CHAPTER II
LITERATURE REVIEW

2.1 Classification of Antibacterial

Antibacterial can be classified in to 2 groups: 1) chemical antibacterial and 2) natural antibacterial.

Chemical antibacterial may be divided into two groups according to their speed of action and residue production. The first group is non-residue-producing antibacterials that act rapidly to destroy bacteria but quickly disappear by evaporation or breakdown and leave no active residue behind. Examples of this type are the alcohols, chlorine, peroxides, and aldehydes (Table 2.1). The second group is residue-producing antibacterials consisting mostly of newer compounds that leave long-acting residues on the surface to be disinfected and thus have a prolonged action. Common examples of this group are triclosan, triclocarban, and benzalkonium chloride (Table 2.2).

Table 2.1 Non-residue-producing antibacterials

Substance group	Substance
Alcohols	Ethanol Isopropanol
Aldehydes	Glutaraldehyde Formaldehyde
Halogen-releasing compounds	Chlorine compounds Iodine compounds
Peroxides	Hydrogen peroxide Ozone Peracetic acid
Gaseous substances	Ethylene oxide Formaldehyde

Table 2.2 Residue-producing antibacterials

Substance Group	Substance
Anilides	Triclocarban
Biguanides	Chlorhexidine Alexidine Polymeric biguanides
Bisphenols	Triclosan Hexachlorophene
Halophenols	Halophenols
Heavy metals	Silver compounds Mercury compounds
Phenols and Cresols	Phenol, Cresol
Quaternary ammonium compounds	Cetrimide Benzalkonium chloride Cetylpyridinium chloride

Example of non-residue-producing antibacterials

Alcohol used to disinfect the skin before injections are given. A solution of 70-85% of ethanol is commonly used as a disinfectant. It kills organisms by denaturing their proteins and dissolving their lipids.

Aldehydes is a high level disinfectant. They inactivate proteins by forming covalent crosslinks with several functional groups. Formaldehyde gas is excellent disinfectant commonly used as formalin. Formalin was used extensively to preserve biological specimens and inactivate viruses and bacteria in vaccines. Whereas glutaraldehyde is less irritating and more effective than formaldehyde. It is one of the few chemical disinfectants that is a sterilizing agent commonly used to disinfect hospital instruments.

Chlorine is used to disinfect swimming pools and is added in small quantities to drinking water to reduce waterborne diseases. When mixed in water, it will form hypochlorous acid which is a strong oxidizing agent.

$$Cl_2 + H_2O \text{ ------> } H^+ + Cl^- + HOCl$$

Iodine is usually dissoved in an organic solvent. It is used in the poultry industry and added to the birds drinking water. Iodine is rapidly neutralised by the presence of organic material, so surfaces must be cleaned prior to disinfection.

Peroxides are considered to be an oxidant disinfectant. They must undergo a complex chemical reaction, not fully understood, forming highly reactive hydroxyl radicals which attack cell membranes. Commercially available preparations of 3% hydrogen peroxide are relatively stable and effective when used on inert surfaces but contact must occur for as long as 20 minutes to have anti-fungal activity.

Example of residue-producing antibacterials

Phenol compounds are used as a scrub for preoperative hand cleansing. They are used in the form of a powder as an antiseptic baby powder, where it is dusted onto the belly button as it heals. Also used in mouthwashes and throat lozenges, where it has a painkilling effect as well as an antiseptic one.

Quaternary Ammonium compounds contain NH_4^+ (Figure 2.1). They carry a strong positive charge that makes good contact with negatively charged surfaces. This characteristic makes most very cleaning agents. They are generally low in toxicity but prolonged contact can be irritating. The quaternaries are commonly used in ordinary environmental sanitation of noncritical surfaces such as floors, furnitures and walls.

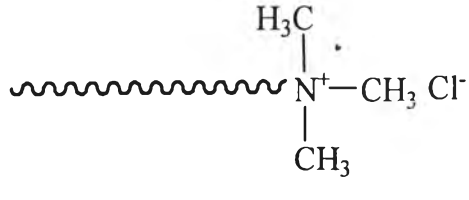

Figure 2.1 Chemical structure of quaternary ammonium compound.

Natural antibacterial has been used in various applications. For example, salt or sodium chloride is used as a flavour enhancer and food preservative in food industry because salt brine dehydrates the bacterial cells causing the cell death.

