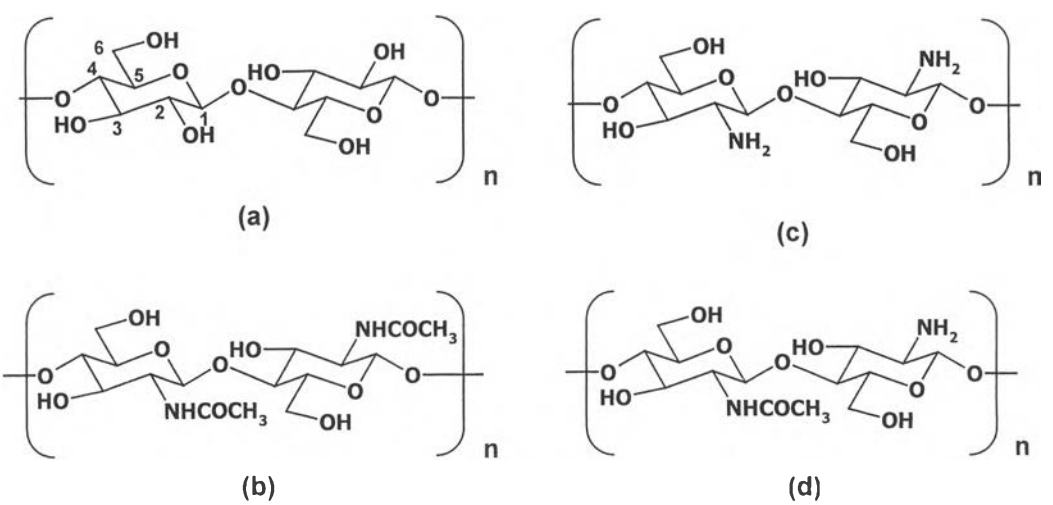
CHAPTER I
INTRODUCTION

1.1 Chitin-Chitosan: the Specific Structure and Unique Properties

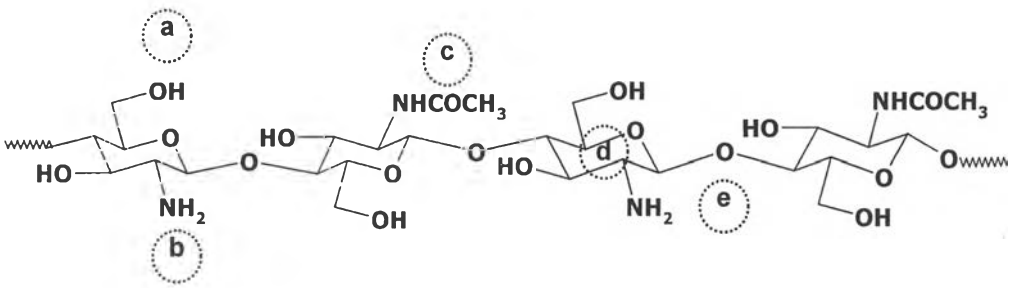
Chitin-chitosan is the second most naturally abundance polysaccharide, next to cellulose, obtained from crustaceans, insects and fungi. The structure of chitin-chitosan is similar to cellulose (Scheme 1(a)), but hydroxyl groups at C-2 are replaced by acetamide groups for chitin units (Scheme 1(b)) and amino groups for chitosan units (Scheme 1(c)). Naturally, chitin-chitosan presents as a high molecular weight copolymer of β-(1-4)-2-acetamido-2-deoxy-β-D-glucose and β-(1-4)-2-amino-2-deoxy-β-D-glucose (Scheme 1(d)).

Chitin-chitosan is classified as an aminopolysaccharide, which the amino group provides unique properties such as metal ion chelating, protonation to cationic species, including the variety of functionalizations. The well accepted applications, thus, are chelating agents (Sakaguchi *et al.*, 1981), high performance adsorbents (Kurita *et al.*, 1988b), ion exchange membranes (Pellegrino *et al.*, 1990), etc.

Scheme 1. Chemical structures of (a) cellulose, (b) chitin, (c) chitosan, and (d) chitin-chitosan copolymer



Scheme 2. Properties of chitin-chitosan relating to the structure



Here, the attractive properties relating to chemical structure are summarized as follows (Scheme 2).

a: Hydroxyl group. Chitin-chitosan has two types of hydroxyl groups, which are primary alcohol group at C-6 and secondary hydroxyl group at C-3. The primary hydroxyl group is more reactive than the secondary hydroxyl group; therefore, most chemical reaction will take place at C-6. These hydroxyl groups impart hydrophilicity to chitosan chains and show the inclusion properties (Shimizu *et al.*, 1995 and Shan-Yang and Ren-Ing, 1992). It can be, thus, formed inclusion and/or host-guest compound with ions or molecules, which is appropriate for industrial wastewater treatment. The lone pair electrons of oxygen atom also forms complex with metal ions, i.e., Ca^{2+}, Ni^{2+}, etc. (Nishi *et al.*, 1987). Chitin-chitosan possesses antimicrobial properties (Suzuki *et al.*, 1986) as the lone pair electrons of oxygen atom initiate the microorganism destruction.

