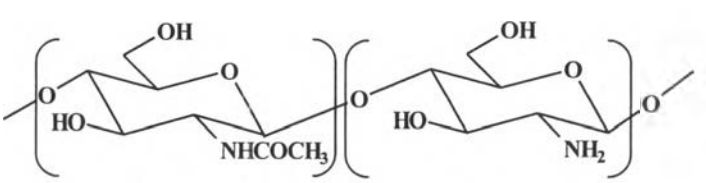
CHAPTER I
INTRODUCTION

1.1 Chitin-Chitosan: An Attractive Biopolymer

Chitin (β-1,4-linked-2-acetamido-deoxy-D-glucopyranose) is the second most natural occurring abundant polysaccharide and is obtained from shells of crustaceans, insects and plant cell walls. Chitosan (β-1,4-linked-2-amino-deoxy-D-glucopyranose) is a deacetylation derivative of chitin. The basic chemical structure of chitin-chitosan (Scheme I) is a co-polysaccharide chain having two hydroxyl groups at C_3 and C_6 positions and an amino or acetamide group at C_2. Basically, the hydroxyl and amino groups of chitin-chitosan act as crosslinkable sites to produce membranes (Qurashi *et al.,* 1992), gels (Hiroyuki and Masato, 1997), and beads (Eric *et al.*, 1998). The glucopyranose ring backbone is nontoxic (Chandy *et al.,*1992), biodegradable (Amano *et al.*, 1978), and biocompatible (Singh *et al.*, 1994), all of which are attractive for biomedical applications. The free amino group can be protonated to give cationic polymer which is effective for chelating with metal ions (J. Guzman *et al.*, 2002) and also has antimicrobial activity (Hirano and Nagao, 1989).

Scheme I



Chitin **Chitosan**

1.2 Limitation of Chitin-Chitosan

For more than three decades, many approaches to apply chitin-chitosan based on its unique properties have been proposed. At present, almost all practical items of chitin-chitosan available in the market are achieved from physical modifications that change the flakes to beads (Eric *et al.*, 1998), membranes (Qurashi *et al.,* 1992), and powders (Sawayanagi *et al.*, 1982).

Although chemical modification is well known, the preparation of chitosan derivatives always faces the problems of high molecular weight and strong inter- and intramolecular hydrogen bonding (Figure 1). Chitosan does not show a glass transition temperature but degrades before melts. The ability to carry out chemical reactions on chitin-chitosan is limited because of its insolubiltiy in common solvents. In most cases, the reactions have to be done in heterogeneous conditions such as, tosylation (Kurita *et al.*, 1992), phthaloylation (Nishimura *et al.*, 1990), and N-acylation (Fujii *et al.*, 1980). The reactions are also accompanied by various problems, such as, low extents of reaction, difficulty in regioselective substitution, product nonuniformity, and partial degradation during the unavoidable harsh reaction conditions. Thus, much attention on chemical modification the chitosan is about improving the solubility for homogeneous effective reaction.