In medical purpose, honey is used for infected wound due to it contain enzyme that produces H_2O_2 which is a strong oxidizing agent. Lemon juice is used to kill bacteria because living organism can not maintain a life below a pH of 3. In textile industry, chitosan is used to reduce various unpleasant odors caused by bacteria.

2.2 Antibacterial Action of Chitosan and its Derivatives

Chitosan was first discovered in 1859 by Rouget. After chitin is boiled in a very concentrated potassium hydroxide (KOH) solution, the product becomes soluble in organic acids (Muzzarelli, 1977). Currently, chitosan is generally prepared by treatment of chitin with 40-50% NaOH or KOH solution at high temperature (No and Meyer, 1997). As a result of treatment, acetamido groups may undergo *N*-deacetylation. Chitosan is a copolymer consisting mainly of of β-(1→4)-2-amino-2-deoxy-D-glucose units and β-(1→4)-2-acetamido-2-deoxy-D-glucose units (Knaul *et al.*, 1999a). Figure 2.2 and 2.3 represent segments of chitin and chitosan.

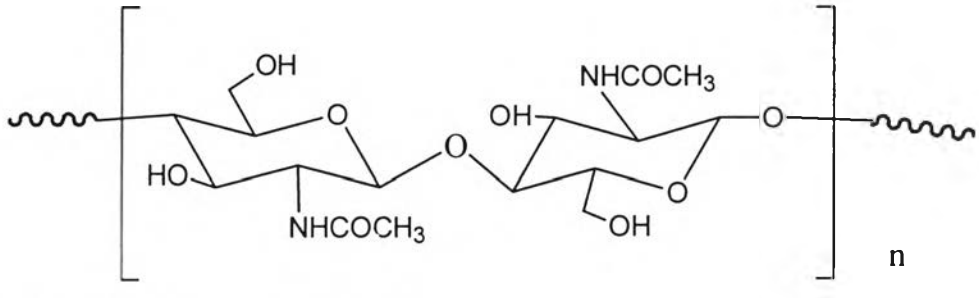

Figure 2.2 Chemical structure of chitin.

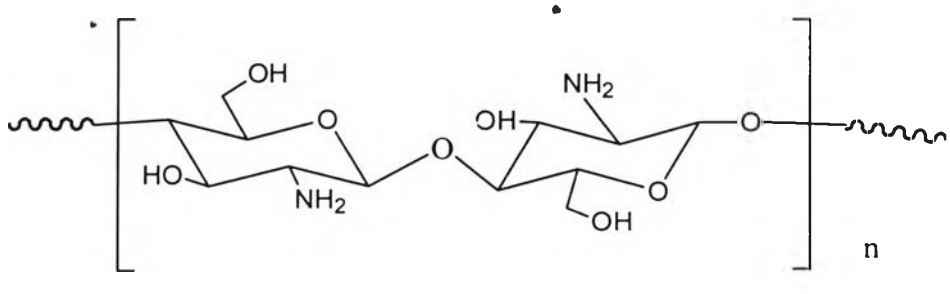

Figure 2.3 Chemical structure of chitosan.

The degree of *N*-deacetylation, which is usually expressed as a percentage to represent the average number of D-glucosamine units per 100 monomers (Sabnis and block, 1997), is one of the important parameters that has a remarkable effect on the solubility and solution properties of chitin and chitosan (Rathke and Hudson, 1994). Most publications use the term of chitosan when the degree of deacetylation is more than 70% (Li *et al.*, 1992). It is soluble in weak acid but poor solubility in other organic solvent due to strong hydrogen bonding between the molecules.

Chitosan inhibits the growth of a wide variety of bacteria as shown in table 2.3 (Liu *et al.*, 2001 and Li *et al.*, 2002).

