CHAPTER II
LITERATURE SURVEY

2.1 Chemical Structure of Chitin-Chitosan and Its Attractive Properties

Chitin-chitosan is the second most naturally abundant biomass next to cellulose. Chitin-chitosan is found as a polysaccharide in the exoskeletons of crustacean, insect, as well as, a cell wall of yeast and fungi. The structure is a copolymer chain of a chitin unit, β(1-4)-2-acetamido-2-deoxy-β-D-glucan, and a chitosan unit, β(1-4)-2-amino-2-deoxy-β-D-glucan (Scheme 1.1).

Chitin-chitosan is unique owing to an additional nitrogen atom of acetamide and amine groups at C_2 position. Combining with the basic structure of polysaccharide, chitin-chitosan has two reactive sites, i.e., acetamide and amino groups at C_2 position and primary alcohol at C_6 position. Both groups are, thus, important for physical and chemical modifications. Recently, chitin-chitosan received much attention for applications either via chemical and physical modification pathway.

The attractive properties of chitin-chitosan can be well understood when we consider the chemical structure. Basically, the nitrogen atom of chitin-chitosan acts as an electron donor and is presumably responsible for selective chelating with metal ions (Tsezos, 1983). The anhydroglucosamine chain makes chitin-chitosan behave as a linear polyelectrolite at acidic pH. The free amino groups in chitosan were considered to be much more effective for binding metal ions than the acetyl groups in chitin (Maruca *et al.*, 1982). Due to the high density of amino groups, chitosan can interact strongly with negatively charged substances, such as proteins, dyes, and polymers to give electric neutrality.

The backbone of chitin-chitosan chain is a saccharide unit, which gives the properties of nontoxicity and biocompatibility. In addition, β(1-4)-

glucosidic linkage gives biodegradability. Therefore, chitin-chitosan received much interest to apply as a material utilized in life science area, i.e., the biomedical, pharmaceutical, agricultural and biotechnological fields.

2.2 Basic Applications of Chitin-Chitosan

One of the earliest applications of chitin-chitosan was for chelating harmful metal ions, such as copper, lead, mercury, and uranium, from wastewater (Hirano *et al.*, 1984). Muzzarelli *et al.* (1973) indicated that chitosan was a powerful chelating agent and exhibited higher collection ability than chitin and cellulose derivatives as a result of the amino group.

Many types of chitosan membranes have been developed for water clarification and filtration. Hirano *et al.* (1980) prepared a series of chitosan membranes and reported about the permeability and stability in dilute acid and alkali solutions, which are related to the cast conditions.

Lang and Clausen (1988) suggested the use of chitin-chitosan and its derivatives in cosmetic. For example, chitosan as a nontoxic cationic polymer is used in hair treatment and skin care. A cationic character of chitosan primarily exhibited clear film adhered to hair or skin.

Chitosan is developed into strong fibers for the use in the chopped fibers. Chitosan fibers are incorporated into a nonwoven matrix to use as a suture material and a wound dressing material (Kibune *et al.*, 1987). In addition, chitosan may be used to inhibit fibroplasias in wound healing and to promote tissue growth and differentiation in tissue culture (Muzzarelli, 1989).

