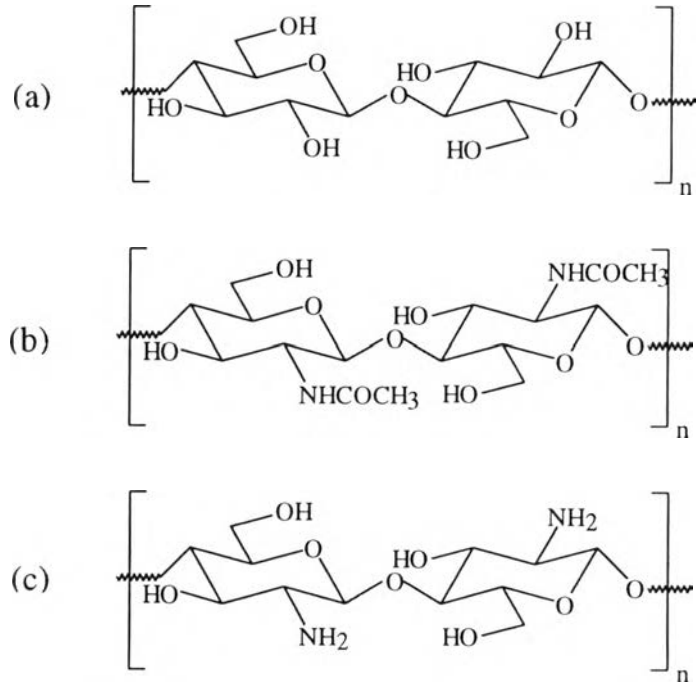
CHAPTER II
LITERATURE SURVEY

2.1 Chitin-Chitosan, Structure and the Unique Properties

Chitin-Chitosan has the same backbone with cellulose but hydroxy group at C-2 position is replaced by acetamide and amino groups in chitin unit and chitosan unit, respectively (Scheme 2.1).

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



Thus, chitin-chitosan, which is aminopolysaccharide, is superior to cellulose for functional group modification. The chemical structure of chitin-

chitosan is unique for the nitrogen belonging to acetamide or amino group at C-2 position in addition to primary alcohol at C-6 position.

Most research works carried out on chitin-chitosan concerns the amino group, which is of course the most important function of the macromolecules. 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 and reduction reaction.

2.2 Practical Research of Chitin-Chitosan

The major applications of chitosan were centered on sludge dewatering, food processing, and metal ion chelation. However, the promissing application is in the form of value-added products, such as cosmetics, drug carriers, feed additives, semipermeable membranes, and pharmaceutics. Recently, much attention has been paid on chitosan as a potential polysaccharide resource owing to its specific structure and properties.

Most practical researches of chitin-chitosan have been emphasized on physical modifications.

Uragami *et al.* (1983) prepared anion exchange membranes containing amino groups from chitosan, poly (vinyl alcohol), and glutaraldehyde. The membrane, insoluble in acidic and alkaline aqueous solution, was fixed in a diaphram, one side being alkaline and the other being acidic, for transportation of actively halogen ions.

Bodmeier *et al.* (1989) prepared sulfadiazine beads by dropping drug-containing solutions of the positively charged polysaccharide, chitosan, into tripolyphosphate (TPP) solutions. The droplets instantaneously formed gelled spheres by ionotropic gelation, entrapping the drug within a three-dimensional

network of the ionically linked polymer. The chitosan beads showed pH-dependent swelling and dissolution behavior. The beads swelled and dissolved in 0.1 N HCl, while they stayed intact in simulated intestinal fluid.

Thacharodi *et al.* (1995) prepared and characterized the composite membranes consisting of collagen and chitosan and studied on the permeability properties of this membrane for propranolol hydrochloride. This properties of composite membranes were found to depend on the concentration of collagen and chitosan in the membranes.

2.3 Limitations of Chitin-Chitosan and the Strategies to solve the Problems

By considering the chemical structure, chitin-chitosan is known to have high intermolecular- and intramolecular-hydrogen bonding with high molecular weight. As a result, chitin chitosan is limited in chemical modification owing to the low solubility and reactivity in most common organic solvents.

Chitin-chitosan chain degradation is the one way to improve many disadvantages as mentioned above. It is known that, polymer chain degradation can be obtained by many methods.

Sashiwa *et al.* (1993) indicated that most of deamination products of various partially deacetylated-chitin (DA-chitin) derivatives prepared under either heterogeneous or homogeneous conditions were oligomers of less than six residues.

Allan *et al.* (1997) and Varum *et al.* (1994) reported the depolymerization of chitosan by the action of HONO for producing chito-oligosaccharides at a desired molecular size. The amine groups on chitosan perform the reaction, but not the N-acetyl moieties, the β-glucosidic linkages

are cleaved. A 2,5-anhydro-D-mannose unit is formed at the reducing end of the cleaved polymer.

Chen *et al.* (1997) studied on the ultrasonic conditions involved with the parameters of chitosan concentration, reaction temperature, type of solvent, and ultrasonic time. The result shows that chitosan was degraded highly in dilute solutions and in lower temperature solutions. Degradation increased with prolonged ultrasonic time, and during storage in an acidic solution at ambient temperatures.