Table 2.3 Antimicrobial activities of chitosan

Bacteria	MIC (ppm)
Agrobacterium tumefaciens	100
Bacillus cereus	1000
Corinebacterium michiganence	10
Erwinia sp.	500
Erwiniacarotovora subsp.	200
Escherichia coli	20
Klebsiella pneumoniae	700
Micrococcus luteus	20
Pseudomonas fluorescens	500
Staphylococcus aureus	20
Xanthomonas campestris	500
Fungi	**MIC (ppm)**
Botrytis cinerea	10
Fusarium oxysporum	100
Drechstera sorokiana	10
Micronectriella nivalis	10
Piricularia oryzae	5000
Rhizoctonia solani	1000
Trichophyton equinum	2500

MIC: minimum growth inhibitory concentration

Several mechanisms were proposed for the antimicrobial activity by chitosan. In one mechanism, the polycationic nature of chitosan interferes with the negatively charged residues of macromolecules at the surface. Chitosan interacts the membrane of the cell to alter cell permeability (Figure 2.4). The other mechanism involves the binding of chitosan with DNA to inhibit RNA synthesis (Figure 2.5).

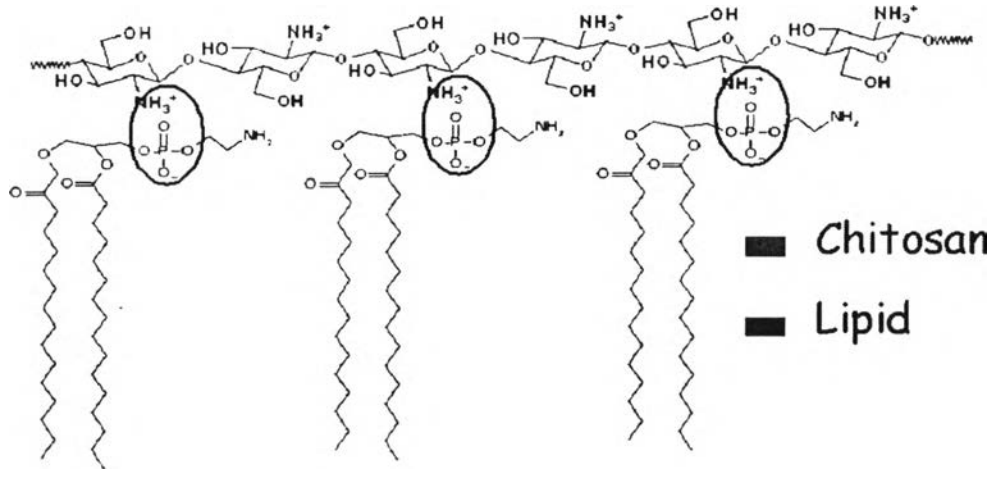

Figure 2.4 Ionic interaction between chitosan and lipid at the cell wall.

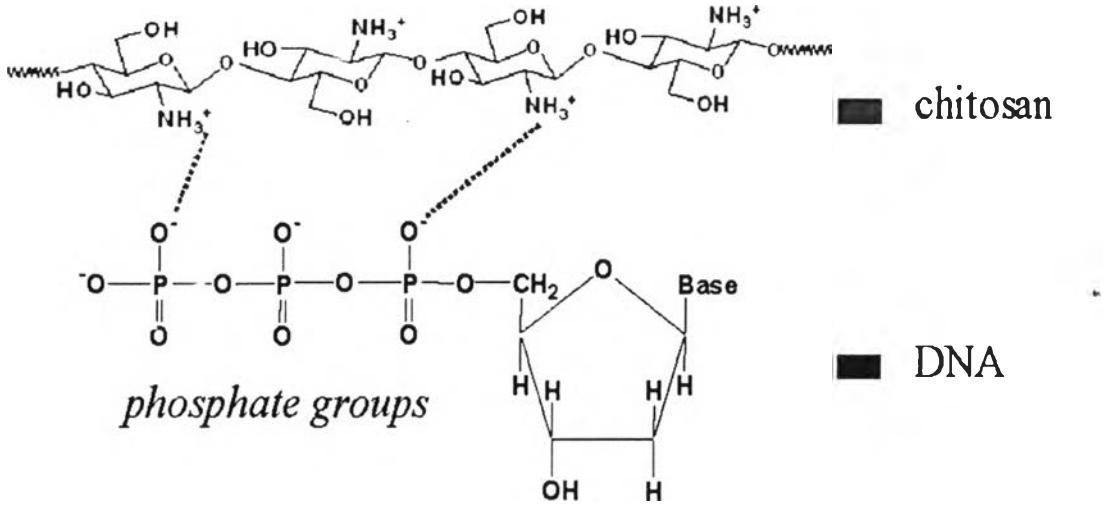

Figure 2.5 Ionic interaction between chitosan and DNA.