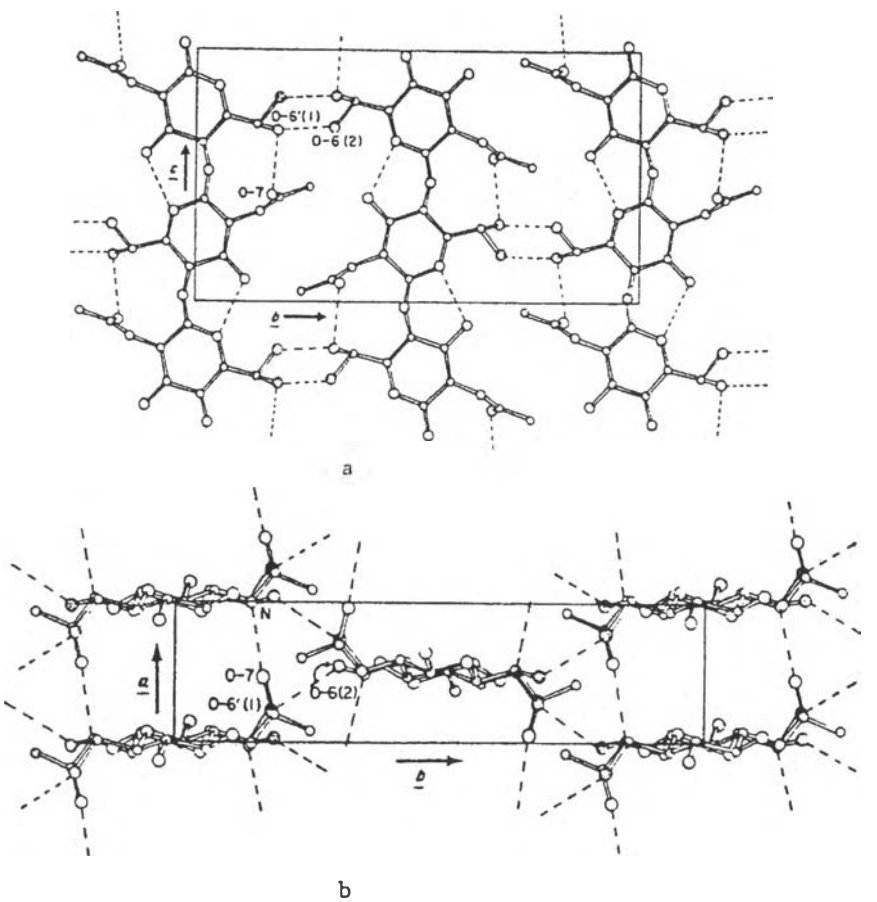


Figure 1. Structure of α-chitin; (a) *bc* projection, and (b) *ab* projection (Minke and Blackwell, 1978).

1.3 Approach to Overcome Limitations

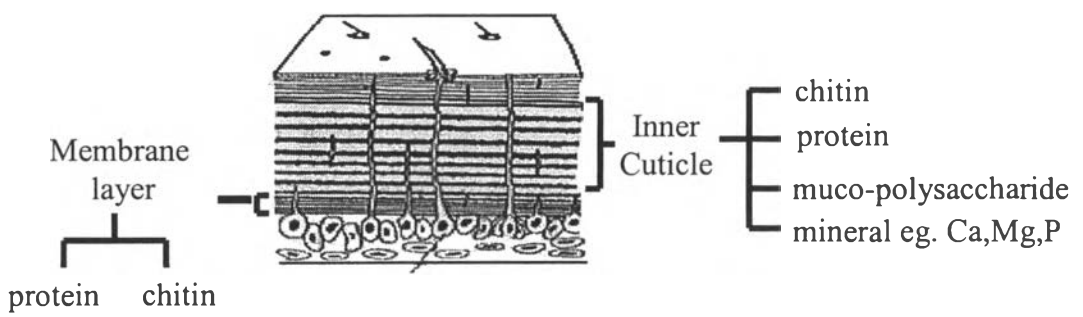
One of the approaches to overcome the solubility problems is to reduce the molecular weight. As a result, the inter- and intramolecular hydrogen bonds are decreased as well as the reactivity is improved. Recently, low molecular weight chitosan has been reported to have versatile functional properties, such as antitumor activity (Tsukada *et al.,* 1990), immuno-enhancing effects (Suzuki, 1996), antifungal activity (Hirano and Nagao, 1989), and antimicrobial activity (Hirano and Nagao, 1989). Thus, the preparation of low molecular weight chitosan has received much attention to clarify the optimal conditions for production of as-designed molecular weight. Low molecular weight chitosan (LMCS) can be prepared by enzymatic degradation, γ ray-irradiation, and chemical treatment. Enzymatic hydrolysis is an attractive method because of the mild reaction conditions and high specific cleavage. For example, endochitinase performs the chain scission of chitin at the β-(1,4) glycosidic linkage of N-acetylglucosamine within the chain whereas exochitinase exhibits the cleavage at the end of the chain. However, this method requires many steps in the enzyme preparation process. Photoirradiation is an approach to apply the high radiation energy with a large scale production whereas no purification step is required. Rangrong *et al.* (2001) determined the optimum γ-ray irradiation condition at 25 kGy to obtain 75% chain degradation with limited crosslinking or changes in structure. It is also found that the reduction of molecular weight reached a steady state of 10^5 gms/mole when the starting molecular weight is above $7x10^5$ gms/mole. Chemical treatment is an effective method to obtain low molecular weights around 10^4 gms/mole although the chemical waste and purification are involved. For example, Allan *et al.* (1997) reported the depolymerization of chitosan by the action of HONO for producing chito-oligosaccharides at a desired molecular size. Moo-Yeal Lee *et al.* (1999) prepared oligochitosan (DP = 5-7) using 35% hydrochloric acid for 2 h. at 80°C.

Although the oligomerization by acid hydrolysis are reported, the depolymerization for low molecular weight and the structural changes are rarely mentioned. Thus, it is important to clarify the effect of hydrolysis process on chitosan chain and structure.

