CHAPTER II
LITERATURE REVIEW

This thesis attempts to synthesize, characterize and fabricated the *N*-carboxyacyl chitosan, one of the water soluble chitosan derivatives for using as biomaterials. As previously studies, several derivatives of chitosan have been modified in order to improve their solubility and enhance their biological properties in neutral and high pH conditions. Therefore, this review can be divided into the following aspects.

2.1 Introduction to Chitosan
2.2 Biological Properties Chitosan
2.3 Fabrication of Chitosan as Biomaterials
2.4 Chemical Modification of Chitosan into Water Soluble Derivatives and some of their Interesting Biological Properties
2.5 Synthesis of *N*-Carboxyacyl Chitosan
2.6 Fabrication of *N*-Carboxyacyl Chitosan Derivatives as Biomaterials

2.1 Introduction to Chitosan

Many polysaccharides have been developed as novel biomaterials for use in medical field because of their unique structure and characteristics as well as their inherent biological properties. Unlike cellulose (Figure 2.1), chitin and chitosan (Figure 2.2) are an amino polysaccharides which are a unique structure for providing distinctive biological properties and for conducting modification reactions therefore, they advantages over cellulose are both abundant resource and unique biological properties. However, chitosan is more interesting utilizable biomaterial than chitin because of their greater solubility, higher reactivity and promoter many biological properties.

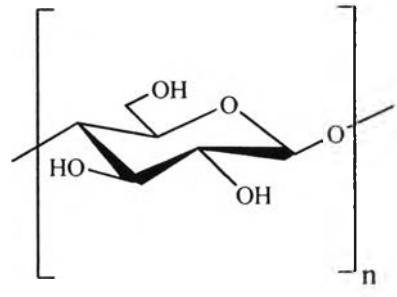


Figure 1. Chemical structure of cellulose (Kurita, 2001).

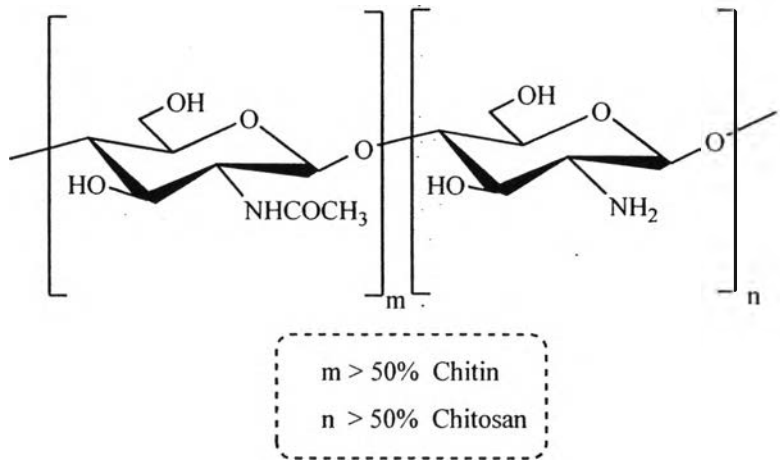


Figure 2. Chemical structure of chitin and chitosan (Kurita, 2001).

2.1.1 Sources and Production of Chitin

Chitin, a crystalline polysaccharide, composed of poly-β-(1,4)-*N*-acetyl-D-glucosamine and mainly found fungi, algae, nematodes and arthropods (insects, crustaceans, arachnids and myriapods). The structural patterns of chitin are presented in three polymorphic forms with different orientations of the microfibrils, known as α -, β- and γ-chitin. The most stable polymorphic form of chitin, α-chitin which has an antiparallel structure is commonly found in crustaceans and cuticles of insects. Unlike α-chitin, β-chitin which was a loose packing parallel structure can be isolated from the squid pens (*Ommastrephes*in *bartrami* and *Loligo* species) and cuttlefish (*Sepia officinalis*). Not common form, γ-chitin is a mixture of antiparallel and parallel structure which was found in the cocoons of insects and cell wall of fungi. Because of their weak intermolecular interaction, β-chitin has higher solubility and greater reactivity for deacetylation and chemical modification than α-chitin. However, the production of chitin is commercially obtained from an exoskeleton of

crab and shrimp shells because of their wastes from many seafood industries. To exact chitin, demineralization and deproteinization have been commonly performed.

2.1.1.1 Demineralization

Demineralization is the process to remove the mineral component of the exoskeleton of the shells. The most of mineral containing in the exoskeleton of the shells is Ca and the others mineral are Na, P, Mg and K which was depend on the sources of chitin. The exoskeleton of the shells was treated with diluted HCl aqueous solution for about three times (Tolaimate, *et al.*, 2003) at room temperature to remove all of these minerals.

2.1.1.2 Deproteinization

To obtain chitin, the dematerialized chitin was further deproteinized by treating with the low concentration NaOH aqueous solution (0.3-2 M) at 65-100 °C which was repeated several times as shown in Table 2.1.

Table 2.1 Production of chitin in various conditions (Tolaimate, *et al.*, 2003).

