo* and *in vitro* antitumor studies of these conjugates were found for cytotoxic activity.

Nishimura *et al.* (1991) reported the facile conversions of phthaloylchitosan into several 6-O-substituted derivatives by the reactions with bulky substituents such as triphenylmethyl(trityl) and (p-tosylsulfonyl)oxy (tosyloxy) groups under mild conditions in homogeneous solution. The products were reported to be the versatile intermediates, which permit the introduction of additional functional group regioselectively.

Sulfonation is expected for bioactivity as seen in the case of heparin. The treatment of chitin or chitosan with sulfuric acid or chlorosulfonic acid were reported to produce sulfonate chitin (Nagosawa *et al.*, 1971) or sulfonate chitosan (Cushing *et al.*, 1954), respectively.

Kurita *et al.* (1992) investigated the tosylation at O-position of chitin to prepare tosylchitin to be a reactive precursor. The tosylchitin performs a nucleophilic reaction, which can change to other derivatives including the high reactive iodochitin.

2.3 Chitin-Chitosan for Drug Delivery System

Owing to the high amount of the material, combining with the bioactivity, biocompatibility, biodegradability and non-toxicity including the capability for chemical modification, chitin-chitosan shows a potential to be a polymer drug with a controlled release system. Generally, two categories of chitin-chitosan for controlled release system are known, i.e., physical conjugation of drug into the matrix and chemical bonding of drug onto the chain.

2.3.1 Physical Conjugation System

Physical conjugation of drug with chitin-chitosan can be achieved either by blending the drug in the powder form or mixing the drug with the solution to prepare as a film, membrane, or bead. Thus, drug delivery system

of chitosan is dependent on the secondary forces between the drug and chitin-chitosan chain and the interaction with the surroundings.

Miyazaki *et al.* (1981) reported the use of chitin and chitosan as drug carriers by dispersing indomethacin and papaverine hydrochloride in chitosan gel to find the release at a constant rate (zero order). Sawayanagi *et al.* (1982) developed a sustained release system of a water-soluble drug using lactose/chitosan as a carrier in the form of a compressed tablet. Bodmeier *et al.* (1989) prepared chitosan beads containing sulfadiazine to find the release profiles is up to the pH and the temperature.

Teixeira *et al.* (1990) studied the assessment of chitosan gels for the controlled release of the herbicide atrazine and the fertilizer urea. Elution studies conducted on chitosan-coated atrazine beads showed an initial rapid release of atrazine followed by a constant release rate while that of chitosan-coated urea beads were found to be a sustain release system.

2.3.2 Chemical Conjugation System

Drug conjugation onto polymer by chemical bonding is known to be a method for producing a polymer drug with a control stability. The effective of drug, thus, is dependent on the bonding between drug and polymer chain while the stability of the drug is related to the stability of polymer chain itself. Up to now, various types of polymer drug delivery system have been proposed. Conceptually, in order to achieve a controlled release system, the polymer chain is designed to have a spacer group in between the drug molecules where the bond can be broken in certain conditions, such as pH, heat, and light. The practical drug for *in vivo* systems also requires the water-soluble property. Thus, most of the reported chemical conjugation drug is focused on carboxymethyl chitin, or O, N-carboxymethyl chitosan.

Ohya *et al.* (1992) reported the conjugation of 6-O-carboxymethyl chitin (CM-chitin) with 5-fluorouracil (5-FU) through pentamethylene and monomethylene spacer groups via amide and ester bonds. The obtained product is water-soluble and allows the release of 5-FU depending on the stability of the spacer groups. Onishi *et al.* (1995) synthesized the monoester of 5-fluorouridine with 4-carboxybutyric acid as a prodrug. The prodrug conjugated with chitosan showed the release of 5-fluorouridine related to the pH of the system.

2.4 Chitosan for Agricultural Purpose

It is noted that most reports on chitin-chitosan for drug delivery system are the polymer drug in medical or pharmaceutical application, while less attention has been paid on the agricultural applications.

Chitin-chitosan is reported to be a fertilizer and bioactive for plants (Hirano *et al.*, 1987). Struszczyk *et al.* (1989) reported the antivirus properties of chitosan for various virus in plants such as alfalfa mosaic, phaseoulus vulgaris, chenopodium amaranticolor.

2.5 The Potential of the Present Work

Up to now, less attention has been paid on chitosan with a toxic drug such as insecticide in either physical or chemical conjugation approach. It can be expected that the conjugation of toxic drug can reduce the amount of using in the real application together with the increase of stability of the drug to prolong the lifetime. The present work, thus, organizes the work on the concept of chemical modification of chitosan and conjugation toxic drug. The work also focuses on the controlled release system of drug and drug stability as expected from the molecular design of polymer drug.