b: Amino group. Comparing to cellulose, chitin-chitosan can be chemically modified, since it has the reactive primary amino groups, which are more reactive than hydroxyl groups. Moreover, a lone pair electron of nitrogen atom tends to form bond with ions and metals. This brings the application for wastewater treatment as ion and/or metal absorbers. The use as a coagulant is also effective since amino group interacts with inorganic species, soil, mud, etc. to accelerate the precipitation (Peniston and Johnson, U.S. Patent). It is important to note that amino group can be protonated in the presence of proton species providing positively charged polymer ($-NH_3^+$, cationic polymer). The antibacterial and antiviral of chitin-chitosan are induced from the formation of ionic bond between positively charged amino group and negative charge of microorganism cell wall, as a result the

growth inhibition of bacteria and virus is achieved (Kendra and Hadwiger,1984).

c: Acetamide group. The functions of acetamide group are mostly similar to those of amino group, but acetamide group is hardly chemically modified. The acetamide group forms strong hydrogen bond network leading to high crystallinity for chitin. The acetamide group and the induced strong hydrogen bond also bring chitin to show poor solubility in almost all solvents.

d: Pyranose ring. Chitin-chitosan consists of pyranose ring either N-acetyl-D-glucosamine or D-glucosamine, which are reported for the detoxification and the combining with fatty acid for lowering cholesterol. Chitin-chitosan oligomer activates the growth of tissue and functions as a fibroblast for reconstruct collagen leading to the effective wound recovery. The activity of chitin-chitosan, a bio-essence saccharide unit, imparts biocompatibility (Richardson *et al.*, 1999 and Risbud and Bhonde, 2000), bioactivity (Dumitriu *et al.*, 1989 and Matsuhashi and Kume, 1997) and non-toxicity (Chandy and Sharma, 1992) under the structure of pyranose ring with N-acetyl-D-glucosamine or D-glucosamine.

e: Glycoside linkage. Glycoside linkage or glucosidic bond (C-O-C) provides biodegradability (Yamamoto and Amaike, 1997 and Tomihata and Ikada, 1997) via enzymatic hydrolysis, i.e., chitinase, chitosanase, and lyzozyme, which presents in nature leading to chain degradation.

1.2 Basic Research of Chitin-Chitosan for Practical Applications

Up to now, much attention has been paid on chitin-chitosan as a potential polysaccharide resource on accounting of its specific structure and unique properties, but most basic ideas are based on physical modifications, such as film and membrane formations, bead preparations, gel productions, fiber extrusions, etc. Generally, the preparations of beads and gels are obtained from partially crosslinking with dialdehyde. In this case, aldehyde reacts with both hydroxyl and amino groups of chitin-chitosan to form ether linkage or Schiff base unit. The degree of crosslinking can be easily verified by controlling the concentration of dialdehyde. Here, the flexibility of matrices can be manipulated by the chain length of aldehyde and degree of crosslinking. Among the dialdehydes, glutaraldehyde is mostly used since it is

water-soluble and give crosslinking in aqueous solution under mild condition. The excess amount of glutaraldehyde can be excluded easily by simply washing or soaking in water before use. However, the use of aldehyde faces some limitations such as (i) aldehyde is toxic agent and the complete removal of the excess is difficult and (ii) the crosslinking reaction and/or degree of crosslinking is hard to control. Yao *et al.* (1994) proposed the preparation of the hydrogels composed of the crosslinked chitosan with glutaraldehyde and polyether N330 for controlled release of chlorhexidini acetas. The hydrogels was prepared by mixing polyether and glutaraldehyde with chitosan solution dissolved in acetic acid. The release of chlorhexidini acetas from the semi-IPN (interpenetrating polymer network) depends on the pH of solution.

Ionotropic gelation for the formation of chitosan bead and membrane is a mild process. Chitosan beads/membranes form with a variety of counterions or polymers, such as pyrophosphate, octylsulfate, and alginate. Bodmeier *et al.* (1989a and 1989b) proposed gel-like beads containing sulfadiazine drug prepared by dropping drug-containing solutions of the positively charged chitosan into tripolyphosphate solution. The droplets instantaneously formed gel-like spheres by ionotropic gelation entrapping the drug within a three-dimensional network. 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.

In addition, gel formation can be successfully prepared by using high molecular weight chitin under the control of concentration and temperature. Bianchi *et al.* (1997) prepared thermoreversible chitin gels by heating the solution of chitin in dimethylacetamide (DMAc) containing 5% of LiCl at $T > 90°C$. The sol-gel transition is thermoreversible depends on the concentration and the molecular weight.

Chitosan membranes/films may be prepared in various ways: evaporation of chitosan solvents, crosslinking with bifunctional reagents, chelating with anionic counterions, or complexing with polymers and proteins. The direct evaporation of a chitosan solution spreading on a glass plate is the simplest technique for the preparation of chitosan films and generally produces a water-soluble type. The

unique multi-layer film formations were successfully prepared from the evaporation and casting layer by layer. Kanke *et al.* (1989) proposed three types of chitosan films containing prednisolone (PD) for controlled release studies. The films were; (i) a monolayer type (ML) film prepared by evaporating the solvent from a chitosan/drug mixture, (ii) a double layer type (DL) film prepared by sticking two ML films together (one ML film contained a drug), and (iii) N-Ac film prepared by the same way as DL film but the first ML film contained drug and the other was N-acetylated. The release of PD was retarded as the films become thicker. The release of the drug from N-Ac films was more depressed than that from DL films.

