CHAPTER II
LITERATURE REVIEW

2.1 Fiber and Fiber Production

Fibers may be defined as units of matter characterized by flexibility, fineness, and a high ratio of length to thickness (Morton and Hearle, 1975).

Fibers can be classified based on the generation process into two categories: 1) natural fibers and 2) man-made fibers (Cook, 1984a; Corbman, 1987; Nakamura, 2000). Natural fibers are those occur in nature in a ready-made fibrous form such as cotton, silk, wool, and flax. On the other hand, man-made fibers are produced by man from either naturally occurring polymers or synthetic polymers that are not previously in a suitable fibrous form (Cook, 1984a).

Man-made fibers can be sub-divided into two distinct groups: 1) synthetic fibers and 2) natural polymer fibers, according to the source of the fiber-forming substances from which they are made (Cook, 1984a).

The synthetic fibers are those made from simple chemicals such as those derived from coal or oil. These chemicals are polymerized into materials capable of forming fibers, and then manipulated into a fibrous form, entirely by man.

The natural polymer fibers, on the other hand, are those in which the fiber-forming substances have been generated by nature. In these natural polymer fibers, nature, which can possibly be both plants and animals, has created a substance capable of taking on a fibrous form. Then, man has only taken a further step by manipulating this substance into a fiber. Examples of the natural polymers that can be used as raw materials for making fibers are proteins and polysaccharides.

The production of man-made fibers is described as 'fiber spinning'. The first step of fiber spinning process is to change the fiber-forming substance from its raw state, which is usually in a solid form, into a liquid or semi-liquid state. This can be achieved either by dissolving the material in

high concentration in a solvent that does not degrade the polymer or by melting it with heat and shear. The liquid containing the fiber-forming material is then filtrated and extruded under pressure out of spinneret, a specific device containing very small holes, to be formed in endless, fine jets of liquid. From this point on, to solidify the extruded liquid jets as filaments, several ways might be applied, and thus fiber spinning process can be classified into three main types: melt spinning, dry spinning, and wet spinning. When the fiber-forming material is rendered liquid by heating it until it melts, extruded through spinneret, and then solidified by being cooled down from its molten state, the process is called melt spinning. In dry spinning, the fiber-forming substance is dissolved in a solvent and then solidified by evaporating the solvent after passing through the spinneret. In the wet system, the solution of fiber-forming material is extruded into a fluid which either extracts the solvent from the filaments or enters into a chemical reaction causing the original polymer to be recovered (Walczak, 1977; Cook, 1984b; Rathke and Hudson, 1994; Knaul and Creber, 1997b).

The wet spinning technique, in spite of its low productivity as comparing to melt or dry spinning process, is the most suitable technique for the production of fibers from polysaccharide materials of which high melting temperatures resulted from a large amount of hydrogen bonds in their molecules.

Polysaccharides, a kind of natural polymer, have received considerable attention across a broad range of fields because they are not only naturally abundant, but also non-toxic and biodegradable (Li *et al.*, 1992). While cellulose is the most abundant renewable polysaccharide known, chitin, which has a very similar molecular structure to cellulose, is considered to be in the second rank (Knaul *et al.*, 1999a) with an annual production of 10^{10} to 10^{11} tons per year (Felse, P.A. and Panda, T., 1999).

2.2 Alginate and Alginate Fiber Preparation

Alginate is a linear polysaccharide made up of α-L-guluronic acid (G) and β-D-mannuronic acid (M) (Wong *et al.*, 2002) as shown in Figure 2.1.

