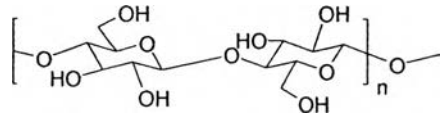
CHAPTER II
LITERATURE REVIEW

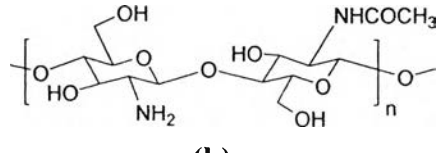
2.1 Chitin-chitosan: Structure and Specific Properties

Chitin-Chitosan is the second most naturally abundance polysaccharide, next to cellulose, obtained from the shells or skeletons of crustaceans, insects and fungi. The structure of chitin chitosan is similar to that of cellulose but hydroxyl group at C-2 is replaced by acetamide group for chitin unit and amino group for chitosan unit. Naturally, chitin-chitosan present 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 2.1).

Scheme 2.1 Chemical structure of (a) cellulose, (b) chitin-chitosan copolymer



(a)



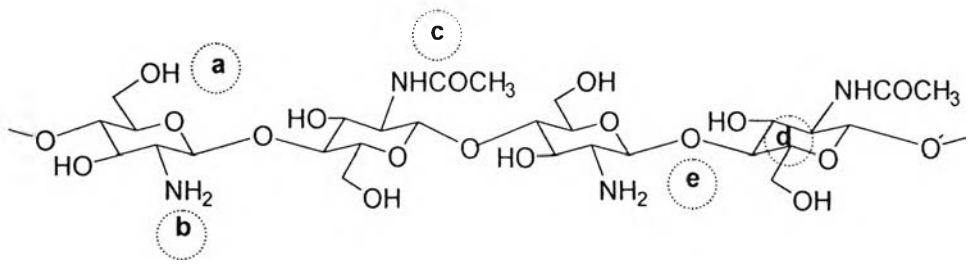
(b)

Chitin-Chitosan is classified as an aminopolysaccharide, which the amino group provides unique properties such as chelation with metal ion, protonation to be cationic species, including the derivatization and functionalization onto ether polymers. The well accepted application, thus are chelating agents (Varma *et al.*,2004), high performance adsorbents (Carol and Matthew, 1999), ion exchange membranes (Pellegrino *et al.*, 1990), etc.

Here, the attractive properties relating to chemical structure (Scheme 2.2) are summarized as follows.

a: Hydroxyl group. Chitin-chitosan has two types of hydroxyl group, 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 takes place at C-6. These hydroxyl groups impart hydrophilicity to chitosan chain and show the inclusion properties (Shimizu *et al.*, 1995). 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 electron 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.

Scheme 2.2 Structure based properties of chitin-chitosan



b: Amino group. Comparing to cellulose, chitin-chitosan can be chemically modified, since it has the reactive primary amino group, which are more reactive than hydroxyl groups. Moreover, lone pair electrons of nitrogen allow the interaction with metal ions. This brings the application for wastewater treatment as ion and/or metal absorbers. The uses as coagulant are also reported since the amino group effectively 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 and species provides the 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, resulting in the inhibition of the growth of bacteria and virus. (Kendra and Hadwiger, 1984).

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

d: Pyranose ring. The pyranose ring, either N-actyl-D-glucosamine or D-glucosamine, is reported for its detoxification ability and the lowering effects for cholesterol. When the pyranose is at the level oligomer, the activity on the cell and issue growth including the functions as a fibroblast for reconstruct collagen leading to the effective wound recovery are significant. The pyranose ring is also the key structure induces the 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 Charma, 1992) under the structure of pyranose ring with N-actyl-D-glucosamine or D-glucosamine.

e: Glycoside Linkage. Glycoside linkage or glucosidic bond (C-O-C) provides biodegradability (Yamoto and Amaika, 1997 and Tomihata and Ikada, 1997) via enzymatic hydrolysis, i.e chitinnase, chitosanase, and lyzozyme, leading to the biodegradation.

2.2 Development of Chitosan Material

According to many specific properties based on its structure, there are numerous reports to develop chitosan in a wide variety of physical and chemical modification.