Source	Deproteinization				Demineralization		
	NaOH (M)	Temperature (°C)	Number of baths	Treament Duration (h)	HCl (M)	T (°C)	Treament Duration (h)
Carb	0.5	65	1	2	1.57	T_{room}	5
Shrimp	0.125	100	1	0.5	1.25	T_{room}	1
	0.750	100	1	-		T_{room}	
Krill	0.875	90-95	1	2	0.6	T_{room}	2
Crab	1	80	1	3	1	T_{room}	12
Crab	1	100	1	36	2	T_{room}	48
Crab	1.25	85-90	1	24	1.37	T_{room}	24
Shrimp	1.25	100	1	0.5	1.57	20-22	1-3
Crab	2.5	Room	3	72	11	-20	4
Lobster	1	100	1	60	2	T_{room}	5,48
Crab	1	100	5	72	1	T_{room}	-
Squid	2	Room	3	1 night	1	T_{room}	1 night
	2	100	2	4		T_{room}	
12 Species of crustaceous and cephalopods	0.3	80-85	2-7	1 h/bath	0.55	T_{room}	15 mn to 1 h by bath repeated 2-5 times

However, the pigments traces of the chitin can be removed by dry the shells with sunlight and/or treating with a mild oxidizing treatment (H_2O_2/33% HCl in a volume ratio 9/1).

2.1.2 Production of Chitosan

Chitosan was produced by *N*-deacetylation process of chitin. There are two general methods to produced chitosan. The first method was studied by Broussignac (Broussignac, 1968). The obtained chitin 500 mg was suspended in the mixed solvent (50 w/w% potassium hydroxide, 25 w/w %96% ethanol and 25 w/w% monoethylene glycol) 30 ml. The mixed solvent is exothermic reaction then the temperature will rise up to 90°C during this step.

The second method which was used to produce chitosan is the method of Kurita and coworkers. (Kurita, *et al.*, 1993). The isolated chitin 500 mg was dispersed in 30 ml of 50% w/v NaOH aqueous solution which was heated up to 80°C, for example, under a nitrogen stream with stirring.

Table 2.2 The deacetylation process in various conditions (Tolaimate, *et al.*, 2003).

Original chitin	Deacetylation process				Acetation degree (DA%)
	NaOH (w/w%)	Temperature (°C)	Duration (h)	Addition $NaBH_4$	
β- Chitin squid pen DA=100%	40	80	9	-	17
	40	80	3h × 3	-	1
	40	80	3h × 3	+	0
	40	80	6	-	20
	40	80	3h × 2	-	3
	40	80	3h × 2	+	5
α –chitin grey shrimp, DA=100%	50	120	12	-	3
	50	120	6h × 2	-	3.9
	50	120	6h × 2	+	1.8
	50	120	4h × 2	+	1
	50	120	3h × 4	+	0
	50	120	3h × 3	+	1

+ the present of $NaBH_4$,- the absent of $NaBH_4$

The condition parameters of these two methods including reaction conditions, reaction time, temperature, concentration and type of alkaline reagent were compared as shown in Table 2.2. Finally, the obtained chitosan was filtrated and neutralized with distilled water and dry in air or an oven at 50°C. Moreover, to prevent polymer degradation, $NaBH_4$ and thiophenol may be added (chitin/$NaBH_4$ or thiophenol weight ratio equal to 1/1) during deacetylation process of chitin.

According to chitosan production process, various degree of deacetylation (%DD) and molecular weight are obtained. These two parameters have an effect on their physicochemical properties as well as biological properties.

2.1.3 Solubility of Chitosan

Chitosan, a semi crystalline structure, can be dissolved in dilute acid aqueous solutions such as hydrochloric acid, diluted acids of formic, acetic, oxalic and lactic acid because the unique amino group of chitosan can be protonated in these solvents. However, an increase in acid concentration can reduce the solubility of chitosan and some of dilute acid aqueous solutions are not a good solvent for chitosan such as citric, sebacic, phosphoric acids, etc.

It was found that the solubility of chitosan is also depended on both molecular weight and %DD of chitosan. It was found that the lower molecular weight of chitosan showed greater water solubility (Feng *et al.*, 2008) because of lower inter molecular interaction (i.e. van der waals forces) of lower molecular weight. Moreover, the %DD around 50% (Sannan and Kurita, 1976; Varum, *et al.*, 1994) can be dissolved readily in distilled water.

2.2 Biological Properties of Chitosan

According to its natural deriving polymer and same structure to glycosaminoglycans as hyaluronic acid Figure 2.3 naturally presented in human skin and body, chitosan has been extensively studied for numerous bioactivities including biocompatibility, biodegradability and would healing properties. Moreover, the unique amino groups of chitosan can exhibit the inherent its special properties as mucoadhesive, antimicrobial and antioxidant properties.