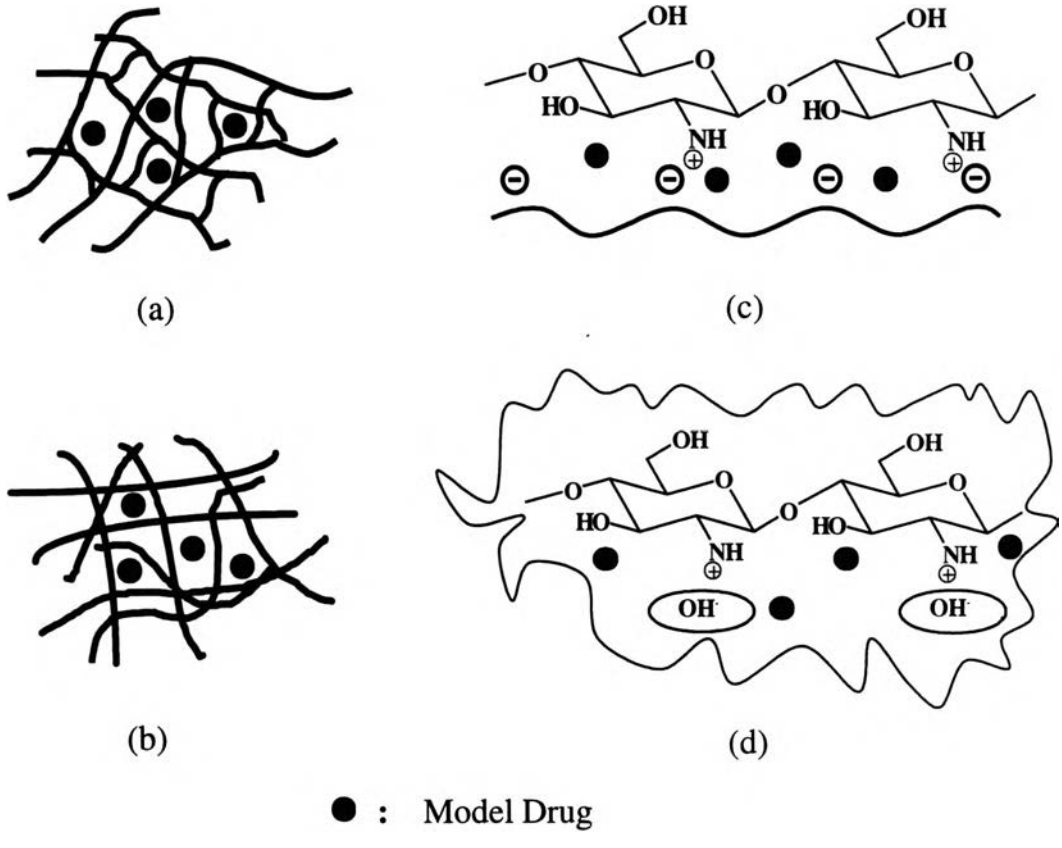
2.3 Advanced Applications of Chitin-Chitosan

Chitin-chitosan is reported for the effectiveness to reduce the cholesterol. Various hypolipemic formulations containing chitin-chitosan,

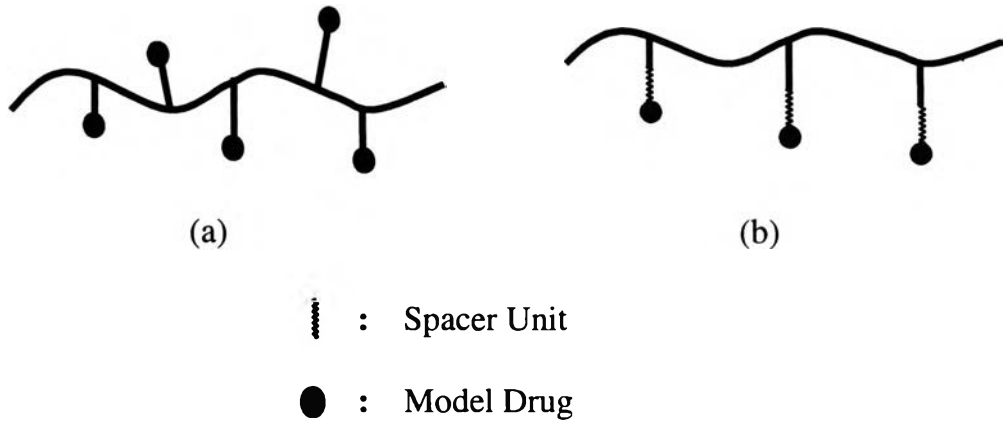
including particles, powders, solutions, and injections, were prepared and studied on oral administration (Suzuki *et al.*, 1988). In oral tests on mice, chitosan effectively decreased the cholesterol level in blood up to 66%. The hypocholesterolemic activity of chitosan has not been clarified but it was probably due to its inhibition of lipid-micelle formation (Muzzarelli *et al.*, 1984).

Sirca and Woodman (1971) showed that chitin-chitosan performed the selective aggregation with leukemia tumor cells *in vitro*, producing a dense aggregate and inhibiting cell growth. The special affinity of chitin-chitosan for biomolecules has been utilized to reduce side effects of drugs. Ouchi *et al.* (1989) reported that the chitosan prodrug exhibited an enhanced inhibition effect against tumor cells without displaying any apparent toxicity.

One of the most interesting and advanced utilizations of chitin-chitosan is the drug delivery system (DDS). There are 2 main polymeric designs for DDS, which are physical blending and chemical conjugation. For physical blending, it can either be bead (Daly *et al.*, 1989), polymer network (Kanke *et al.*, 1989), interpenetrating network (IPN) (Yao *et al.*, 1994), or ionic interpolymer complex (Mi *et al.*, 1997) as shown in Scheme 2.1. The physical modification, generally, concerned with the blending and reforming as a gel, membrane, or bead. Thus, it is easy to prepare but difficult to achieve the controlled release system. In another way, a chemical modification is an approach based on the conjugation of drug molecule, thus, drug can either be directly conjugated onto chitosan chain (Ouchi *et al.*, 1989) or conjugated via spacer group (Ohya *et al.*, 1992). Although, these designs are aimed to control the drug release mechanism, in practical, we need a chemical reaction of which the condition must not deactivate or denature the drug.