2.2.1 Physical Modification

As chitosan is in the hydrogen bond networked structure, its linear polysaccharide does not show the melting temperature. As a result, the materialization of chitosan can not be achieved from the thermoplastic melting process but has to rely on the solution methods, especially chitosan acetic acid solution. Most practical researches of chitin-chitosan have been emphasized on physical modifications such as casting into membranes or films, dropping in the poor solvent for beads, crosslinking to form gels, etc. Uragami *et al.* (1983) prepared

anion exchange membranes containing amino group from chitosan, poly(vinyl alcohol), and glutraldehyde, and proposed 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 the 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 propranoyl hydrochloride. These properties of composite membranes were found to depend on the concentration of collagen and chitosan in membranes.

2.2.2 Chemical Modification

Since the amino as well as the primary and the secondary hydroxyl groups in chitin-chitosan are reactive for chemical reactions, the chemical modification is the approach to develop novel derivatives. In many cases, the chemically modified chitin-chitosan show unique properties than the original polymer.

Owing to the reactive amino group, chitosan has received much more attention in chemical modification than chitin. The amino groups on chitosan chain, can act as a nucleophile, since it contains nitrogen atom with an unshared pair of electrons. N-Acylation (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 and in aqueous solution of fatty acid. Kurita *et al.* (1982) proposed the preparation of N-phthaloylated organo-soluble chitin in DMSO. 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 base derivative in gel form (Hirano

et al., 1983). These derivatives have been used as an intermediate for the chemical modification of chitosan.

The chemical modification at hydroxyl group is also reported. Alcohols are versatile starting materials for the preparation of alkyl halides, alkenes, carbonyl compound, ethers, etc. The regioselective modification of chitosan derivatives shows much improved properties. The primary hydroxyl group at C-6 position is more reactive than the secondary hydroxyl group at C-3 position. Nishimura *et al.* (1991) reported the facile conversion of phthaloylchitosan into several 6-O-substituted derivatives such as triphenylmethyl(trityl) and (p-tolylsulfony)oxy(tosyloxy) groups under mild conditions in homogeneous solution. 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 swell in organic solvents.

2.3 Chitosan for Surface Functionalization

Surface functionalization is a technique to introduce the functional groups on a material surface. In this way, functional materials can be designed based on the various forms of the products. Surface modification can induce the multifunctionality, such as mechanical properties, bio-related properties, optical, and electrical properties, etc. It is important to note that surface functionalization is a possible way for the combination of two different materials, i.e. organic/iorganic material, or hydrophobic/hydrophilic material. Silane coupling agent is one of the surface functionalization methods to overcome the limitation of organic/inorganic component, whereas radiation grafting technique is the most widely used for the combination of hydrophobic/hydrophilic one.

Radiation-induced grafting reaction have receive much attention the the field of surface functionalization technology. The use of technique started in 1950s and continued to be a subject of an intensive research with the objective of obtaining modified materials for various applications. The radiation enables the changes of polymer in wettability, adhesion, metallization, anti-fog properties, anti-static properties, and biocompatibility (Betz *et al.*, 2003).

Radiation-induced surface functionalization is known for the simplification of the whole treatment process, without the detrimental residue and cost reduction (Abdel-Barry and El-Nesr, 1997). The ability to initiate surface functionalization in a wide range of temperatures including no limitation in state of functional substance such as in bulk, solution, and emulsion and even at solid state (Chapiro, 1997) is also attraction.

The surface modification of polymer membrane becomes important when polymeric material is contacting with the physiological component such as blood and living tissues. Liu *et al.* (1999) fabricated a platelet-compatible polymer by surface modification of polyethylene membrane with phosphorylcholine derivatives. Kwon *et al.* (1999) produced polyethylene glycol methacrylate-*graft*-polyethylene film by using γ-ray irradiation. The adsorbed protein and platelet adhesion on the polyethylene film surface decreased rapidly with the grafting yield. In addition, the methyl methacrylate grafted onto ultra high molecular weight polyethylene to modify its surface adhering with human bone and organic or inorganic material also has been studied (Kwon and Nho, 2002).

It is known that radiation-induced grafting reaction offers a unique way to combine two highly incompatible polymers and imparts new properties (Guilmeau *et al,* 1997). As chitosan is a hydrophilic bio-polymer and most of the commercial thermoplastics are hydrophobic materials, radiation induced grafting might be possible for surface functionalization. Chitosan as a bio-additive for surface functionalization with commodity polymer might be good material for biocompatible medical material, such as artificial blood vessel.

