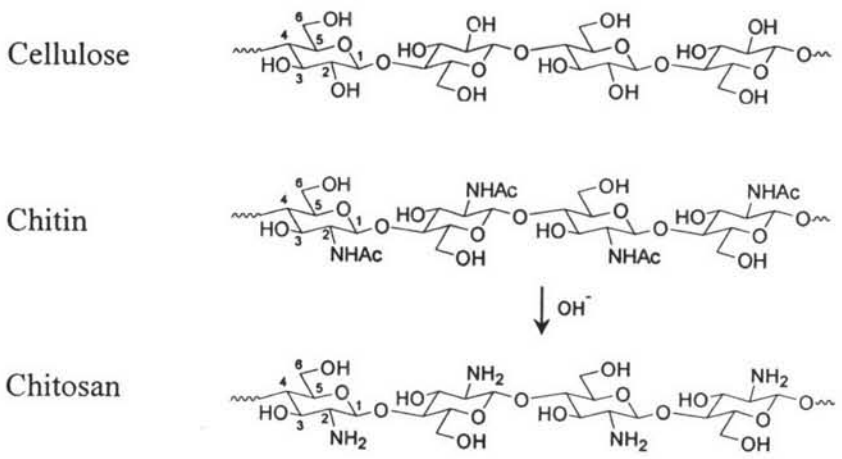
CHAPTER II
LITERATURE REVIEW

2.1 Chitin-Chitosan: the Specific Structure and Unique Properties

Chitin-chitosan is the second-most abundant naturally-occurring polysaccharide next to cellulose obtained from crustaceans, insects and fungi. 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-deoxy-β-D-glucose (Scheme 1). The structure of chitin-chitosan is similar to that of cellulose, but the hydroxyl group at C-2 is replaced by an acetamide group to be chitin units and an amino group to be chitosan units. It is known that the chitosan unit is achieved from the deacetylation of the chitin unit by alkaline hydrolysis under heterogeneous conditions (Scheme 1).

Scheme 1



Based on the chemical structure, the chitin-chitosan copolymer has the following attractive properties:

a) Hydroxyl group. Chitin-chitosan has two types of hydroxyl groups, which are a primary alcohol group at C-6 and a secondary hydroxyl group at C-3. In general, the primary hydroxyl group is more reactive than the secondary one, and

therefore most chemical reactions are considered to be at C-6. These hydroxyl groups impart hydrophilicity to chitosan chains and show the inclusion properties. It can, thus, form inclusion and/or a host-guest compound with ions or molecules, which is appropriate for industries in wastewater treatment (Shimizu *et al.*, 1995). The lone pair electrons of the oxygen atom are also reported for the complexation with metal ions, e.g. Ca^{2+}, Ni^{2+}, etc. (Nishi *et al.*, 1987). Chitin-chitosan possesses antimicrobial properties (Suzuki *et al.*, 1986) as the lone pair electrons of the oxygen atom initiate the microorganism destruction.

b) Amino group. Compared to cellulose, chitin-chitosan is more reactive and can be chemically modified since it has the reactive primary amino groups. The lone pair electrons of the nitrogen atom form an interaction with metal cations or accept the proton to be a protonated species. Hence, the protonation of the amino groups causes the electrostatic attraction of anionic compounds, including metal anions or anionic dyes (Guibal, 2004). This brings about the applications for wastewater treatment as a metal entrapment resin (Peniche-covas *et al.*, 1987), and dyes sorption (Knorr, 1983). The antibacterial and antiviral properties of chitin-chitosan are known to be from the formation of the positively charged amino group and the negatively charged microorganism cell-wall to result in the inhibition of bacteria and viruses (Kendra and Hadwiger, 1984).

c) Acetamide group. The function of the acetamide group is similar to the amino group but is rather weak in reactivity. The acetamide group forms a strong hydrogen bond network leading to the high crystallinity of chitin. The hydrogen bond via the acetamide group brings about poor solubility for chitin in most solvents.

d) Pyranose ring. Chitin-chitosan consists of a pyranose ring, either *N*-acetyl-D-glucosamine or D-glucosamine, which is reported for its bioactivity, such as detoxification ability and the cholesterol or fatty acid interaction (Muzzarelli, 1996). Chitin-chitosan oligomer activates tissue growth and functions as a fibroblast for collagen reconstruction leading to effective wound recovery (Ueno *et al.*, 2001). The activity of chitin-chitosan, a bio-essence saccharide unit, imparts the biocompatibility (Richardson *et al.*, 1999), bioactivity (Dumitriu *et al.*, 1989) and

non-toxicity (Rao and Sharma, 1992) under the structure of the pyranose ring with *N*-acetyl-D-glucosamine or D-glucosamine.

e) Glycoside linkage. The glycoside linkage or glucosidic bond (C-O-C) provides biodegradability via enzymatic hydrolysis, i.e. chitinase, chitosanase, and lysozyme. Biodegradability in nature leads to chain degradation (Yamamoto and Amaike, 1997 and Tomihata and Ikada, 1997).