Cross-linked chitosan membranes can be prepared by the addition of bifunctional reagents, such as aldehydes, carboxylic anhydrides, and glutaraldehyde, to the chitosan solution. Uragami *et al.* (1983) proposed crosslinked chitosan membrane for transportation of active halogen ions. The anionic exchange membranes were prepared from amino groups of chitosan, poly(vinyl alcohol), and glutaraldehyde. The membrane, insoluble in aqueous acidic and alkali solution, was fixed in a diaphragm cell to transport halogen ions from the acidic side to alkali side against the concentration gradient, pH difference, and electric potential difference between both sides of the membrane.

1.3 Advanced Research of Chitin-Chitosan for Value added Applications

For decades, many researches about chitosan have been actively done; however, the practical uses of chitosan products were hardly seen in the daily life. This may be due to the raw material consistency or quality control including the cost performance. In recent years, chitosan for advanced applications received much attention as it is an alternative way to produce value added product in which the price and amount invested for quality control are not the problem. Many researchers reported on the structural modification of chitosan at molecular level to propose novel products.

The self-assembly of chemically modified chitosan into nanoparticles has been investigated for the delivery of macromolecules. Lee *et al.* (1998a and 1998b) designed a new carrier for DNA delivery by conjugating chitosan with deoxycholic

acid (hydrophobic moiety). The amphiphilic macromolecule formed self-assemblies of self-aggregates after sonication to associate with DNA and transfer *in vitro*.

Ohya *et al.* (1999) conjugated PEG to soluble chitosan via an amide linkage. The conjugated product formed self-aggregates at basic pH and could entrap insulin due to electrostatic interactions between the unconjugated chitosan and the anionic residues of the protein. The release rate of insulin was controlled by the degree of PEG substitution: the higher the PEG substitution, the more rapid release of insulin.

Calvo *et al.* (1997 and 1998) and Fernandez-Urrusuno *et al.* (1999) modified the surface of chitosan nanoparticles by coating poly(ethylene glycol) to the surface of pre-formed nanoparticles via covalent amide bonds between the free amine group of nanoparticles and methoxyPEG. These chitosan nanoparticles showed the effective association and protein delivery such as bovine serum albumin (BSA), tetanus toxoid, diphtheria toxoid, and the peptide insulin.

1.4 Limitations and Strategies to Overcome

As can be analyzed from the chemical structure, chitin-chitosan exhibits high crystallinity through inter- and intramolecular hydrogen bond network. Combining with the high molecular weight developed naturally, chitin-chitosan faces the weak points about solubility and reactivity. However, chitosan is much more attractive than chitin for chemists to propose novel derivatives owing to its reactive amino group. In research laboratories, chitosan derivatives are reported for the potential uses in pharmaceutical (Bodmeier *et al.*, 1989a and 1989b), biotechnological (Tsigos *et al.*, 2000 and Damodaran, 1996), agricultural (Bittelli *et al.*, 2001), and food processing (Suntornsuk *et al.*, 2002) fields. Most chemical reactions of chitosan have to be done in heterogeneous system (Kurita *et al.*, 1992) or in some specific acid solutions (Hirano *et al.*, 1976), which are difficult to control or repeat. Therefore, most chitin-chitosan products available in the market are mainly obtained from the simple physical modifications, such as film formation (Xu *et al.*, 1996), membrane casting (Hirano, 1978), gel formation (Bianchi *et al.*, 1997), powder blending (Sawayanagi *et al.*, 1982), fiber extrusion (Tokura *et al.*, 1979), and

bead preparation (Bodmeier *et al.*, 1989b and Sezer and Akbuga, 1995), to produce the products used in basic applications, for example, water treatment agents (Huang *et al.*, 2000 and Selmer-Olsen *et al.*, 1996), papermaking additives (Muzzarelli, 1983), cosmetics ingredients (Kurita *et al.*, 1992), etc. As a result, the up-to-date research of chitin-chitosan is about the approach for advanced applications to achieve value added products.

The structural modification of chitin-chitosan at molecular level is the way to overcome the limitations and develop the products to be more valuable. There are two points to be considered for achieving chitin-chitosan modified at molecular level: (i) the designs of reaction for organic solvent and/or aqueous soluble derivatives for the effective reaction in the following step and (ii) the designs of the process for lowering molecular weight of chitosan to enhance the solubility.

1.4.1 Chemical Modification to Improve Chitosan Solubility

The conjugation of bulky group (sometimes, as a protecting group) onto chitosan chain at either amino or hydroxyl groups is a chemical modification pathway to improve the solubility. In this way, the hydrogen bonds will be obstructed; as a result, the solubility in organic solvents can be achieved. Nishimura *et al.* (1991) proposed N-phthaloylation of chitosan as an intermediate step. After the bulky phthalimido group is introduced to chitosan chain, the N-phthaloylchitosan dissolves in some organic solvents, i.e., *N,N*-dimethylfomamide (DMF), dimethyl sulfoxide (DMSO), dimethylacetamide (DMAc), and pyridine. The further chemical reactions of this compound are homogeneous whereas the deprotection of N-phthalimido group may be carried out at final.