Wang *et al.* (2003) demonstrated that chitosan and heparin can covalently immobilized onto a poly(lactic acid-*co*-glycolic acid) (PLGA) surface using *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide(EDC), *N* hydroxysuccinimide (NHS) in

a 2-morpholinoethane sulfonic acid (MES) buffer system. Mao *et al.* (2004) improved the blood compatibility of polyethylene (PE) film by grafting O-Butyrylchitosan (OBCS) on to film surface. They found that the blood compatibility of OBCS-*graft*-PE film is better than that of blank PE film. Zhu *et al.* (2005) developed a novel method of binding of chitosan/heparin (CS/Hp) complex to the surface of vascular graft to reduce platelets adhesion. The binding of chitosan was achieved by coating the azide modified chitosan on the PTFE surface and irradiating with ultraviolet light. Zhu et al. (2006) prepared poly (ethylene terephthalate) (PET) film treated by argon plasma and followed by graft copolymerization with acrylic acid (AAc). The obtained PET-surface grafted PAA (PET-g-PAA) was coupled with chitosan (CS) and *o*-carboxymethylchitosan (OCMCS) molecules. The PET-g-PAA surface containing carboxylic acid, CS immobilized PET surface containing amino and OCMCS immobilized PET surface containing both carboxylic acid and amino groups, exhibited a hydrophilicity. The anticoagulation of PET-OCMCS is ascribed to the suitable balance of hydrophobicity/hydrophilicity, and the low protein adsorption. They suggested that this method could be applied to not only PET films but also poly(vinyl chloride) (PVC), polyurethane, cellulose, silicon rubber and the other polymeric biomaterials.

2.4 Chitosan Bio-additive for Antioxidant Compounding in Commodity Polymer

As chitin and chitosan are natural polymers and they exhibit the non-toxic, biodegradability and biocompatibility, the use as a bio-additive is an approach to change chitosan to value-added products. Chtiosan have been studied as bio-additive in various applications.

One of the earliest applications of chitin-chitosan is the chelating agent for harmful metal ions, such as copper, lead, mercury, and uranium from wastewater. Hirano *et al.* (1983) and 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.

The use in cosmetic is also proposed. For example, Lang and Clause (1988) suggested 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.

Using chitosan as a plant growth promoter is another simple applications. Chitosan has been investigated for the effects on the growth and development of plant, i.e. orchid plant meristemic tissue in culture (Nge *et al.*, 2006). Ribeiro *et al.* (2007) demonstrated the ability of chitosan based coating to expand the shelf-life of strawberry fruit (*Fragaria ananassa*) mainly for industrial applications. The effects coatings on fresh strawberries were assessed by determining the color change, firmness, weight loss, soluble solids and microbiological growth over 6 days.

Qin *et al.* (2006) indicated that water-insoluble chitosan in acidic medium exhibited inhibitory effect against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.* The water-insoluble chitosans with M_w around 5×10^4 were the optimum for antimicrobial activity in the tested samples.

Chitin-chitosan is reported for the effectiveness to reduce the cholesterol. Various hypolipemic formulations containing chitin-chitosan, including particle, powders, solutions and injections, were prepared and studied on oral administration (Suzuki *et al.*, 1988). In oral test on mice, chitosan effectively decreased the cholesterol level in blood up to 66%. The hypercholesterolemia activity of chitosan has not been clarified but it was probably due to its inhibition of lipid-micelle formation (Muzzarelli *et al.*, 1984). .

Chitosan is developed into strong fibers for the use in the chopped fibers. Chitosan fibers are incorporated into a nonwoven matrix used as suture material and a wound dressing material (Alemdaroglu *et al.*, 2006).

Recently, chitosan has received much consideration as a bio-additive in compounding process. Trong-Ming *et al.* (2006) studied the increase in physicochemical compatibility between chitosan and poly (vinyl alcohol), chitosan-*graft*-poly(vinyl alcohol)/poly(vinyl alcohol) (CS-g-PVA/PVA) blends were prepared. They demonstrated that the cellular compatibility of PVA was improved due to the incorporation of chitosan.

In the recent year, chitosan have been considered as a bio-additive based antioxidant to expand a broad range of its applications.

Xie *et al.* (2001) prepared the water-soluble chitosan derivatives by grafting copolymerization of maleic acid sodium onto hydroxypropyl chitosan and carboxymethylchitosan sodium. The chitosan derivatives obtained shows the antioxidant activity against hydroxyl radicals.