2.2 Development of Chitin-Chitosan for Value-Added Applications

For decades, a lot of research about chitin-chitosan has been actively done; however, the practical uses of chitin-chitosan products are barely seen in daily life. This might be due to the raw material consistency or quality control, including the cost performance. In recent years, chitin-chitosan for advanced applications has received much attention as it is an alternative way to produce advanced applications in which the consumers are concerned with the specific properties and an acceptable price. Many researchers reported on the structural modification of chitosan at the molecular level to propose novel products, mostly for pharmaceutical and biomedical applications.

a) Applications in controlled drug release. A drug delivery system (DDS) is one of the advanced and value-added applications and is considered for most bipolymers. As most biopolymers are naturally excreted from the body via the kidneys and/or are digested by microorganism in the environment, the DDS offers numerous advantages compared to conventional dosage form such as increasing therapeutic activity, reducing toxicity and reducing the number of drug administrations required during treatment. Before making use of these polymers as drug carriers, drugs will thus be attached to polymer chains via either covalent or non-covalent bonds. The conjugation of the drug onto the polymer is successfully prepared via two major methods, i.e. physical modification and chemical modification conjugation.

The physical modification for a non-covalent bond drug carrier is mostly in the form of films, membranes, beads, and gels. For example, Bodeier *et al.* (1989)

prepared sulfadiazine beads by dropping drug-containing solutions of chitosan into a tripolyphosphate (TPP) solution. The drug was entrapped within a three-dimensional network of the ionotropic gel-like spheres and the release was involved with pH. The beads were swelled and dissolved at low pH and remained intact at high pH. The release decreased with an increasing of TPP concentration. Although, up to present, a large number of drug carriers prepared by the physical modification method has been reported, the disadvantages about the sizes of product, the instable sustained release, and the toxicity of crosslinkers are involved.

For chemical conjugation, the drugs are covalently bonded to polymer chains via a spacer molecule where the bond cleavage is induced by external stimuli, e.g. pH, temperature, etc. For example, 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 remarkable antitumor activity against leukemia. Although this method exhibits high cost as the neat reaction has to be considered, the obtained product has a more systematic controlled release than that of physical modification. In most cases, the drug carriers prepared by chemical conjugation are taking risks about the losses of drug active sites during the conjugation step owing to the severe conditions.

b) Tissue engineering applications. Tissue engineering has been defined by Laurencin *et al.* (1996) as the multidisciplinary principles of biological, chemical, and engineering fields toward the repair, restoration, or regeneration of living tissues. In recent years, tissue engineering has emerged as a potentially effective approach to repairing, damaged skin and to bone replacement. A common and practical method is to use biodegradable and scaffold porous materials. The important point of biodegradable materials is non-toxicity and biodegradability during the skin or bone tissue growth. A porous structure is needed to increase the surface area, allowing a great number of fibroblasts or osteoblasts to attach to the matrix and promote tissue regeneration. It should be noted that chitosan can be considered for supporting the expression of extracellular matrix proteins in human fibroblasts, and osteoblasts for skin and bone replacement as its structural characteristics are similar to

glycosaminoglycans, which are major components of the extracellular matrix of skin, and bone (Lahiji *et al*, 2000). In addition, the lone pair electrons of the nitrogen atom tends to react with metal cations by a chelating mechanism, which is effective for binding with calcium ions in hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), the main inorganic component of bone. Yamaguchi *et al.* (2003) reported that hydroxyapatite may form complexes between amino groups of chitosan and calcium ions.

2.3 Limitations and Strategies to Overcome

As can be analyzed from the chemical structure, chitin-chitosan exhibits high crystallinity through its inter- and intra-molecular hydrogen bond network. Combined with the high molecular weight developed naturally, chitin-chitosan faces weak points about its solubility and reactivity.

Structural modification of chitin-chitosan at the molecular level is the way to overcome these limitations and develop more valuable products. There are two points to be considered for achieving chitin-chitosan modifications at the molecular level; (i) lowering the molecular weight of chitosan to enhance solubility, and (ii) development of organo-soluble and water-soluble chitosan derivatives, which are expected for the effective reaction in the further derivatization step.

2.3.1 Lowering Molecular Weight of Chitosan to Enhance the Solubility

For the past few years, several research efforts for low molecular weight chitosan (LMWC) and/or oligochitosan have been done; for example, acid or base hydrolysis, enzymatic degradation, and photoirradiation. 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 multiple steps, especially, enzyme preparation and purification. This brings about a hesitation to develop in large-scale production. Photoirradiation is considered as a specific technique in terms of operating system and capital cost, and it is attractive since it is an easy

process with large-scale production in a single step without waste generation or 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). Lee *et al.* (1999) studied the hydrolysis of chitosan with hydrochloric acid (HCl). To enhance acid hydrolysis, Chen *et al.* (1997) proposed pre-treatment with ultrasonic followed by acid hydrolysis. It was concluded that the degradation increased with sonication time and the chain scission was significant even in dilute solutions stored in low temperature.