Fujii *et al.* (1980) illustrated that the derivative achieved from the complete N,O-polyacylation of chitosan with an excess of long-chain acid chloride can be soluble in some organic solvents such as pyridine, DMAc, and DMSO.

Although there are many derivatives proposed for the organosoluble chitosan, the reaction faces the problems of low reproducibility, multi-step reaction, purification, and deprotection. For this reason, the conjugation of bulky group to enhance the solubility is not quite practical for industrial scale.

1.4.2 Chain Degradation to Improve Chitosan Solubility

For the past few years, several efforts for low molecular weight chitosan (LMWC) and/or oligochitosan have been done, for example, acid or base hydrolysis (Allan and Peyron, 1995a and 1995b and Domard and Cartier, 1989), enzymatic degradation (Nordtveit *et al.*, 1994 and Aiba, 1994a and 1994b), and photoirradiation (Andrady *et al.*, 1996, Lim *et al.*, 1998, Wenwei *et al.*, 1993, and Domard and Cartier, 1989). Chemical treatment is an easy process with low cost performance, but chemical waste and reproducibility are the main problems. Enzymatic hydrolysis is an effective way to achieve specific cleavage to obtain oligochitosan. However, it requires multi-steps, especially, enzyme preparation and purification. This brings a hesitation for large-scale production. Photoirradiation is considered as a specific technique in terms of the operating system and the capital cost, but it is attractive since it is an easy process with a large-scale production in a single step without waste generation and no product purification required.

In the case of chemical treatment, examples are as follows. Allan and Peyron (1995a and 1995b) proposed the depolymerization of chitosan by nitrous acid (HONO). The rate of depolymerization was rather dependent on the degree of deacetylation of chitosan than the molecular weight. Nitrosating species attacked the amine groups, but not the *N*-acetyl moieties, and subsequently cleaved the β-glycosidic linkages. A mole of HONO was consumed for the reaction with a mole of amine group reacted, and 2,5-anhydro-D-mannose unit was formed at the reducing end of the polymer. This deamination was carried out under homogeneous system with selective, rapid, and easily controlled reaction without side reactions.

Lee *et al.* (1999) studied on the hydrolysis of chitosan with hydrochloric acid (HCl) and the optimum conditions to recover chitosan oligomers. Chitosan was hydrolysed with 10 volumes of 35% HCl (ml of HCl /g of chitosan) for 2 h at 80°C. The mixture was diluted with an equal amount of water and then stored at –20°C for 2 days to obtain the mainly precipitated oligomer with DP 5-7. Belamie *et al.* (1997) studied on the chemical depolymerization of chitosan in solid state by

means of gaseous HCl. This method avoided the complicated purification steps, such as isolation of the products from acidic solutions.

To enhance acid hydrolysis, recently, Chen *et al.* (1997) proposed the pre-treatment with ultrasonic following by acid hydrolysis. It was concluded that the degradation increased with sonication time and the chain scission was significant even in dilute solutions storing in low temperature.

Hsu *et al.* (2002) studied on the free radical degradation of chitosan with potassium persulfate (KPS) to find that the solution viscosity and the molecular weight of chitosan decreased in a very short time after adding KPS into chitosan solution at 70°C. When KPS is thermally dissociated into radicals, the radicals are the cationic amino group on chitosan. Subsequently, the radical attacks the C-4 carbon to result in the breakage of the glycosidic bonds (C-O-C).

Kabalnova *et al.* (2001) studied on the oxidative destruction of chitosan affected by ozone and hydrogen peroxide. Destruction of chitosan by hydrogen peroxide leads to the formation of oligosaccharides in contrast to ozonolysis. Hydrogen peroxide oxidizes functional groups of chitosan only under tough conditions.

Owing to biodegradability, chitin-chitosan can be degraded by natural occurring enzymes such as chitinase, chitosanase, and lysozyme. Lysozyme and chitinase are clarified for chitin hydrolyzation to produce $(GlcNAc)_n$. The hydrolysis rate is low because of the heterogeneous conditions where the oligomers (less than 6 repeat units) are not obtained in good yield. Lysozyme hydrolyses partially N-acetylated chitosans (PNACs) under homogeneous conditions (Aiba, 1994a and 1994b). The function of digestibility to PNACs increases with an increase in the degree of N-acetylation of PNACs because lysozyme recognizes a GlcNAc sequence with more than three residues (Nordtveit *et al.*, 1994). Chitinase recognizes a single GlcNAc residue in chitosan (Aiba, 1993). The hydrolysis rate by chitinase depends on the N-acetylation group of chitosan.