2.4 γ-Radiation for Oligochitosan

An energy saving, environmentally friendly, and effective method to produce oligochitosan has been expected for years. γ-Radiation is an effective way to treat a bulk of chitin-chitosan at one time. However, since γ-radiation gives the chain degradation as well as crosslinking of polymer, the condition for each reaction and the structure of the obtained polymer are the main problems to be studied.

2.4.1 The Condition on γ-Radiation for Oligochitosan

The studies on the effects of high energy exposed to chitosan had not been done only in the case of γ-radiation but also other energy sources. Ionizing-radiation-induced changes in high-performance polymers are of continuing interest because of the potential utility of chitosan in many fields. Polymer under long-term exposured to ionizing radiation will be degraded. Depending on the radiation environment conditions, particularly the temperature and the radiation dose, they typically undergo chain scission, sometimes with simultaneous crosslinking, leading to progressive reduction in molecular weight.

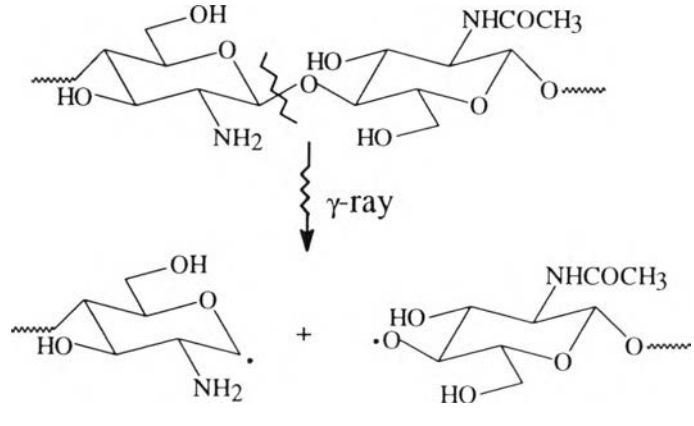
The condition of γ-radiation, i.e., the amount or the dose of γ-ray, the state (liquid or solid state) of chitin-chitosan raw materials to be irradiated were discussed by many researchers.

Aiba *et al.* (1988) studied on the effect of γ-ray irradiation on chitin and chitosan. They found that the irradiation on the wet state of chitosan, i.e., aqueous solution or water-swollen film, the molecular weight decrease 10 times with dose 1.0 Mrad whereas irradiation in the dry state shows the decrease in viscosity 13.4 times with dose 1.0 Mrad.

Ulanski and Rosiak (1992) examined radiation-induced changes in chitosan irradiated in solid state and in aqueous solution. Radiation yields of the scission in solid state are 0.9 mol/J in vacuum, 1.1 mol/J in air, and 1.3 mol/J in oxygen while the corresponding yields of crosslinking are equal to zero. They found that molecular weight decrease as an increasing the amount of γ-ray.

Lim *et al.* (1997) indicated the amount of γ-ray (doses) up to 25 kGy caused main chain scission as shown in Scheme 2.2. The viscosity average molecular weight of the polymer decreased with increasing irradiation dose, the radiation yields of scission being 1.16 mol/J in air and 1.53 mol/J in anoxia.

Scheme 2.2 Mechanism of main chain scission



2.4.2 Structural Changing of Oligochitosan after γ-Radiation

The changes in structure of irradiated chitosan have been studied by many researchers.

Ulanski *et al.* (1992) examined the changes of chitosan by UV spectrum after irradiation with various doses. The increase in an absorbance at 247 and 290 nm are probably due to the formation of carbonyl and carboxyl groups. These changes are most pronounced for samples irradiated in oxygen.

Wenwei *et al.* (1993) indicated that $-NH_2$ group is more sensitive to γ-irradiation than $-NHCOCH_3$ group. The hydroxyl group was observed to be increased with increasing radiation dose while C-O-C group decreased.

2.5 Chemical Modification of Oligochitosan

Since the reactive amino as well as primary and secondary hydroxyl groups in chitin-chitosan are readily available for chemical reactions, much emphasis has been paid on the chemical modifications of chitin-chitosan in developing new derivatives from the viewpoint of the high potential of the amino polysaccharide. In many cases, the chemically modified chitin-chitosan shows unique properties than the original polymer.

Chitin-chitosan can react with other reactive functional group such as carboxylic acid, acid chloride, and alkyl halide. These chemical modifications of amino and hydroxyl groups in the molecule give rise to novel molecular and biological functions. Chitin-chitosan can undergo etherification, esterification, cross-linking, and graft copolymerization reactions (Aiba *et al.*, 1985). It should be noted that the reactions of chitin-chitosan face the problem of dissolvation in organic system. In some cases, the heterogeneous reaction has to be operated (Kurita *et al.*, 1992).