Kamil *et al.* (2002) studied the antioxidant activity of chitosan of different viscosity in preventing lipid oxidation in the herring flesh model system. The oxidative stability of treated flesh fish was determined and compared with those treated with conventional antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) at a level of 200 ppm. They found that the low viscosity chitosan (14 cP) exhibited the strongest antioxidative effect.

Xing *et al.* (2005a) studied the antioxidant potential of the differently regioselective chitosan sulfates. The data obtained in vitro models clearly establish the antioxidant potency of all kinds of sulfated chitosan. These in vitro results suggested the possibility that sulfated chitosan could be effectively employed as ingredient in health or functional food, to alleviate oxidative stress.

Xing *et al.* (2005b) demonstrated that low molecular weight chitosan and chitosan sulfate derivative had stronger scavenging effect on $O_2^{\bullet -}$ and $^{\bullet}OH$ than high molecular weight chitosan. For example the $O_2^{\bullet -}$ scavenging activity of low molecular weight chitosan (9 kDa) and high molecular weight chitosan (760 kDa) were 85.86% and 35.50% at 1.6 mg/mL, respectively.

Xing *et al.* (2005c) prepared high-sulfate content chitosan (HCTS) derivative. The HCTS obtained show IC50 for superoxide and hydroxyl radicals 0.012 and 3.269 mg/mL, respectively. Moreover, HCTS had significant reducing power and low chelating activity. They concluded that HCTS exhibits a potential antioxidant.

Based on the literature reports about chitosan and its antioxidant potential, it is important to challenge chitosan for bio-additive based antioxidant which the

compounding with the commodity polymers might offer a unique material with antioxidant ability.

2.5 Motivation of the Present Research

2.5.1 Chitosan Surface Functionalization with Commodity Polymer and its Metal ion Implantation

In the previous reports (section 2.3), chitosan surface functionalization on to commodity polymer was carried out via several steps in preparation. Some derivatives of chitosan have to be prepared for the functionalization. The modification of material surface with chitosan was undertaken mainly focused on the biocompatibility property of chitosan for medical application. Taking into our consideration, chtiosan exhibits ion chelating ability as well as biocompatibility. The functionalization of chitosan with commodity polymer such as polyethylene would provide a new practical material in term of metal ion complex polymer.

In the present work (Chapter III), a method to take advantage of many unique properties of chitosan by attaching it to a common industrial polymer, polyethylene, using gamma radiation grafting technique is mainly demonstrated. One step grafting reaction via the simple, effective, efficient γ-ray irradiation method in aqueous solution is our approach for chitosan surface functionalization onto polyethylene film. According to excellent metal complex of chitosan, this new commodity polymer is able to adsorb the toxic cupric ion for appropriate applications, e.g. ion exchange resin, , toxic metal separator for wastewater treatment, pesticide complex material in preventing some pests species in agricultural application, etc.

2.5.2 Chitosan Bio-additive for Antioxidant Compounding in Commodity Polymer

Although there were many reports to develop chitosan bio-additive as a natural antioxidant according to section 2.4, there is no report about chitosan based antioxidant for compounding in commodity polymer.

It is important to note that polymers require the protection against the effects of heat, oxygen, light, high-energy radiation and so on. Recently, natural antioxidant has been paid much attention as alternative stabilizers for polymers since the toxicity of traditional synthetic antioxidant, e.g. BHA, BHT (Bran, 1975), Tinuvin 770 (Glossmann, *et al.*, 1993), etc., has been concerned. Natural antioxidants are biologically degradable in nature rendering them attractive for use in the stabilization of food packaging, medical packaging, and medical device. In addition, most of synthetic antioxidants are small or low molecular weight molecule resulting in the instability and the loss of antioxidant from polymer matrix. For this reason, to develop even more stable natural antioxidant for compounding in commodity polymer still require further study.

For another view point of bio-additive chitosan (Chapter IV, V, and VI), the present work originally proposes a new approach in chtiosan materialization based natural antioxidant. Here, the reducing agent or H-atom donor introduction to chitosan is an attractive approach to achieve the antioxidant anchoring polymer. Based on this approach, the molecular design of chitosan will be expanded to accomplish the bio-additive based natural antioxidant for compounding with polyethylene via additional functionalization of hydrophobic side chain on the chitosan.