Photoirradiation can be achieved by the high-energy electron such as UV light, X-ray, or γ-ray. Generally, photoirradiation gives chain scission and crosslinking, after exposure to high-energy electron. For biopolymer, chain scission

is mainly occurred since C-O-C is weak bond. Andrady *et al.* (1996) studied on UV irradiation onto chitosan to find that acetamide groups were cleaved and changed to amino side groups when using low energy ($\lambda > 360$nm), while glucosidic linkages were broken at high energy level ($\lambda < 360$nm) to form carbonyl at the chain end. In addition, the decrease of molecular weight was directly propotional to the energy used.

Although many of chitosan degradation methods have been proposed, most are related to the effectiveness of the conditions to degrade chitosan. The uses of the obtained low molecular weight chitosan for further chemical modifications are rarely reported.

1.5 γ-Ray Irradiation of Chitin-Chitosan

The γ-ray irradiation process can be carried out in both dry and wet states. There are many researchers studied on the effect of γ-ray on chitosan properties such as the decreasing of molecular weight in various conditions and the formation of new functional groups.

Aiba and Izume (1988) studied on the γ-ray irradiation on the wet state of chitosan, i.e., aqueous solution or water-swollen film. The molecular weight was found to decrease for 10 times with dose 1.0 Mrad for wet state whereas irradiation for dry state showed the decrease in viscosity 13.4 times with the same dose.

Ulanski and Rosiak (1992) examined the changes of chitosan structure induced by radiation. It was concluded that the radiation yielded the chain scission in solid state at 0.9 mol/J in vacuum, 1.1 mol/J in air, and 1.3 mol/J in oxygen while the corresponding yielded crosslinking was equal to zero. The studies also clarified that the molecular weight decreased and the carbonyl and carboxyl groups were formed as the γ-ray dose increased.

Wenwei *et al.* (1993) reported that the hydroxyl group increased with an increasing in radiation dose while C-O-C bond was cleaved. Kume and Takehisa (1982) investigated the change in physico-chemical properties of chitosan in dilute solution and in dry state by γ-ray irradiation. In the case of dilute solution,

molecular weight, viscosity and surface charge of chitosan, which are important factors on coagulation property for protein suspension, were decreased as irradiation increased. On the other hand, chitosan was stable for irradiation in dry state.

Lim *et al.* (1998) applied γ-irradiation for sterilizing chitosan fibers and films before use as a material in biomedical applications. The sterilizing dose of up to 25 kGy caused main chain scissions leading to the reduction in viscosity. The radiation yielded the chain scission being 1.16 in air and 1.53 in anoxia (a negative pressure of 100 kPa). Irradiation in the air improved the tensile strength of chitosan films, while T_g decreased.

Though γ-ray irradiation on chitosan has been reported for decades, the γ-ray irradiation of chitosan in the solution of initiator including the comparative studies under various conditions both dry and wet states either heterogeneous or homogeneous solution was not proposed. In addition, the clarification of structural changing of irradiated products and its mechanisms were not verified.

1.6 Chemical Modification of Chitosan

In order to develop chitin-chitosan by means of chemical modification, it is known that chitosan is more practical than chitin owing to the reactive amino groups. On the viewpoint of organic chemistry, chitosan can be considered as a nucleophile species to react with other reactive functional groups such as carboxylic acid, acid chloride, and alkyl halide. As shown in Scheme 3, a series of chitin-chitosan derivatives were proposed via etherification, esterification, cross-linking, and graft copolymerization. It is important to note that the reactions of chitin-chitosan face the problem of dissolvation in organic system. In most cases, the reactions are carried out in heterogeneous system.

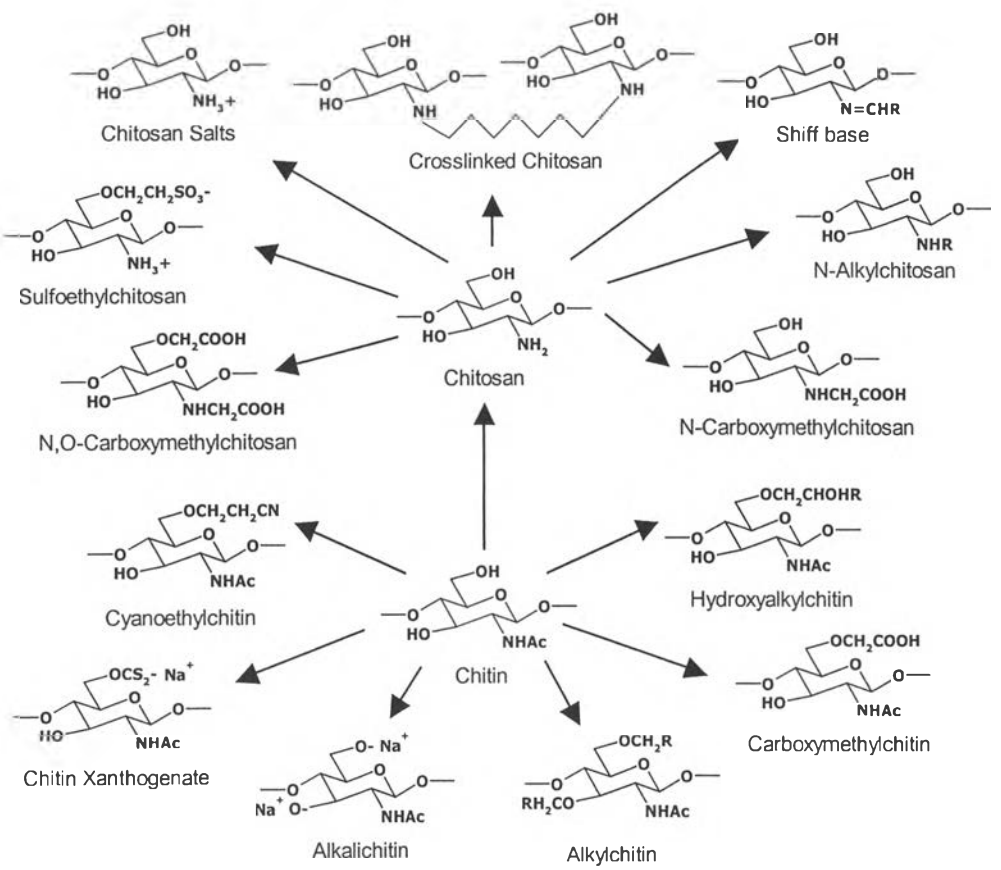
1.6.1 Chemical Modification at Amino Group