2.5.1 Chemical Modification on Hydroxyl Groups

There are two hydroxyl groups, primary alcohols at C-6 position and secondary alcohols at C-3 position, on the pyranose ring. Alcohols are versatile starting materials for the preparation of alkyl halides, alkenes, carbonyl compounds, ethers, and etc. The regioselective modification of chitosan derivatives shows much improved properties. The primary hydroxyl group is more reactive than the secondary hydroxyl groups. Up to now, most reactions will be, thus, paid on hydroxyl groups at C-6 position.

Nishimura *et al.* (1991) reported the facile conversions of phthaloylchitosan into several 6-*O*-substituted derivatives were carried out by the reactions 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.

Kurita *et al.* (1992) investigated the tosylation of chitin at hydroxyl group to prepare tosylchitin as a reactive precursor for facile chemical modification. The obtained tosylchitin efficiently underwent a nucleophilic reaction, which could change to other derivatives including the high reactive iodochitin. Furthermore, iodochitin was evaluated as a precursor for graft copolymerization of styrene both by cationic and a free-radical mechanisms. Though the grafting percentages were not high in the radical graft copolymerization, a small amount of homopolystyrene could be achieved. The resulting chitin-graft-polystyrene was proven to be more soluble or swellable in organic solvents.

2.5.2 Chemical Modification on Amino Group

Basically, acetamide group of chitin is known to change to amino group, by deacetylation with alkali solution (Morton *et al.*, 1965) to produce

the reactive chitosan. Owing to the reactive amino group, chitosan has received much more attention in chemical modification than chitin.

Amino groups on chitosan chain, which is reactive primary amine, can act as a nucleophile, since it contains nitrogen atom that bears an unshared pair of electrons. There are many researchers studied on the reaction of this reactive groups.

N-Acylation (Fujii *et al.*, 1980, and Kurita *et al.*, 1988) is known to overcome the intermolecular hydrogen bonding which arises from the close packing of the polymer chains. Moore *et al.* (1981) reported this reaction would disrupt the interchain packing and making the hydroxyl groups more accessible. Hirano *et al.* (1976) prepared *N*-acylation of chitosan both in aqueous, methanolic acetic acid and in aqueous solution of fatty acid. They found that the presence of both *N*- and *O*- acyl groups were evident in prepared using 10% acetic acid as solvent. The acylchitosans from the higher fatty acids exhibit IR absorptions for *N*-acyl but not for *O*-acyl groups.

Kurita *et al.* (1982) proposed the preparation of *N*-phthaloylated water-soluble chitin in DMSO. The phthaloyl group is effective to impart solubility owing to the bulky nature, and complete removal of *N*-attached hydrogens that form intermolecular hydrogen bonds. The *N*-phthaloylated chitosan shows high solubility in organic solvents.

Chitosan was reported to react with phthaldehyde in aqueous acetic acid-methanol at room temperature to give the schiff's base derivative in gel form (Hirano *et al.*, 1983). These derivatives have been used as an intermediate for the chemical modification of chitosan.

Recently, Nishimura *et al.* (1991) reported *N*-phthaloylation of chitosan by using phthalic anhydride in *N,N*-dimethylformamide (DMF) at 130°C. The resulting phthaloylchitosan exhibited much improved solubility in common organic solvents. *N*-Phthaloylchitosan can also act as starting material for several 6-*O*-substituted derivatives. The preparative procedures

based on *N*-phthaloyl-chitosan are useful for regioselective and quantitative introduction of substituents.

After desired modification reactions, the phthaloyl groups are easily removed with hydrazine to regenerate the free amino groups.

2.5.3 Limitations of Chemical Modification on Chitin-Chitosan

The modification of chitin-chitosan is generally difficult owing to the problem of material solubility in most solvents. Many efforts have thus been focused on the development of facile modification reactions to prepare derivatives with well-defined structures. Recently, the chemical modification of chitin-chitosan has received much attention not only for improving the low solubility but also for achieving some expected novel properties that cannot be found in the natural chitin-chitosan. Of various attempts, to develop solubility of this rigid intractable polysaccharide, destruction of the crystalline structure by the addition of the functional groups or highly reactive groups to the main chain in the structure as a concept of functional polymer has been proposed.

2.6 The Scope of the Present Work

Up to now, chitin-chitosan has received much attention owing to its specific properties of biodegradability, biocompatibility, bioactivity, and non-toxicity including the possibility for physical and chemical modifications. Owing to these properties and its structures as well as the cost performance, it is attractive and on the expectation for various fields of applications.

Owing to the lack of solubility, most practical utilizations of chitin-chitosan are mainly focused on physical modification. To overcome this problem, the combination of two technologies, γ-irradiation and chemical modification become an alternative way. The idea of the present work is to

prepare chitosan derivatives for medical purposes based on oligo chain of chitosan. Here, the oligochitosan is concerned to be achieved by γ-radiation. Point of interest is also cover on the molecular weight of chitosan as a function of its solubility and the amount of γ-ray. The successful of the project will lead to an understanding on the structure or morphology of chitosan and oligochitosan. In order to achieve the reactive oligochitosan precursors, the present work will be extended to the chemical modification of oligochitosan.