2.2.1 Biodegradability

Chitosan shows similar structure to HA which can be degraded by lysozyme into mono and disaccharides in our body and further converted into ammonia, carbon dioxide and water via the Krebs cycle (Al-Assaf, *et al.*, 2003). Evidently, chitosan can be degraded by *in vitro* and *in vivo* studies. *In vitro* studies, chitosan can be degraded by various types of enzymes such as chitinase, lysozyme and peptien (Nordtveit, *et al.*, 1996) and, *In vivo* studies, chitosan is also degradated by lysozyme though the hydrolysis of acetyl residual groups. It was suggested that the lysozyme susceptibility depends on solubility and %DD of chitosan (Varum, *et al.*, 1994; Freier, *et al.*, 2005). Therefore, an increase in %DD exhibited lower lysozyme susceptibility. It was found that chitosan at %DD around 50% showed the highest lysozyme susceptibility because chitosan which was %DD around 50% have high solubility than other conditions.

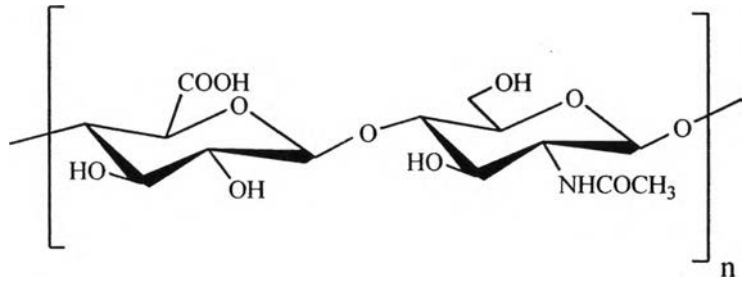


Figure 3. Chemical structure of hyaluronic acid (Freier, *et al.*, 2005).

2.2.2 Would Healing Properties

Due to its similar structure to the hyaluronic acid presented in skin, chitosan can be act as a wound healing accelerator. The several type of animals (rat, rabbit, dog and cat) were tested for would healing properties of chitosan and the result suggests that chitosan can induce granulation tissue without an excessive tissue and without the formation of scar (Chandy and Sharma, 1990; Okamoto, *et al.*, 1993a; Ueno, *et al.*, 1999). In addition, chitosan can promote the functions of inflammatory cells such as polymorphonuclear leukocytes (PMN), macrophages and fibroblasts cells chitosan induce fibroblasts to release interleukin-8, which is involved in migration and proliferation of fibroblasts and vascular endothelial cells (Ueno, *et al.*, 2001). The following study of linear incisional wound in rats was also

supported that the lower molecular weight (oligomer and monomer) and the higher degree of deacetylation of chitosan can accelerate the wound healing by increase the wound break strength and collagenase activity which can observed more activated fibroblast cell (Minagawa, *et al.,* 2007). Moreover, chitosan treated with burn wound of rat can reduce the healing and complete recovery of the skin and hair covers occurs without the formation of scars (Antonov, *et al.,* 2008).

2.2.3 Antimicrobial Properties

It is known that chitosan has effective property to inhibit the growth of several microbial cells. The important factor of the antimicrobial activity of chitosan is the positive characteristic of chitosan (pKa <6.5) can interact with negative substances of microbial cells such as DNA molecules (Kendra and Hadwiger, 1984), phospholipids (Liu, *et al.,* 2004) and teicolic acid (Raafat, *et al.,* 2008) of cell membranes that lead to the leakage of intracellular constituents of microbial cell (Papineau, *et al.,* 1991; Sudarshan, *et al.,* 1992; Helander, *et al.,* 2001).

The antimicrobial properties of chitosan also depend on concentration of chitosan, molecular weight of chitosan and type of microbial cell. It was indicated that the antimicrobial activity mechanism of chitosan is different on different types of microbial cells (Zheng and Zhu, 2003). It was found that the higher concentration of chitosan can enhance the antimicrobial activity. Chitosan can promote the antimicrobial activity against a gram negative *Escherichia coli* (*E. coli)* when the molecular weight of chitosan decreases because the lower molecular weight can enter to the bacterial cells and interact with the negative substance which disturbed the metabolism of the cell. In contrary to that of *E. coli*, chitosan can promote the antimicrobial activity against a gram positive *Staphylococcus aureus* (*S. aureus*) when the molecular weight of chitosan increases because the higher molecular weight of chitosan can forms a film which inhibits nutrient adsorptions of this cell.

2.2.4 Mucoadhesive Properties

Because of its cationic nature, chitosan has been evaluated for its mucoadhesive property (Lehr, *et al.,* 1992). Chitosan exhibited much higher detachment force with the pig intestinal mucosa than other types of natural polymers

such as non-ionic polymers (i.e., hydroxypropylcellulose, scleroglucan and hydroxyethyl starch) and anionic polymers (i.e., pectin, xanthan gum and carboxymethylcellulose). This result revealed that the important factor to enhance the mucoadhesive properties of chitosan is the electrostatic interaction between cationic charge of chitosan and negative charge of mucosal surface. Chitosan has been incorporated with polyacrylic acid a well known for excellent mucoadhesive polymer in order to overcome its solubility problem. The polyacrylic acid-chitosan complex can improve the adhesive force and reduce aqueous solubility of polyacrylic acid itself that can prolong the duration time for the drug to permeate across the membrane (Ahn, Choi and Cho, 2001). Moreover, thiolated modified chitosan showed the grater adhesion time to porcine small intestinal mucosa than unmodified chitosan 20 fold due to the immobilization of thiol groups. The adhesion time of thiolated chitosan was improved about 80 fold, whereas that of thiolated poly (acrylic acid) was 17 fold and that of thiolated polycarbophil (a high molecular weight poly acrylic acid copolymer) improved only 2.9 fold (Grabovac, *et al.*, 2005).