The amino group on chitosan, which is primary amine, can act as a nucleophile due to the nitrogen atom with an unshared electron pair.

1. Increasing amino group by deacetylation

Up to now, a series of studies on deacetylation of chitin have been succeeded. Acetamide groups in chitin units are changed to amino groups by deacetylation with alkali solution (Horton and Lineback, 1965). Hirano *et al.* (1976) reported a mild procedure for the selective N-acylation of chitosan by treating the solution in aqueous or methanolic acetic acid with carboxylic anhydrides at room temperature. N-acylchitosans are interesting in terms of the selective aggregation of some cancer cells (Williamson *et al.*, 1964).

Scheme 3. Chemical derivatives of chitin and chitosan



Kurita *et al.* (1988a) studied on the chitosan N-acetylation in a swollen state with acetyl chloride and acetic anhydride to find that under mild conditions, acetic anhydride is generally more appropriate in protic solvents, whereas acetyl chloride is effective and advantageous especially in pyridine containing only small amounts of

protic solvents.

Sashiwa and Shigemas (1999) prepared N-acylated partially deacetylated chitin (DAC-88) derivatives via ring-opening reactions with various cyclic acid anhydrides in aqueous MeOH system. N-Alkylation of DAC-88 was also performed in aqueous MeOH with various aldehydes, monosaccharides, and disaccharides. These two products showed the water-soluble property at various pHs.

2. Increasing the solubility by conjugating bulky group at C-2 amino group

Nishimura *et al.* (1991) prepared N-phthaloylchitosan from the reaction of chitosan with phthalic anhydride in DMF at 130°C. The removal of two hydrogen atoms of amino groups by the introduction of phthalimido groups induced the destruction of inherent crystalline structure resulting in the improvement of solubility. The N-phthaloylchitosan, thus, exhibited much improve in solubility in some organic solvents. The product can be applied as a key starting material for preparation of several O-substituted derivatives.

3. Host-guest derivatization by conjugating crown ether onto amino group

Yang *et al.* (2000) synthesized azacrown ether chitosan derivatives by reacting aryl mesocyclic diamine with the C-6 hydroxyl group (CTS-OC) or C-2 amino group (CTS-NC) on chitosan chain. The CTS-OC was obtained by protecting amino group on chitosan with benzaldehyde to form N-benzylidene chitosan. After the reaction, the shiff base was removed by reacting with a diluted ethanol hydrochloride solution. The selectivity properties of CTS-OC were better than those of CTS-NC for Pb^{2+}, Cd^{2+}, and Hg^{2+}.

1.6.2 Chemical Modification at Hydroxyl Groups

There are two kinds of hydroxyl groups on pyranose ring, which are primary alcohol at C-6 and secondary alcohol at C-3. Alcohols are versatile starting materials for the preparation of alkyl halides, alkenes, carbonyl compounds, ethers, etc. Most reactions have been paid on the hydroxyl group at C-6, since it is more reactive than the one at C-3.

1. Carboxylation at C-6

Horton and Just (1973) proposed C-6 carboxylation of chitosan perchlorate salt using chromium trioxide in acetic acid. The product obtained was N-sulfated with chlorosulfonic acid in pyridine to yield a (1→4)-2-sulfoamino-β-D-glucopyranuronan, which displays moderate blood-anticoagulant activity.

2. Iodo-chitin: an intermediate for graft copolymerization with polystyrene

Kurita *et al.* (1992) synthesized iodo-chitin via tosylation and iodination of chitin, respectively. The tosylation proceeded by the interfacial reaction between the two immiscible solutions of an aqueous alkali chitin solution and a chloroform solution of tosyl chloride. The tosyl derivative was converted to iodo-chitins by reacting with sodium iodide. The iodination brought the successful graft copolymerization of styrene.