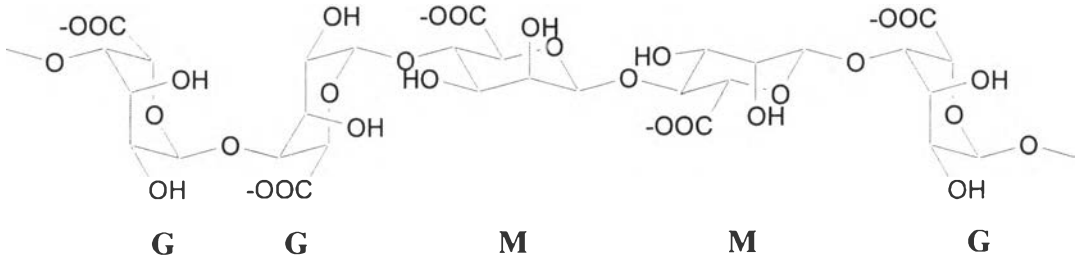

Figure 2.1 Chemical structure of alginate.

Alginates are extracted from various species of brown algae (seaweed). The alginates have different characteristics of viscosity and reactivity based on the specific algal source and the ions in solution. The common salts used in the food and pharmaceutical industries are sodium alginate, potassium alginate, and propylene glycol alginate. Among alginate's characteristics, its gelation behavior is unique. It forms ionic gels in the presence of calcium or other multivalent metal cations and acid gels at pH below the pK_a value of the uronic acid residues (Kim *et al.*, 2000).

In the presence of calcium, alginate chains associate to form hydrated networks (Lloyd *et al.*, 1998). In alginate, the structurally regular homopolymeric sequences are interrupted by the occurrence of other residues. These interruptions act to terminate intermolecular associations through structural junction zones, with the consequent exchange of partners building three-dimensional networks. The primary mechanism of interchain association on calcium-induced gelation of alginate is dimerization of polyguluronate chain sequences with interchain chelation of Ca^{2+}, like eggs in a box (Figure 2.2). Calcium polyguluronate junctions are terminated by D-mannuronate residues. In the presence of excess Ca^{2+} uninterrupted polyguluronate chains form a solid precipitate.

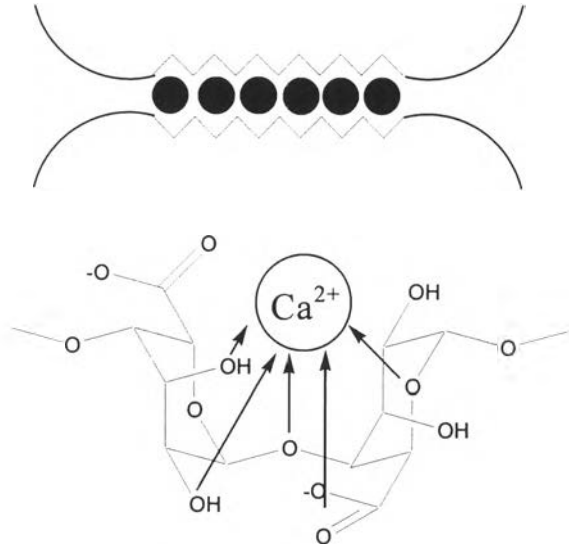

Figure 2.2 Egg-box model for binding of divalent cation to alginates.

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.

Kim *et al.* (2000) produced alginic acid fibers by wet spinning process. Spinning dope was prepared by dissolving 3% by weight of alginate in water and aging overnight at room temperature to remove all the air bubbles. The dope was then placed in a stainless-steel vessel and 1 kg/cm^2 pressure was applied with nitrogen gas. The dope passed through a 100 mesh stainless-steel filter to a constant volume metering pump and then to a spinneret with twelve 200-μm diameter holes. With the extrusion rate fixed at 0.53 cc/min, the dope was coagulated in a 1% HCl (35%) coagulation bath and the gel fibers were wound at a take-up speed of 2.0 m/min. The alginic acid gel fibers were dehydrated by exchanging water with dioxane, crosslinked with glutaraldehyde, then neutralized for better absorbency. Crosslinked alginate filaments exhibited a high saline solution and synthetic urine absorbencies, maintaining the integrity of the fiber structure. Maximum synthetic urine absorbency was obtained with the fiber crosslinked at a lower

glutaraldehyde concentration compared with that required for maximum saline solution absorbency. This appears to be due to the crosslinking effect of calcium ions in the synthetic urine solution being absorbed. Strain and tenacity of the crosslinked alginate fibers decreased with an increasing amount of glutaraldehyde used in the crosslinking reaction. The decrease in tenacity was not significant while the strain showed an extensive decrease.