2.2.5 Antioxidant Properties

Chitosan has been proved as a natural antioxidant due to their active hydroxyl and amino groups. It was suggested that the scavenging activity of chitosan was occurred due to its residual free amino groups and hydroxyl groups. These reactive groups of chitosan can react with various free radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, superoxide anion radicals and hydrogen peroxide radicals. The hydroxyl groups of chitosan can react with free radicals by the typical H-abstraction reaction (Xue, *et al.*, 1998) to form stable macromolecule radicals and the amino groups can form ammonium groups by absorbing a hydrogen ion from the solution (Xie, Xu and Liu, 2001). Therefore, the antioxidant activity on various radicals depends on degree of deacetylation. The studies suggested that the increase of degree of deacetylation can enhance this antioxidant activity (Park, Je and Kim, 2004; Yen, Yang and Mau, 2008). Moreover, it was found that the antioxidant activity was also related to its molecular weight. The low molecular weight of chitosan has stronger antioxidant activity than that of high molecular weight of chitosan (Yin, *et al.*, 2002; Feng, *et al.*, 2008) because the higher molecular weight

of chitosan has stronger intramolecular hydrogen bond which can limit the reactivity of their active hydroxyl and amino groups to interact with free radicals (Xing, *et al.*, 2005).

2.3 Fabrication of Chitosan as Biomaterials

According to these biological properties, chitosan has been fabricated into many forms for specifical applying in medical applications such as film for wound dressing (Aoyagi, Onishi and Machida, 2007; Khan and Peh, 2003; Sezer, *et al.*, 2007), bead for drug carrier (Aydin and Akbuğa,1996; Shu and Zhu, 2002), fiber (Sangsanoh, *et al.*, 2007; Heinemann, *et al.*, 2009) and hydrogel (Martino, Sittinger and Risbuda, 2005;Griffon, *et al.*, 2006) for tissue engineering scaffold.

Due to its available free amino groups, chitosan has been crosslinked by various methods such as chemical agents (i.e. gultaraldehyde, gyloxal, tripolyphosphate), polymer complexation (i.e., chitosan/alginate, chitosan/ polyaspartic acid, chitosan/gum kondagogu), and radiations (UV: Ono, *et al.*, 2000,γ-ray: Yoshii, *et al.*, 2003) and electron beam; Gryczka, *et al.*, 2009). Howerver, the degradation of chitosan can be presented at its low concentration or high radiation dose condition (Jaroslaw, *et al.*, 2005). Therefore, low molecular weight can be produced by using the radiation methods (Hai, *et al.*, 2003). The crosslinking method of chitosan can be generally divided into two types: physically crosslinked chitosan and covalently crosslinked chitosan.

2.3.1 Physically Crosslinked Chitosan

Because of cationic character, chitosan can be crosslinked by various types of anionic crosslinking agents and polyanionics. As previously studies, tripolyphosphate (Akbuğa, 1996; Mia, *et al.*, 2003; Bhumkar and Pokharkar, 2006), citrate and sulfate (Shu and Zhu, 2002) have been used to crosslink between amonium groups of chitosan. Several types polyanionics (i.e., alginate (Lawrie, *et al.*, 2007), polyaspartic acid (Zheng, *et al.*, 2007), gum kondagogu (Naidu, *et al.*, 2009). containing ionizable carboxylated groups can complex with ammonium groups of chitosan known as polyelectrolyte complex (PEC). Moreover, hydrophobic crosslink chitosan exhibiting of hydrophobic interaction between its chains has been produced

by using these hydrophobic groups which are palmitic acid *N*-hydroxysuccinimide (Noble, *et al.,* 1999) and pluronic (Park, *et al.,* 2009).

2.3.2 Covalently Crosslinked Chitosan

To improve the stability and strength of crosslinked chitosan, several chemical agents have been used to crosslink with chitosan at available free amino groups. Glutaraldehyde (Jameela and Jayakrishnan, 1996; Gupta and Jabrail, 2006) and glyoxal (Khalid, 1999; Yang, *et al.,* 2005; Gupta and Jabrail, 2006) are common and effective agents to crosslink chitosan by schiff base reaction. Because of the low biocompatibility and high biodegradability of using these crosslinking agents, (Noble, *et al.,* 1999) chitosan has been crosslinked with genipin derived from natural substance (*Gardenia jasminoides Ellis* fruit) for improving these bioproperties and suitable for medical applications (Mi, Sung and Shyu, 2000; Mia, *et al.*, 2003; Bhattarai, *et al.,* 2005; Yuan, *et al.,* 2007). In addition, radiation can also induced the covalently crosslinked chitosan (Felinto, *et al.,* 2007).