It was also found that for *S. aureus*, a gram-positive bacteria, as the MW of chitosan increased, the antimicrobial effect was enhanced (Table 2.4). The main reason might be the chitosan of higher MW forms a film which inhibits nutrient adsorptions. But for *E. coli*, a gram-negative bacteria, as the MW of chitosan decreased, the antimicrobial effect was enhanced (Table 2.5). The main reason might be that the chitosan of lower MW enters the microbial cell more easily, which disturbed the metabolism of the cell. In addition, when the chitosan concentration reached 1.0%, the inhibition rate reached 100% for both *E. coli* and *S. aureus* (Zheng and Zhu, 2003).

Table 2.4 The antimicrobial effect of chitosan on *E.coli*

MW (kDa)	Inhibition rate (%)			
	Concentration of chitosan			
	0.25%	0.5%	0.75%	1%
<5	50	60	90	100
48.5	30	80	90	100
72.4	5	10	50	100
129	0	5	90	100
166	0	40	80	100
305	0	40	50	100

Table 2.5 The antimicrobial effect of chitosan on *S.aureus*

MW (kDa)	Inhibition rate (%)			
	Concentration of chitosan			
	0.25%	0.5%	0.75%	1%
<5	0	0	0	0
48.5	0	95	99	100
72.4	0	96	99	100
129	40	100	99	100
166	95	100	100	100
305	99	100	100	100

Chitosan, however, shows its antibacterial activity only in an acidic range because of its poor solubility above pH 6.5. Thus, water soluble chitosan derivatives which are soluble in both acid and basic physiologic circumstances might be good candidates for a polycationic biocide (Jia *et al.*, 2001). *O*-carboxymethylated chitosan (*O*-CM chitosan), a water-soluble chitosan derivative (Figure 2.6), plays an important role in the antimicrobial activity of chitosan because it is the substitution of chitosan with CH_2COOH only to-OH; its number of $-NH_2$ is not changed (Kim *et al.*, 2002).

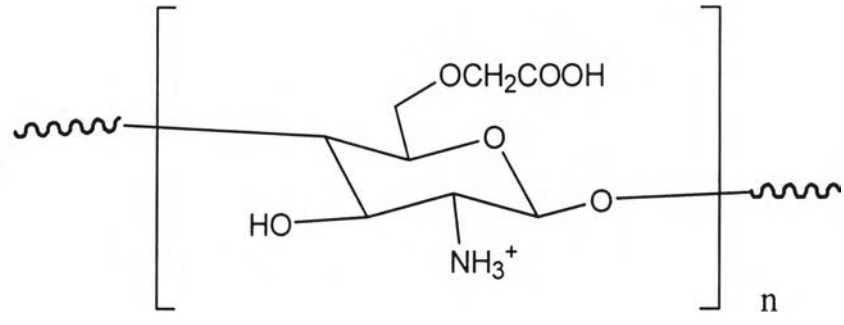

Figure 2.6 Chemical structure of *O*-CM chitosan.

Besides *O*-CM chitosan, *N*-Carboxybutyl chitosan, a water-soluble chitosan derivative (Figure 2.7), displayed inhibitory, bactericidal, and candidacidal activities when tested against 298 cultures of various pathogens. Examination by electron microscopy showed that microbial cells exposed to *N*-carboxybutyl chitosan underwent marked morphological alterations (Muzzarelli *et al.*, 1990).

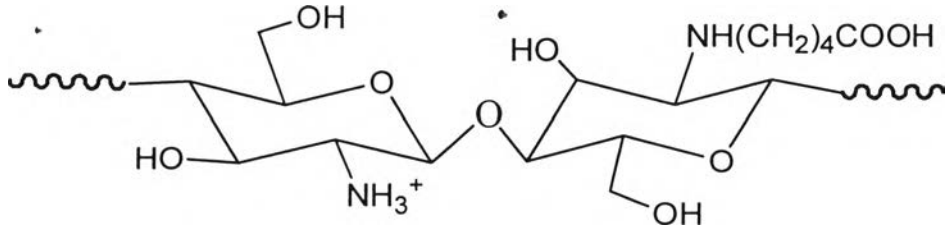

Figure 2.7 Chemical structure of *N*-Carboxybutyl chitosan.