1.4 Approach to develop Chitin-Chitosan for Biomimetic composite material

Crab shell is a superb nano- and biocomposite material consisting of organic polymer chain of polysaccharide (chitin-chitosan) and protein while the inorganic minerals of calcium and magnesium are interpenetrating (Scheme II). Weiner and Addadi (1988) extracted proteins from mineralized crab shells and clarified the proteins that existed between the surfaces of the calcium carbonate crystals and chitin. Naturally, the layer structure of chitin-chitosan and protein harden after binding with calcium and magnesium.

Scheme II



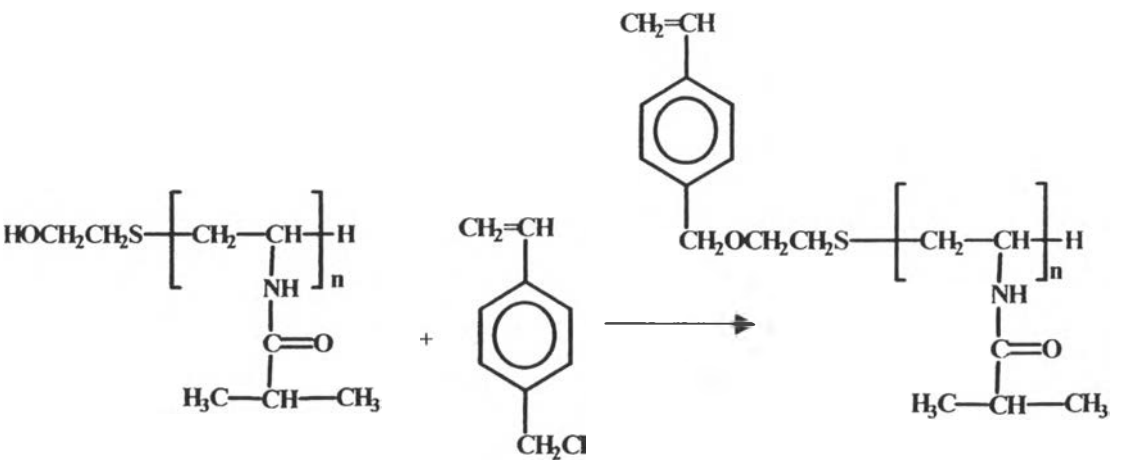
Zhang and Gonsalves (1995) prepared $CaCO_3$-chitosan composites by dipping chitosan in a supersaturated $CaCO_3$ solution for the crystal growth of $CaCO_3$ on chitosan. Recently, our group has paid attention to the layered structure of crab and shrimp shells in order to design chitosan chain layer polymers where we expect for high adsorptivity with controlled hardening properties. It is a challenge to develop chitin-chitosan layer structure with synthetic polymer chain that mimics that of the crab and shrimp shell.

1.5 From Macromonomer Concept to Chitosan Macromonomer

For the past few years, macromonomers have been applied for preparation of novel polymeric materials. A macromonomer is an oligomer or polymer chain conjugated with one or more reactive monomer. The reactive species are vinylic, acetylenic or heterocyclic. The reactive vinyl terminal groups can be introduced by end-capping or living polymer deactivation.

Takeshi *et al.* (1998) prepared poly (N-vinylisobutyramide) macromonomer by N-vinylisobutyramide oligomer conjugated with a reactive monomer of p-chloromethylstyrene. (Scheme III).

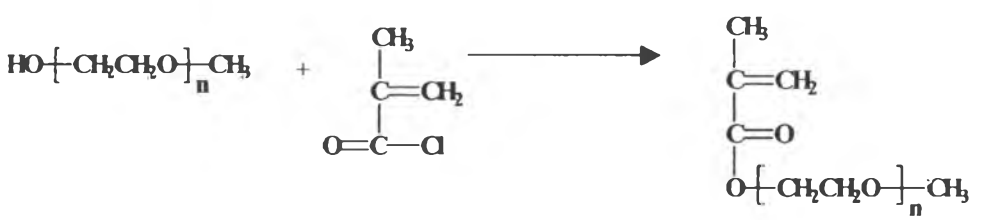
Scheme III



NVIBA Oligomer p-Chloromethylstyrene PNVIBA Macromonomer

Hiwatari (2001) used macromonomer approach to prepare nanospheres by conjugating the methacryloyl chloride (MA) with poly(ethylene glycol) (PEG) (Scheme IV).