2.4 Chemical Modification of Chitosan into Water Soluble Derivatives and some of their Interesting Biological Properties

It was known that chitosan can not be dissolved in neutral or higher pH conditions. The poor solubility of chitosan was shown due to the inter and intra molecular hydrogen bonding between its structure (Yui, *et al.,* 1994) which can limit the use or reduce the bioactivities of chitosan in these regions. To overcome these disadvantages of chitosan, modified chitosan is an important key to improve their chitosan properties. Due to its available of reactive amino and hydroxyl groups, chitosan can be able to modify with several chemical agents. Modification of chitosan into water soluble not only improve its bioactivities but also safe for directly use or apply to human.

Several water soluble derivatives of chitosan have been synthesized according to the several chemical modifications (i.e., reductive alkylation, quaternization, sulfonation and carboxyalkylation) and evaluated for their biological properties as shown in Table 2.3 and 2.4.

2.5 Synthesis of *N*-Carboxylation of Chitosan and their Characterization

Due to the unique carboxylic group substitution, *N*-carboxylation chitosan showed the anionic properties which posses interesting properties for using as a biomedical material.

N-carboxyacryl chitosan has been firstly synthesized by the method of Hirano and Moriyasu (Hirano and Moriyasu, 1981) as shown in Figure 2.4. Chitosan 0.16 g was dissolved in 2% aqueous acetic acid and the solution was diluted with methanol 20 ml and follow with the addition of anhydride (3–5 mol/gulcosamine unit) which was also dissolved in methanol. This mixture was stirred at 50 °C for about 5 min and allowed to retain at room temperature overnight to obtain this derivatives. The degree of substitution of these derivatives was found between 0.53–0.80.

The water solubility of these derivatives which was degree of substitution (DS) between 0.20-0.43 was found that even in low pH (pH 1) or high pH (pH 13) but they precipitated in solution between pH ~3.5-pH 7.0 due to the isoelectric point of their carboxylated and ammonium groups. It was found that the water solubility of *N*-maleoyl chitosan and *N*-itaconyl chitosan, vinyl containing derivatives, was decreased after keeping them for more than 1-2 weeks because of the reformation of their structures (Sashiwa and Shigemasa, 1999).

N-carboxyacyl chitosan was prepared via ring opening reaction of succinic anhydride in dimethyl sulfoxide system (Yan, *et al.*, 2006) as shown in Figure 2.5. Chitosan was firstly dissolved in dimethyl sulfoxide before adding the succinic anhydride,stir at 60 °C and stand at room temperature for 24h. The obtained product which was degree of substitution 0.33 can not be dissolved in the range of pH 4.5-6.8 due to the interaction between COO^- and NH_3^+. Moreover, the Tg of this derivative was found at 304.16 which was higher than that of chitosan.

Moreover, *N*-succinyl chitosan and *N*-phthaloyl chitosan was prepared via ring opening reaction of these anhydrides in pyridine system (Aiedeh and Taha, 2006) as shown in Figure 2.6.

Table 2.3 The water soluble derivatives of chitosan and some of their interesting biological properties.

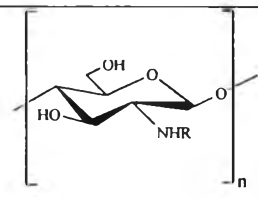
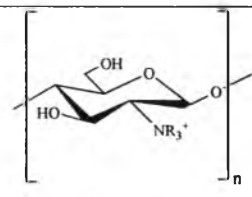
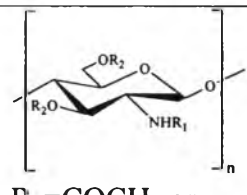
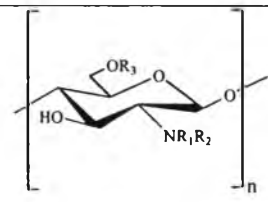
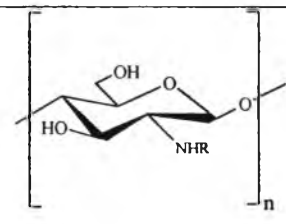
Modification of chitosan	Example of derivatives	Structural repeating unit	Biological activities
Reductive alkylation	-*N*-alkylated disaccharide chitosan	 R=disaccharide (lactose,maltose and cellobilose)	-Antibacterial activity (Yang, Chou and Li , 2004), -Antioxidant activity (Lin and Chou, 2004), etc.
Quaternization	-*N*-Trimethyl chitosan (TMC) -*N*-Diethyl methyl chitosan (DEMC)	 R=CH_3 or R=CH_3,CH_2CH_3, CH_2CH_3	-Mucoadhesivity (Kotze, *et al.*, 1998; Avad, *et al.*, 2004) -Antibacterial activity (Jia, *et al.*, 2000; Avadi, *et al.*, 2004), etc.
Sulfonation	- Sulfate chitosan	 R_1=$COCH_3$ or SO_3H R_2=H or SO_3H	-Anticoagulation (Vongchan, *et al.*, 2002) -Antioxidant (Zhong, *et al.*, 2007), etc.