3. Acetylation at C-6 and C-3

Nishimura *et al.* (1991) studied on the facile conversions of N-phthaloylchitosan into several O-substituted derivatives. Two derivatives of 6-O–substitution carried out by reacting with chlorotriphenylmethane and *p*-tolylsulfonyl chloride in homogeneous solution under mild conditions. In addition, subsequent 3-O-acetylation of the secondary hydroxyl groups gave rise to regioselectively modified chitosan to show better solubility.

1.7 Application of Chitin-Chitosan as a Material for Drug Delivery System (DDS)

For many decades, chitin-chitosan has been reported to be materials suitable for applications in pharmaceutical, biomedical, and agricultural areas. Drug delivery system (DDS) is an advanced and value-added application for most biodegradable polymers, which are naturally excreted directly from the body via the kidneys and/or digested by microorganism in the environment. The DDS offers numerous advantages compared to conventional dosage forms such as increasing the therapeutic activity, reducing the toxicity and the number of drug administrations required during treatment, including the convenient treatment. Before making use of these polymers, drug will be, thus, attached to polymer chains via either covalent

or non-covalent bond to achieve the polymeric prodrug, which can be successfully prepared via two major methods, i.e., physical modification and chemical conjugation.

1.7.1 Chitin-Chitosan based Prodrugs obtained from Physical Modification Method

The preparation of prodrugs via physical modification is the easiest and simplest method since drugs are attached to polymer chains via non-covalent bonds. Most prodrugs are in the forms of films, membranes, beads, and gels with the random crosslinking. Up to now, many researchers have prepared the prodrugs using physical modification method.

Mi *et al.* (1999) prepared the acylchitosan microspheres by modifying the microencapsulation process from spray-drying to spray in-liquid coagulating which provides the variation of chemical properties from a hydrophilic chitosan microsphere to a hydrophobic acylchitosan microsphere. The physical structure of microspheres was varied from a porous chitosan microsphere to a dense acylchitosan microsphere. As a result, drug release rate of acylchitosan microspheres was depressed, and the long-acting release of antibiotic drug was possible.

Thanoo *et al.* (1992) prepared chitosan microspheres (<10 μm) with a smooth surface by cross-linking with glutaraldehyde in an aqueous acetic acid containing chitosan, paraffin oil, and dioctyl sulphosuccinate as a stabilizer. Theophylline, aspirin or griseofulvin was incorporated into microspheres and the release was depending on the cross-linking density, size, and initial drug content in microspheres.

Koseva *et al.* (1999) prepared chitosan gel beads as carriers of 8-hydroxy-7-iodoquinoline-5-sulfonic acid (SQ) and 2,5-dihydroxybenzenesulfonic acid (DHBSA). The release was controlled by crosslinking agent (epichlorohydrin or glutaraldehyde) and pH of the medium. The release of SQ decreased when changing from alkali to acidic condition.

Mi *et al.* (2002) prepared chemically modified porous chitosan beads by introducing of quaternary ammonium and aliphatic or aromatic acyl groups in order

to interact with an anti-inflammatory drug, indomethacin, via the electrostatic interaction and hydrophobic interaction. The pore size and the porosity of beads were depending on the synthesis conditions, i.e., initial polymer concentration, pH value, and concentration of the casting agent (tripolyphosphate aqueous solution).

Bodmeier *et al.* (1989b) prepared sulfadiazine beads by dropping drug-containing solutions of chitosan into tripolyphosphate (TPP) solution. The drug was entrapped within a three-dimensional network of the ionotropic gel-like spheres and the release involved with pH. The beads swelled and dissolved at low pH and stayed intact at high pH. The release decreased with increasing of TPP concentration.

Fang *et al.* (1998) studied on the *in vitro* controlled release of different molecular weights of drugs, which were urea, 5-fluorouracil (5-Fu), sodium benzoate, sodium salicylate, sodium mandelate, and sulfecetamide sodium from chitosan/gelatin hybrid membrane to find that the release of these drugs follows zero-order kinetic. The transport process of drug molecules in the hydrogel membrane was presumed to be predominantly of the pore mechanism.

Watanabe *et al.* (1990) prepared 6-O-carboxymethyl-chitin gel by adding iron (III) chloride under mild condition without any organic solvent. The bovine serum albumin (BSA) and the anticancer drug doxorubicin (DOX) were incorporated inside the gels. The releases of BSA and DOX were increased by lysozyme digestion in a time-dependent manner.

Although, up to present, a large number of prodrugs prepared by physical modification method have been reported, the disadvantages about the size of prodrug, the unstable sustained release, and in some cases the toxicity of crosslinkers are involved. For pharmaceutical and biomedical applications, (i) the size of the prodrug should be as small as nanoscale to avoid irritation, (ii) the surface area of carrier should be high to enhance drug incorporation efficiency, and (iii) the water solubility as it is the nature of biosystem, including the basic requirements about non-toxicity, biocompatibility, and biodegradability.

1.7.2 Chitin-Chitosan based Prodrug obtained from Chemical Conjugation Method

For chemical conjugation, the drugs are covalently conjugated to polymer chains via a spacer molecule where the bond cleavage from external stimuli, e.g., pH, temperature, etc. are possible. Although this method exhibits high cost, as the neat reaction has to be considered, the obtained prodrugs perform more systematic controlled release than that of physical modification.