Hirano *et al.*, (1981) prepared *N*-(carboxyacyl) chitosans by reactions with intramolecular carboxylic anhydrides (Figure 2.8). This derivative has hydrophilic and acidic properties. The structure of this water-soluble chitosan is similar to *N*-carboxybutyl chitosan. Thus, it would be interesting to investigate if *N*-(carboxyacyl) chitosan, which possesses positive charges on the ammonium groups, could show any antimicrobial activities.

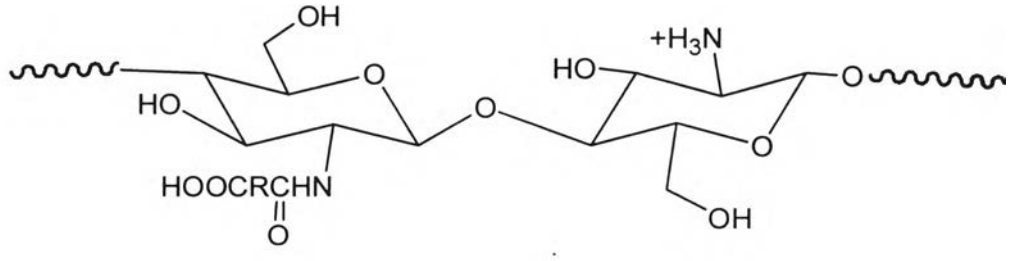

Figure 2.8 Chemical structure of *N*-(carboxyacyl) chitosan.

2.3 Alginate and Chitosan Fiber

Alginates are unbranched binary co-polymers of (1→4)-linked residues of β-D-mannuronic and α-L-guluronic acids (Figure 2.9) (Wong *et al.*, 2002). They are located in the cell wall and in the matrix of the algae such as *Laminaria* sp. and *Ascophyllum* sp. (Lloyd *et al.*, 1998), cementing the cells together and giving certain mechanical properties to the algae. Alginates are prepared by alkaline extraction of seaweed cell walls. The resultant colloidal solution of sodium alginate is precipitated by addition of calcium chloride. Following precipitation the calcium alginate is redissolved using sodium carbonate to generate sodium alginate prior to final preparation of either calcium alginate or sodium/calcium alginate for dressing manufacture. In the presence of multivalent cations such as Ca^{2+}, an aqueous solution of alginate will become a gel. Gel formation occurs due to the ionic interaction between guluronic acid residues from two or more alginate chains and cations, yielding a three-dimensional network of alginate molecules well described by the ' egg-box model ' (Figure 2.10) (Goth *et al.*, 2004).

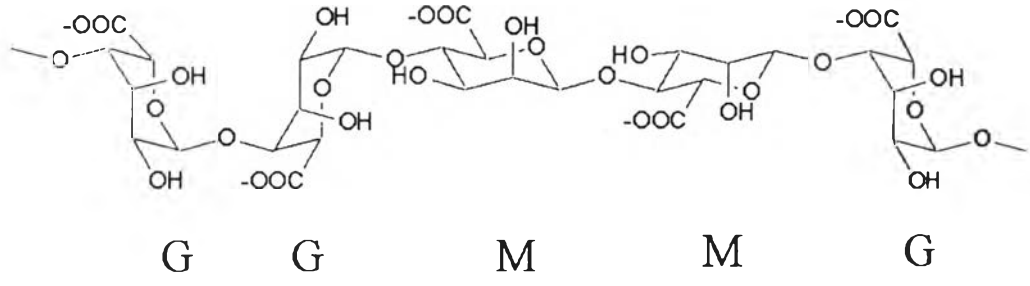

Figure 2.9 Structure of alginate.

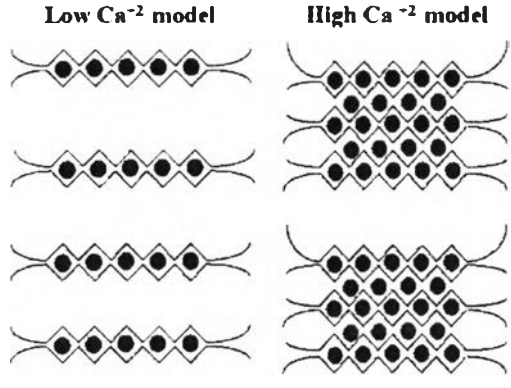

Figure 2.10 'Egg-box model'.