Table 2.4 The water soluble derivatives of chitosan and some their interesting biological properties

Modification methods	Example of derivatives	Structural repeating unit	Biological properties
Carboxy alkylation	-Carboxymethyl chitosan (*O*-CM chitosan), -Carboxyethyl chitosan (CE-chitosan), -Carboxybutyl chitosan, etc.	 R_1= CH_2COOH, R_2= H or CH_2COOH R_3= H or CH_2COOH	-Wound healing ability (Chen, *et al.*, 2002) -Antioxidant activity (Guo, *et al.*, 2005) -Antioxidant and antimutagenic activity (Kogan, *et al.*, 2004) -Antibacterial activity (Muzzarelli, *et al.*, 1990),etc.
Carboxy acylation	-*N*-succinyl chitosan	 R=$COCH_2CH_2COOH$	-In vivo low toxicity (Song Onishi and Nagai, 1993) -High biological stability (Kamiyama, Onishi and Machida, 1999) Low biodistribution and accumulation (Kato, Onishi and Machida, 2004), etc.

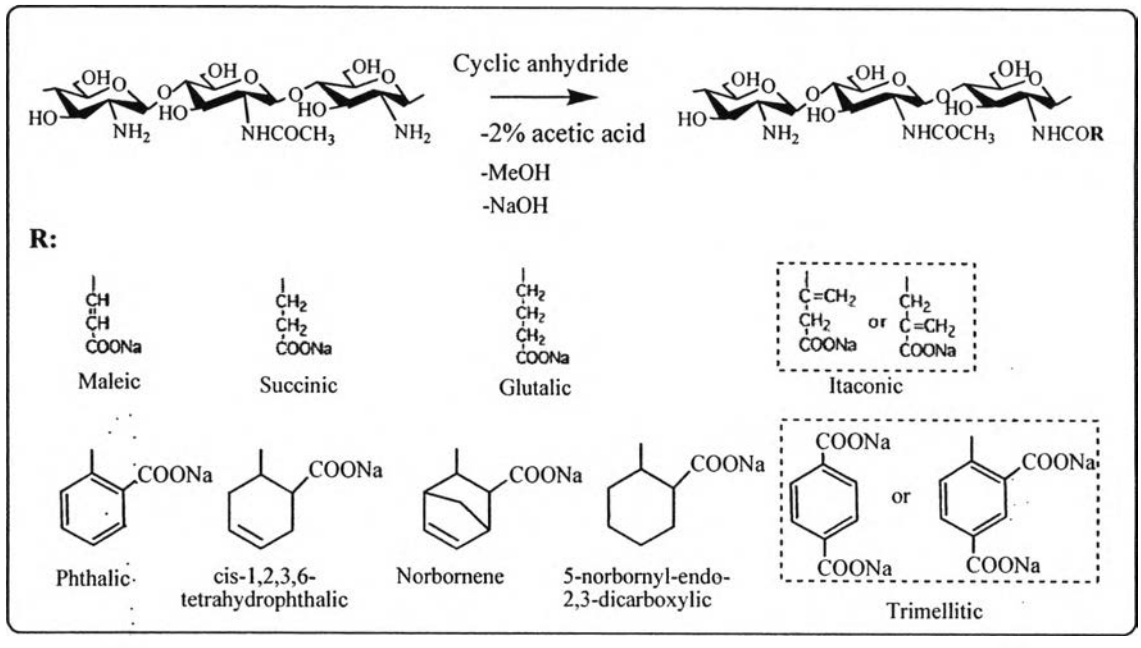


Figure 2.4 Synthesis of *N*-carboxyacyl chitosan in methanol system (Hirano and Moriyasu, 1981).

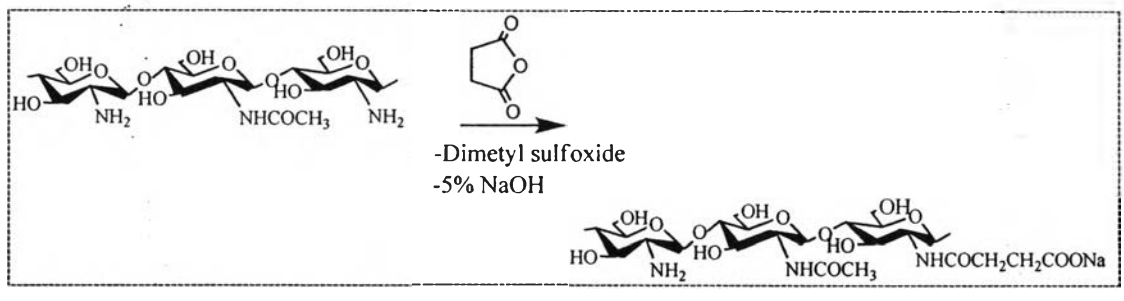


Figure 2.5 Synthesis of *N*-succinyl chitosan in dimethyl sulfoxide system (Yan, *et al.*, 2006).