Owing to its biodegradability, chitin-chitosan can be degraded by naturally occurring enzymes such as chitinase, chitosanase, and lysozyme. Lysozyme and chitinase are clarified for chitin hydrolyzation to produce $(GlcNAc)_n$. The hydrolysis rate is rather low, which might be due to the heterogeneous conditions where the oligomers (less than 6 repeat units) are not obtained in good yield. Lysozyme hydrolyzes partially *N*-acetylated chitosan (PNACs) under homogeneous conditions (Aiba, 1994). The function of digestion ability to PNACs was increased with an increase in the degree of *N*-acetylation of PNACs because lysozyme was found to recognize a GlcNAc sequence with more than three residues (Nordtveit *et al.*, 1994). Chitinase was identified for its recognization of 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 high-energy electrons such as UV light, X-ray, or γ-ray. Generally, photoirradiation gives chain scission and crosslinking, after exposure to the high-energy electron. For a biopolymer, chain scission mainly occurs since C-O-C offers the radical species. For example, Andrady *et al.* (1996) studied 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 proportional to the energy used.

Although many 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 modification are still a challenge.

2.3.2 Development of Organo-Soluble and Water-Soluble Chitosan Derivatives

From the structural 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, alkyl halide, etc. However, the fact that chitosan is inert and stabilized by inter- and intra-molecular hydrogen bonding brings about difficulty in functionalizing chitosan. Most reactions of chitosan have to be done in a heterogeneous system or in some specific acid solutions, and this limits the satisfactory completion of the substitution degree and/or the variety of the reactions. At present, various derivatives to achieve chitosan soluble in organic or water solvents so called "organo-soluble chitosan" or "water-soluble chitosan" have been proposed. Chitosan in a homogeneous system is accepted as an effective pathway to functionalize and develop the material, especially when one considers the function at the molecular level.

a) Organo-Soluble chitosan. The conjugation of bulky group (sometimes, as a protecting group) onto the chitosan chain is a chemical modification pathway for overcoming the solubility problem. In this way, the hydrogen bonds will be disturbed; as a result, solubility in organic solvents can be achieved. In the following step, the chemical reactions can be done as desired followed by deprotecting of the bulky group. The disadvantage of this pathway is that the conjugation with the bulky group consumes some particular functional group of chitosan, which limits the variation of the reaction in the derivatization step. Nishimura *et al.* (1991) proposed *N*-phthaloylchitosan from the reaction of chitosan with phthalic anhydride in DMF at 130°C. The resulting phthaloylchitosan exhibits much improved solubility in common organic solvents such as DMF, DMAc, DMSO, and pyridine. This organo-soluble derivative is accepted as a key starting material for the preparation of several

O-substituted derivatives. 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.

b) Water-Soluble chitosan. In the point of developing materials for advanced functions (such as scaffolds for tissue engineering, nanoparticles for drug targeting, etc.), water-soluble chitosan derivatives, especially the reaction via an aqueous media, have received more interest than organosoluble derivatives. This might be due to the safety level without concerning for a toxic-organic solvent remaining in the purification step. Up to now, several efforts to achieve water-soluble chitosan derivatives have been made by using various chemical modification techniques, such as, PEG-grafting (Sugimoto *et al.*, 1998), sulfonation (Holme and Perlin, 1997), quaternarization (Jia *et al.*, 2001), *O*-hydroxylation (Machida *et al.*, 1986), and carboxymethylation chitosan (CM-chitosan) (Chen *et al.*, 2003).

Although there are many derivatives proposed for organosoluble and water-soluble chitosan, some reactions face problems of low reproducibility, multi-step reactions, purification, and harsh conditions which might increase the production cost and not be practical for safety reasons to use in the human body.

2.4 Scope of the Present Work

The present work stands on the homogeneous system derivatization and materialization of chitosan via organo-soluble and water-soluble chitosan derivatives. For organo-soluble chitosan, we focus on *N*-phthaloylchitosan as the organo-soluble derivative and its chemical homogeneous modification in the dimethylformamide (DMF) system. The introduction of an epoxy group onto chitosan via *N*-phthaloylchitosan and the crosslink network via the ring opening reaction of the epoxy group at room temperature are demonstrated to show the success of chitosan-epoxy gel. The work also shows the mineralization step to grow hydroxyapatite crystals in the chitosan-epoxy gel by an alternate soaking method. For water-soluble chitosan, the present work originally shows a simple chitosan organic complexation

between chitosan and conjugating additives such as 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), *N*-hydroxysuccinimide (HOSu). The complexes are expected for not only providing water solubility to chitosan but also promoting the conjugation in a water-based system. The present work demonstrates an example of conjugating using 1-ethyl-3-(3-dimethylaminopropyl-carbodiimide) (EDC), a water-soluble conjugating agent, to functionalize chitosan with amino acid and ethylene glycol. The work is also extended to show the preparation of water-based gel from the water-soluble chitosan functionalized with dipoly(ethylene glycol).