Scheme 2.1 Schematic chitosan based controlled release system by (a) crosslinked polymer network, (b) interpenetrating network, (c) ionic interpolymer complex, and (d) drug entrapped chitosan bead.



Scheme 2.2 Schematic chitosan based controlled release system by (a) polymer-drug direct conjugation, and (b) polymer-spacer-drug conjugation.

2.4 Limitations of Chitin-Chitosan and the Strategies to Overcome the Problems

Chitin-chitosan is a semicrystalline polymer. Because of its strong intra- and intermolecular hydrogen bonding network, chitin-chitosan dose not perform glass transition temperature as a thermoplastic but degrades before it melts. This physical property makes it difficult to dissolve in any solvents even water. However, protonating the acetamide and amino groups using dilute acid, chitosan solution can be prepared. As a result, carrying out the chemical reaction of chitin-chitosan is limited owing to its insolubility in all common organic solvents. In most cases, the reactions have to be done in heterogeneous condition such as tosylation (Kurita *et al.*, 1992), *N*-phthaloylation (Nishimura *et al.*, 1990), N-acylation (Fujii *et al.*, 1980). The reactions are accompanied by various problems, such as low extents of reaction, difficulty in regioselective substitution, product nonuniformity, and partial degradation due to harsh reaction condition. As a result, the chemical modification of chitin-chitosan cannot be quantitatively achieved.

To overcome these limitations, Tokura *et al.* (1979, 1983) converted insoluble chitin-chitosan into an organic-soluble derivative, such as benzyl and benzoyl chitins, or a water-soluble derivative, such as carboxymethyl chitin (CM-chitin) as well as CM-chitosan. Chain scission is another good way to improve the solubility and reactivity of chitin-chitosan. In another point of view, currently, there are reports about the bioactivity of high molecular weight oligochitosan. Yamada *et al.* (1993) reported that oligosaccharides with the units more than the hexamer show high physiological activity at a very low concentration.

Typically, chain scission can be achieved by 3 main methods, i.e., chemical treatment, enzymatic hydrolysis, and photoirradiation. Allan *et al.* (1997) and Varum *et al.* (1994) reported the depolymerization of chitosan by

the action of HONO or HCl for producing chitooligosacchrides at a desired molecular size. The amino group of chitosan was the key in the reaction, but not to cleave the β-glucosidic linkages.

Chitin-chitosan is susceptible to be hydrolyzed by lysozyme, chitinase, or chitosanase to produce low molecular weight oligomers. These oligomers show an antitumor effect. Water-soluble *N*-acetylchitohexose was reported to show the immuno enhanced growth-inhibitory effect of tumor cells (Suzuki *et al.*, 1986).

Photoirradiation causes the changes in the physico-chemical properties of chitosan (Kume and Takahisa, 1982). The radiation sterilization leads even to the improvement of some biocompatibility factors (Dutkiewicz *et al.*, 1989).

2.5 γ-Ray Irradiation of Chitin-Chitosan

γ-Ray irradiation is one of the photoirradiation methods. Although using γ-ray is known as a hazardous and dangerous pathway, the utilization of γ-ray in polymer chemistry can be claimed as a peaceful and environmental friendly one. Moreover, the ease of sample preparation and the achievement of quantitative reaction make the γ-ray irradiation be an attractive application in the next era. Recently, the effects of γ-ray on the degradation of the biowaste have been proposed (Kaesu *et al.*, 1987), while the biotechnological application of polymerized or grafted materials has gained a widespread use (Gombotz *et al.*, 1985).

Kume and Takehisa (1982) investigated the effect of γ-ray irradiation on physico-chemical properties of chitosan in solution and in dry states. Chitosan was radiosensitive when irradiated in 0.1% solution at pH 6.0. The viscosity, surface charge, and protein coagulation property decreased significantly at high dose. On the other hand, chitosan was stable for

irradiation in dry state. The solubility, surface charge, and protein coagulation property were hardly changed. However, the viscosity decreased significantly at low dose.

Principally, thermal degradation of chitosan depends upon the molecular weight and degree of acetylation of the polymer, and both of these two factors were affected by irradiation. Wenwei *et al.* (1993) reported that the starting decomposition temperature of chitosan drops markedly as the radiation dose is increased, indicating that this polysaccharide becomes less thermal stability on irradiation. In addition, the scission of glucosidic bonds leads to the formation of hydroxyl groups as confirmed by the increasing of hydroxyl group concentration with radiation dose.