Scheme IV



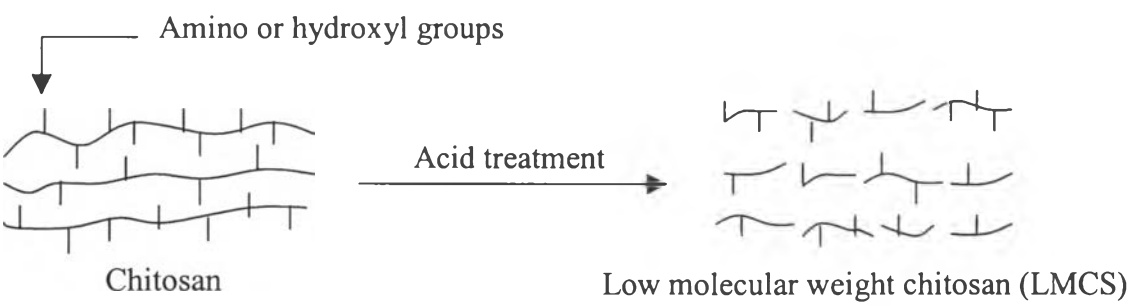
M-PEG Methacryloyl chloride MA-PEG macromonomer

1.6 The Scope of the Present Work

In order to achieve chitosan-based biomimetic composite materials, the present work applies the concept of macromonomers by using low molecular weight chitosan as a polymer chain conjugated with synthetic monomer. In this case, a reactive vinyl group can be attached on chitosan, either at the amino group or

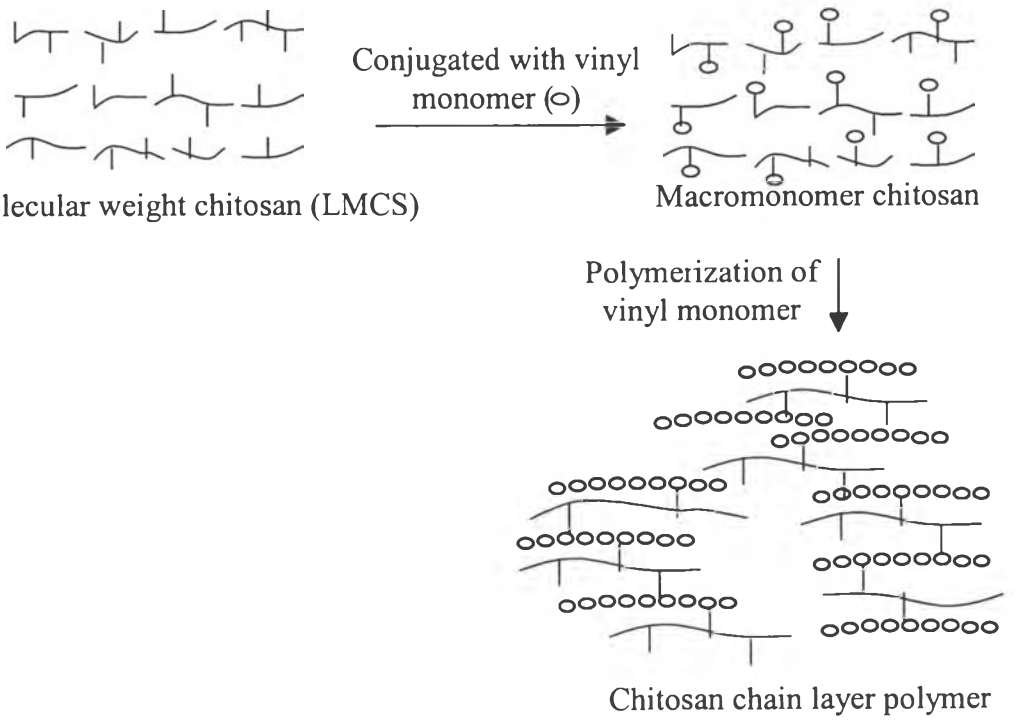
hydroxyl group, to obtain a macromonomer chitosan. The polymerization of chitosan macromonomer with vinyl monomer may possibly bring a successful of chitosan chain layer polymer. Thus, as shown in Scheme V, chapter 2 deals with the preparation of low molecular weight chitosan. The optimal conditions are aimed at achieving water-soluble chitosan without a significant change in structure.

Scheme V



Chapter 3 (Scheme VI) proposed an original concept of macromonomer chitosan. The main studies are about the conjugation of monomer onto low molecular weight chitosan to obtain macromonomer. The studies also extend to polymerization of chitosan macromonomer for controlled structure chitosan-synthetic polymer. The properties of the polymer are also studied to evaluate the structure of the polymer indirectly.

Scheme VI


Conjugated with vinyl
monomer (○)
Low molecular weight chitosan (LMCS)
Macromonomer chitosan
Polymerization of
vinyl monomer
Chitosan chain layer polymer