Ohya *et al.* (1992) prepared 6-O-carboxymethyl chitin (CM-chitin) conjugated 5-fluorouracil (5FU) via pentamethylene and monomethylene spacer groups. The obtained CM-chitin/5FU prodrug showed the slow release of 5FU and exhibited a emarkable antitumor activity against leukemia.

Ohya *et al.* (1995) employed two different spacer groups, a lysosomal digestible tetrapeptide (Gly-Phe-Leu-Gly) and a simple hydrophobic pentamethylene (C5) to immobilize doxorubicin (DXR) molecules to CM-chitin in order to study on the *in vivo* and *in vitro* antitumor activities. The CM-chitin/Gly-Phe-Leu-Gly/DXR showed slightly higher *in vitro* cytotoxic activity against tumor cells than that of CM-chitin/C5/DXR.

It should be noted that the controlled release technology is not only confined to pharmaceutical and biomedical applications but has also proven the beneficial in agricultural applications. The replacement of conventional agrochemical formulations by controlled release systems not only avoids the treatment with an excess amount of active substances, but also offers ecologic and economic advantages.

Chirachanchai *et al.* (2001) proposed two types of chitosan conjugated with 1-naphthyl methylcarbamate or carbaryl (CBR) insecticide: i) without spacer via iodochitosan to obtain chitosan-carbaryl (CHI-CBR) and ii) with spacer by using N,N'-carbonyldiimidazole (CDI) to obtain chitosan acetate-carbonyl imidazole-carbaryl (CA-CDI-CBR). The conjugation of CBR onto iodochitosan was achieved by alkylation of N-substituted amide, while the coupling reaction of carbaryl onto chitosan acetate was carried out by using carbonyl imidazolide via nucleophilic substitution.

However, in most cases the prodrugs prepared by chemical conjugation are taking the risk of loss of drug active sites during conjugation step owing to the severe condition.

1.8 Micro- and Nanosphere Chitosan for DDS

Recently, sphere-like structured polymer has been proposed for the advantage about the effective drug incorporation without crosslinker and chemical reaction. The drug molecules are incorporated into micro- or nanospheres via secondary forces such as hydrophobic/hydrophobic and hydrophilic/hydrophilic interactions. However, the control of particle size and the effective drug incorporation should be considered. Akashi *et al.* (1998) proposed a polystyrene core-corona sphere, which showed the ability to immobilize peptide drugs and antibodies. A core-corona sphere was achieved from the controlling of hydrophobic/hydrophilicity on polymer chain leading to the difference in interfacial free energy in medium.

In the case of chitosan, Ouchi *et al.* (1998) proposed the self-aggregation of chitosan obtained from grafting methoxy poly(ethylene glycol) (mPEG) onto chitosan chains. However, there has been no report about sphere-like structured chitosan.

1.9 Scope of the Present Work

The present work stands on two viewpoints, which are, (i) the low molecular weight chitosan production, and (ii) the use of low molecular weight chitosan for chemical modification at molecular level. For the first point, γ-ray irradiation is mainly considered. Photoirradiation with γ-ray is an effective pathway when we consider that it is a single procedure that allows extensive modification without waste. Although some studies of γ-ray irradiation of polysaccharides have been done previously, it is always the question whether the product obtained after γ-ray irradiation maintains chitosan structure that can be uside a starting low

molecular weight species material for further chemical modification or not. For the second viewpoint, the present work originally proposes low molecular weight chitosan obtained from γ–ray irradiation for further derivatization related to hydrophobic and hydrophilic groups introduction. Here, the hydrophobic and hydrophilic group introduction is an attractive approach, especially when aiming for the complexation and/or aggregation with specific molecules, i.e., drugs, pesticides, or toxic agents through micelle formation. To achieve the above goals, the present dissertation is divided into the chapters as follows.

Chapter II deals with the studies of the γ-ray irradiation effecting on chitosan in dry state. The studies extended to the chemical modification via the selective hydrophobic chain conjugation on hydroxyl group at C-6 are summarized.

Chapter III reports about the comparative studies of γ-ray irradiation conditions effecting on chitosan properties. The work aims to clarify how γ-ray changes the structure of chitosan including the related mechanisms. The work also proposes the optimal conditions to achieve low molecular weight chitosan without the destruction of the backbone.

Chapter IV bases on the molecular design and the optimum reaction conditions to change chitosan flake into sphere-like structured chitosan via the controlling of hydrophobic/hydrophilicity on the chains. The work declares how chitosan spheres are achieved at nanoscale size without any specific processing technique.

Chapter V clarifies the colloidal phenomena of chitosan nanospheres. The work focuses on the self-aggregation chitosan under the effects of media. The nanosphere formation and the factors affected to the nanosphere size are discussed.

Chapter VI is about the structural clarifications of chitosan nanospheres and the feasible applications in pharmaceutical, biomedical, and agricultural areas where the effective aggregation system of drug/pesticide/toxic agent can be expected.

In overall, the present work achieves the guideline to develop chitosan via controlling hydrophobic/hydrophilicity for a potential material to be used in DDS.