Ulanski and Rosiak (1992) studied the structural changes in chitosan induced by irradiation to find that carbonyl and carboxyl groups were formed after irradiation in oxygen. It is known that scission of β(1-4) glucosidic bond is caused by rearrangement of radicals localized on C_1, C_4, and C_5 carbon atoms. These transformations can be described as the reactions of hydrolysis or fragmentation.

2.6 Modifications of Chitin-Chitosan

2.6.1 Physical Modification

Chitin-chitosan has been developed into beads, gels, powders, fibers, sheets, tablets, and sponges, which can be one of the basal materials for cosmetics and drugs. Ion interfacial ability makes chitosan attractive as an ion extraction material for wastewater treatment. The chelating ability of chitin-chitosan could be improved by crosslinking. Muzzarelli (1984) reported that the chelating capacity of crosslinked-glycine chitosan for copper uptake was 22 times higher than that of chitosan.

Chitin can be made into strong flexible fiber by spinning from a chitin-formic acid solution prepared through a repeated freezing process (Tokura *et al.*, 1979).

Hadwiger *et al.* (1984) studied the application of chitosan on post-harvest to find that chitosan coating showed inhibition of fungal pathogeus in the vicinity of seeds including the enhancement of plant-resistant responses against diseases.

Malette *et al.* (1986) exhibited the effects of chitosan on wound healing in dogs. It was concluded that chitosan-treated wounds did not display classic healing, but simply regenerated the normal tissue elements, leaving no visible scars.

2.6.2 Chemical Modification

Chitin-chitosan is more useful than cellulose for developing advanced functions owing to the presence of reactive amino groups and primary hydroxyl groups. Many efforts have, thus, been focused on the development of facile modification reactions to prepare derivatives with well-defined structures and novel properties. Considering the chemical structure, chitin-chitosan can react with other reactive functional groups such as carboxylic acid, acid chloride, and aldehyde or it can undergo esterification, etherification, crosslinking, and graft copolymerization reactions (Hon, 1992). Of various attempts to develop solubility of this rigid intractable polysaccharide, destruction of the crystalline structure by incorporating appropriate substituents has been provided to be an effective pathway.

2.6.2.1 Chemical Modification at N-Position

Partial conversion of acetyl groups of chitin unit into reactive amine groups on the backbone of chitin-chitosan copolymer was known to promote its solubility and enhance its reactivity. This deacetylation

was operated by using NaOH treatment with control of the reaction time (Li *et al.*, 1997).

The nitrogen atom of an amino group on chitosan unit, which is reactive primary amine, acts as a nucleophile. Hence, many derivatives of chitosan have been proposed and the practical uses of chitosan have been developed in more number than that of chitin.

The introduction of acyl groups with longer aliphatic chains to the chitin chain can improve its solubility in organic solvents, since acetylation of chitin increases its solubility property in formic acid (Kaifu *et al.*, 1981). Hirano *et al.* (1976) reported a mild procedure for the selective *N*-acetylation of chitosan by treating the solution in aqueous methanolic acetic acid with carboxylic anhydrides at room temperature. *N*-acetylations are of interest, as they effect the selective aggregation of some cancer cells.

2.6.2.2 Chemical Modification at O-Position

Chitin-chitosan has 2 types of hydroxyl groups on the pyranose ring, i.e., primary alcohol at C_6 position and secondary alcohol at C_3 position. Since the primary hydroxyl group is more reactive than the secondary hydroxyl group, most reactions are paid on C_6 hydroxyl group. Attempts at a disrupting intra- and intermolecular hydrogen bonds in order to prepare organic soluble and/or specific properties induction chitin-chitosan derivatives are known as acylation, alkylation, or carboxymethylation.

Kurita *et al.* (1992) investigated tosylation of chitin accomplished by interfacial condensation to give tosylchitin. The compound is obtained under mild condition and showed the good solubility in organic solvent. The tosylation is effective even at low temperature. It should be noted that Kurita *et al.* mentioned that hydroxyl group is generally much more nucleophilic than amino group under the strong alkaline condition as an

evidence in the tosylation of aminophenol. The resulting tosylchitin is a useful precursor to undergo some reactions such as acetylation and iodonation.

Muzzarelli *et al.* (1984) reported the preparation of chitin heparinoid by the sulfation of *N*-carboxymethyl chitosan as a new type of heparinoid. The obtained compound was reported to give no adverse effect on cellular structure. However, the toxic behavior of chitin heparinoid has not been established.