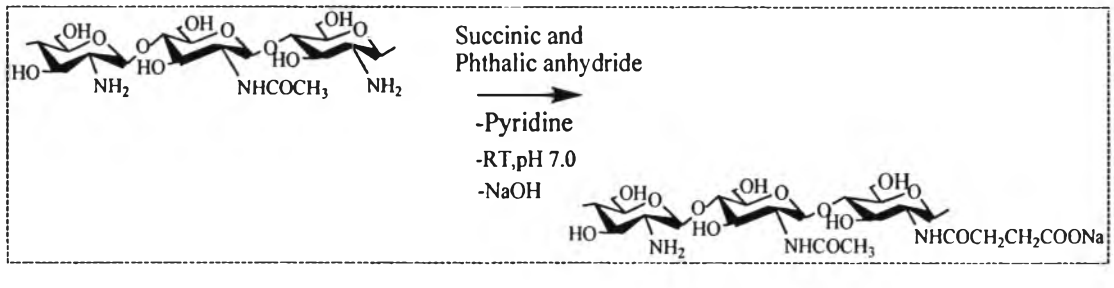


Figure 2.6 Synthesis of *N*-carboxyacyl chitosan in pyridine system.

2.6 Fabrication of *N*-Carboxyacyl Chitosan and their Medical Applications

2.6.1 *N*-succinyl Chitosan

Among of these derivatives, *N*-succinyl chitosan is extensively used for medical proposes. Because of their unique hydrophilic and anionic properties, *N*-succinyl chitosan has been initially used as wound dressing, drug carrier and bioabsorbent.

2.6.1.1 Wound Dressing

N-succinyl chitosan has initially used as a wound dressing (Kuroyanagi, *et al.*, 1994) and this dressing was also developed by blending with gelatin (Tajima, *et al.*, 2000). The result found that this *N*-succinyl chitosan/ gelatin wound dressing provides a suitable moist wound conditions and consequently forming highly vascularized granulation tissue along with decreasing the wound area. Moreover, commercial cosmetic products (Moistfine liquid®) have been produced (Izume, 1998).

2.6.1.2 Drug Carrier

N-succinyl chitosan have been used as macromolecule drug carrier especially for antitumor drug carrier (Song, Onishi and Nagai,1993) because of their low interaction to the blood and tissue which can prolong its half life in the circulating system (Kato, Onishi and Machida, 2000) greater than other carriers (dextran and serum proteins) reported to exhibit relatively long systemic retention. *N*-succinyl chitosan contains many carboxylic groups with can be greatly conjugated with the functional groups of antitumor drug (mitomycin C) and prolong this drug to the specific target site which can enhance the maximum antitumor efficiency. In addition, the lactosaminated *N*-succinyl-chitosan has been modified for liver-specific drug carrier in mice (Kato, Onishi and Machida, 2001). The result suggested the low distribution and low accumulation of this lactosaminated *N*-succinyl-chitosan into the liver as well as long-term retention in the liver because of its negative charge characteristic. Additionally, *N*-succinyl chitosan has a potential for use as a pH-sensitive controlled release or colon-specific drug carrier because they can limit the release of drug in acid condition but they can control the drug release in the basic condition (Aiedeh and Taha, 2006; Dai, *et al.*, 2008).

2.6.1.3 Bioabsorbent

Due to its anionic function, *N*-succinyl chitosan can be served as bioabsorbent which can adsorb several types of heavy metal ions such as Cu^{2+}, Hg^{2+},Zn^{2+}, Pb^{2+},Cd^{2+},Co^{2+}. *N*-succinyl chitosan was modified by using Pb^{2+} (Sun, and Wang, 2006) and CU^{2+} (Sun, Wang and Wang, 2007) as template ions. *N*-succinyl chitosan with Pb^{2+} and CU^{2+} as template ions showed the high selectivity for Pb^{2+} and CU^{2+} adsorption from a mixture of ions.

2.6.2 The Fabrication of *N*-Succinyl Chitosan

According to its interesting properties, *N*-succinyl chitosan has been fabricated into water insoluble soluble forms by using several crosslink agents. *N*-succinyl chitosan can be crosslinked by several means (Figure 2.7) because this derivative contains both COOH and NH_2 groups.

For previously studies, both of ionic crosslink and covalent crosslink have been used for *N*-succinyl chitosan fabrication. For ionic crosslink, tripolyphosphate (TPP) has been used to crosslink at amino residues (Bhumkar and Pokharkar, 2006) and Ca^{2+} has also used to crosslink at carboxylic residues (Dai, *et al.*, 2008; Nobile, *et al.*, 2008)

For chemical crosslink, *N*-succinyl chitosan can form amine bond linkage between carboxylic and amino residues by using 1- ethyl- 3- (3-dimethyl aminopropyl)(EDC)(Ymiaguchi, *et al.*, 1981) as a crosslinking agent. Like the practical method to crosslink chitosan, gultaraldehyde (GA) has been also used to form imine linkages at the amino residues (Suna and Wang, 2006) of this chitosan derivative.

2.7 Gamma Radiation Processing for Chitosan and its Derivatives

Gamma radiation, ionizable energy, is an advantage method to process the materials without using of chemical agents. According to the previous researches, the gamma radiation is useful for crosslinking, degradation and sterilization of chitosan and its derivatives which can also improve their physicochemical and some of biological properties.