The alginates can be processed into various forms such as membrane, film, and fiber forms. Because of their good biocompatibility, biodegradability, and non toxicity (Hermes and Narayani, 2002), much interest has been paid on them.

When the alginate dressing comes into contact with wound exudate, ion exchange occurs between the calcium ions of the dressing and the sodium ions in the exudate resulting in the formation of a gel on the surface of the wound. This gel absorbs moisture and maintains an appropriately moist environment which is considered to promote optimal healing.

The cationic property of chitosan offers an opportunity to take advantage of its electrostatic interaction properties. Then, chitosan has been used to coat calcium alginate filaments utilising the cationic interaction of the chitosan with the anionic nature of the alginate to produce a tight interaction (Figure 2.11).

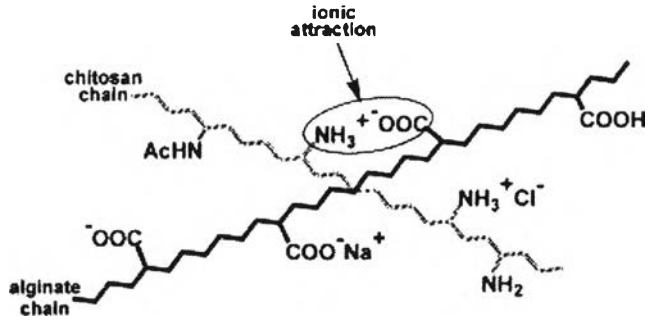

Figure 2.11 Potential ionic interactions between sodium alginate/alginic acid fibers treated with chitosan.

Tamura *et al.*, (2002) prepared chitosan-coated alginate filament. The results showed that the chitosan of high molecular weight tended to precipitate in the presence of calcium ion, then a minimum amount of chitosan should be applied to coagulation bath. For the chitosan of molecular weight $4.0*10^4$, significant increase of wet/dry ratio of knot strength was observed. In general, filament is stronger in dry condition than in wet condition. However, the present chitosan-coated alginate filament showed higher strength in wet condition. For chitosan of molecular weight $1.6*10^5$,the tensile strengths in dry state and wet state were improved compared with the filaments coated by chitosan of lower molecular weight. The optimum concentration of chitosan was suggested to be 0.014% (w/v) in the first coagulation bath.

Knill *et al.*, (2004) reported the results of alginate fibers modified with unhydrolysed and hydrolysed of chitosans. Modification of fibers with unhydrolysed chitosans generally resulted in a significant reduction in tenacity (and a reduction in % elongation if a water washing stage was not used), implying that the unhydrolysed chitosan is more like a coating rather than penetrating/reinforcing the alginate fiber. Reduction of chitosan molecular weight had a positive effect on its ability to penetrate the alginate fibers, not only increasing fiber chitosan content, but also reinforcing fibre structure and thus enhancing tensile properties (compared with unhydrolysed chitosan/alginate fibers). Hydrolysed chitosan/alginate fibers demonstrated an antibacterial effect (in terms of bacterial reduction) with initial use and had the ability to provide a slow release of antibacterially active components.

Due to its high molecular weight, the chitosan must be used in very low concentrations because precipitation occurs in the presence of calcium ions which results in very low levels of chitosan incorporation into the fibers. In addition, chitosan is a semi-crystal polymer and has lower gas permeability. The gas exchange through a wound dressing is important because a high CO_2 pressure increases the acidity and slows down the healing process and a low oxygen concentration decreases the regeneration of tissue cell or makes possible the proliferation of anaerobic bacteria leading to infection of the wound. Problems in production of chitosan/alginate fibers can be overcome by blending with some modified chitosan derivatives.

Zhang *et al.*, (2000) prepared the blend membranes from *O*-carboxymethylated chitosan and alginate. They were miscible in the ratio from 1:1 to 1:5 for CM-chitosan/alginate due to the strong electrostatic force and hydrogen bonding between two polymers. Moreover, the tensile strength and thermo stability were markedly higher than that of nonblend and cellulose/alginate blend membranes.