2.7 Concept of Polymer-Micelle

Micelles are small aggregates formed from surfactant molecules and are thermodynamically stable. Here, surfactants are usually made up of low-molecular weight molecules, which aggregate in water mainly by hydrophobic interaction, which are weak van der Waals forces. The structure of micelle is brittle and difficult to maintain. In addition, the surfactant molecules can interact with the tissues and dissolve cell membranes or even cause protein denaturation. Thus, surfactants have never been reported to use intravenously, although some have been orally administered (Yoshioka *et al.*, 1995).

A typical example of associative polymers is the aqueous solutions of block copolymers. The associative polymers usually contain a water-soluble hydrophilic backbone and insoluble hydrophobic groups located at the ends of the polymer chain or distributed along the polymer backbone. When the polymer dissolves in water, clusters of hydrophobic domains are formed, yielding a network structure. Such structure induces a large viscosity increase of the solution, producing a viscous and often elastic and gel-like fluid behavior. Hence, the associative polymers are good candidates for thickening agents in environmentally friendly coating applications (Dai *et al.*, 2000).

It has been reported that polymer-micelle is convenient compared with other carriers such as liposomes for use as a passive targeting carrier of

anticancer drugs. Moreover, the polymer-micelle could not to be captured by the reticuloendotherial cell system (RES), the body screening system, because of its small particle size (20-100 nm) (Kataoka *et al.*, 1993).

The polymer used as a drug carrier needs to be nontoxic, biodegradable, and metabolized in the body. Thus, chitin-chitosan is known to meet the requirements. However, when the concept of drug delivery system is based on micelle structure, an approach to modify chitin-chitosan is necessary. Recently, chitosan derivatives with both hydrophobic group (long acyl group) and hydrophilic group (sulfate group) were proposed as micelles, which solubilized hydrophobic compounds such as azobenzene (Yoshioka *et al.*, 1995).

2.8 Hydrophobic Introduction at *N*- and *O*-Positions

Yoshioka *et al.* (1995) prepared sulfated *N*-acetyl-chitosan possessing various lengths of alkyl chains to investigate a polymer micelle concept. The chitosan unit attached with various lengths of normal alkyl chain from C_{12} to C_{16} to the amino groups by amide bonding was reported and the specific interaction induced by the hydrophobic parts was clarified. It was concluded that sulfated *N*-acetyl-chitosan with long alkyl chains was successfully formed as micelles via the aggregation of the alkyl chains.

Nishimura *et al.* (1993) investigated the artificial glycolipid-type polymers with the monolayer membranes via regioselective chemical manipulations of chitosan. The intermediate, (1-4)-2-amino-2-deoxy-6-O-trityl-β-D-glucopyranan, was first synthesized. The treatment with excess palmitoyl chloride in pyridine introduces a lypophilic moiety regioselectively at C_2 and C_3 positions. In the following step, *O*-sulfate group at C_6 position of the derivative was introduced to obtain a polysaccharide amphiphile. The

product exhibited better solubility in common organic solvents such as chloroform, dichloromethane, and benzene.

Miwa *et al.* (1998) studied novel chitosan derivatives with both hydrophobic and hydrophilic groups to solubilize taxol, a hydrophobic anticancer agent. Several chitosan derivatives with lauryl groups attached to the amino groups to provide hydrophobic moieties and carboxymethyl groups attached to the hydroxyl groups to provide hydrophilic moieties were synthesized. It was found that the chitosan micelles solubilized taxol and did not show toxicity. Here, the micelle-entrapped taxol was found to be more effective than the free taxol in an *in vitro* assay.

2.9 The Potential of the Present Work

Up to now, polymer-micelle has received much attention owing to its efficient properties of thermodynamic stability and uncaptured ability by the body screening system. Chitin-chitosan is a nontoxic, biodegradable, and biocompatible natural occurring polymer appropriate for being a polymer-micelle carrier. Unfortunately, the chemical modification of chitin-chitosan cannot be quantitatively achieved owing to the lack of solubility and reactivity. As a result, chitin-chitosan faces the problem to develop a progressive application. To overcome this problem, the combination of two concepts, i.e., γ-ray irradiation and polymer-micelle modification, is a novel approach proposed in the present work. Here, the γ-ray irradiated chitosan derivatives were prepared as polymer-micelles by conjugation with hydrophobic molecules. The γ-ray irradiated chitosan is achieved by γ-ray irradiation in water to induce more radicals to break chitosan chains. The effectiveness of γ-ray irradiation was evaluated by molecular weight and chemical structure investigations. The introduction of hydrophobic chains on

γ-ray irradiated chitosan backbones is expected to lead to a novel chitosan micelle to apply in the controlled release system.