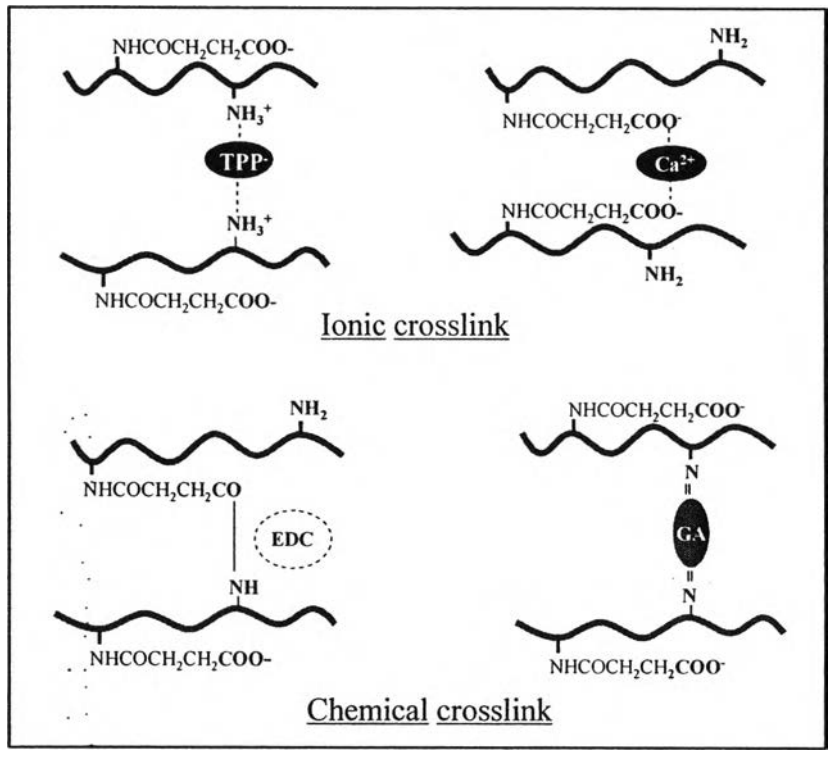


Figure 2.7 The crosslinking process of *N*-succinyl chitosan. (TPP=Tripolyposphate, EDC= 1- ethyl- 3- (3-dimethyl aminopropyl) Carbodiimide, GA=Gultaraldehyde).

2.7.1 Degradation

Gamma radiation can cause degradation in the dilute aqueous solution stated (<10%) and solid stated of various polysaccharides such as chitosan (Choi, *et al.*, 2002; Hai, *et al.*, 2003) and its water soluble derivatives (carboxymethylchitin (Yoshii, *et al.*, 2003), carboxymethylchitosan (Huang, *et al.*, 2007) as well as other types of water soluble polysaccharides (i.e., carboxymethylcellulose, carboxymethylstarch) (Yoshii, *et al.*, 2003). It was found that the reduction of molecular weight occurred at glycocidic linkage (Wasikiewicz, *et al.*, 2005) in dilute aqueous solution is higher than in solid powder due to the greater effect of water radiolysis. The grater reduction of molecular can be more induced by the presence of hydrogen peroxide (Kang, *et al.*, 2007) and nitric oxide (Huang, *et al.*, 2007). After degradation, the improvement of physicochemical as well as biological properties of the low molecular weight of chitosan and its derivatives were found such as the water solubility (Kang, *et al.*, 2007; Feng, *et al.*, 2008), antibacterial activity (Matsuhashi and Kume, 1997), antioxidant activity (Feng, *et al.*, 2008), antifungal

acitivity (Lam and Diep, 2003), phytoalexin eilicitor activity and plant promotion (Kume, Nagasawa and Yoshii, 2002).

2.7.2 Crosslinking

The crosslink structure of chitosan can be generated by using gamma radiation. It was found that carboxymethylchitosan (Yoshii, *et al.*, 2003), as well as other water soluble polysaccharide derivatives (i.e., carboxymethylcellulose, carboxymethylstarch and carboxymethylchitin) can be formed hydrogel at high concentration (>10 %, paste-like state) when radiated with this gamma radiation from 10 kGy to 80 kGy and concentration between 20%-30% is the maximum range to achieve high gel fraction due to its homogeneity of these polymer in the solution. In addition, this carboxymethylchitosan hydrogel also showed the antibacterial activity against *E. coli*.

2.7.3 Sterilization

As a potential use in various biomedical applications, the final products of chitosan have to be sterilized prior to use. Gamma irradiation is become a commercial uses for the sterilization of final medical products instead of using ethylene oxide, a conventional method, which can produce the pollutant gas (Takahashi, 2004). The medical products are often sterilized with standard dose (ISO 11137-2:2006) at 25kGy (Masson, *et al.*, 1997). Chitosan and its derivatives product have been sterilized by using gamma radiation such as chitosan bandage (Siekman, 2006), chitosan microparticles, and carboxymethylchitosan powder. After radiation at low dose (5kGy - 25kGy), microparticles of chitosan exhibited a slightly high release rate of diclofenac sodium and low swelling capacity than the nonirradiated microparticles (Desai and Park, 2006). It was also suggested that carboxymethylchitosan powder can be sterilized by using gamma radiation at low dose because of its retained structures and its high radiation stability up to 25 kGy (Huang, *et al.*, 2007).