Tamura *et al.* (2002) prepared chitosan coated alginate filament. 4.0 g of sodium alginate was dissolved in 100 mL of deionized water and debubbled by standing at room temperature and filtered through a funnel. The dope was extruded through the stainless steel spinneret (0.1 mm Ø x 50 holes) into the first coagulation bath containing chitosan solution. The coagulated filament was then wound up through a stretching procedure (rate was 7.5 m/min for first roller and 9.0 m/min for second roller) and then to cassette at wet state. The length of coagulation baths was 50 cm and volume was ca 700 mL. The filament was dried in air on the cassette following extensive washing with ethanol. The smooth and uniform chitosan coatings were confirmed both by the microscopic picture of ninhydrin treated filament and the brightness of filament. The enhancement of tensile strength was also observed by the coating of alginate filament by chitosan through ionic interaction. There was significant molecular weight dependency on the tensile strength of chitosan coated alginate filament especially in wet properties suggesting the tight interaction of chitosan to alginate filament.

Knill *et al.* (2004) produced alginate/alginic acid fibers by a conventional wet spinning technique using a multi-functional laboratory extruder. Spinning dope solutions (sodium alginate, 1-6% w/v, depending on type and viscosity) were extruded under pressure through a spinneret (200 holes, 76 μm diameter) into a coagulation bath containing either hydrochloric acid (0.2 M) and/or calcium chloride (1-3% w/v) to afford the corresponding alginic acid and/or calcium alginate fibers. The resultant fibers/filaments were then drawn between a first and second set of rollers and were then passed through a water washing bath. The fibers were then squeezed (between rollers) to remove excess liquid, wound up, removed from the extruder and

placed in a treatment bath containing unhydrolysed and/or hydrolysed chitosan (0-5% w/v in AcOH, 1% v/v) for 10 min, rinsed with deionised water and dried using acetone baths of increasing concentrations (50-100% v/v). Chitosan treatment, water washing and acetone drying can also be performed as continuous processes directly after production of initial fibers. Finally, fibers were separated by hand and conditioned (24 h at ambient temperature). Levels of chitosan incorporated onto/into base alginate fibers were estimated by elemental analysis. Tensile properties (% elongation and tenacity) of resultant chitosan/alginate fibers were determined in order to assess their suitability for potential application in wound dressings. A broad range of chitosan contents (~0-6% w/w) and hydrolysed chitosan contents (~7-25% w/w) were obtained using a variety of alginate and chitosan starting materials. Modification of fibers with unhydrolysed chitosans generally resulted in a significant reduction in tenacity, i.e. no increase in fiber strength was observed, implying that the unhydrolysed chitosan is more like a coating rather than penetrating/reinforcing the alginate fibers. 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 fiber 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/leaching of antibacterially active components (presumably hydrolysed chitosan fragments).

2.3 Antibacterial Action of Chitosan and its Derivatives

Chitosan, a group of polymers deacetylated from chitin (Li *et al.*, 1992), was first discovered in 1859 by Rouget who found that, 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 (100°C or higher) (No and Meyers, 1997).

As a result of such treatment, acetamido groups adjacent to *cis* related hydroxyl groups may undergo *N*-deacetylation (Muzzarelli, 1977). However, to achieve a completion of *N*-deacetylation of chitin is almost impossible even under harsh treatment (Li *et al.*, 1992), chitosan is a copolymer with the main repeating units of (1,4)-linked 2-amino-2-deoxy-β-D-glucopyranose and some units of (1,4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (Knaul *et al.*, 1999a). Figure 2.3 and 2.4 represent segments of chitin and chitosan, respectively.

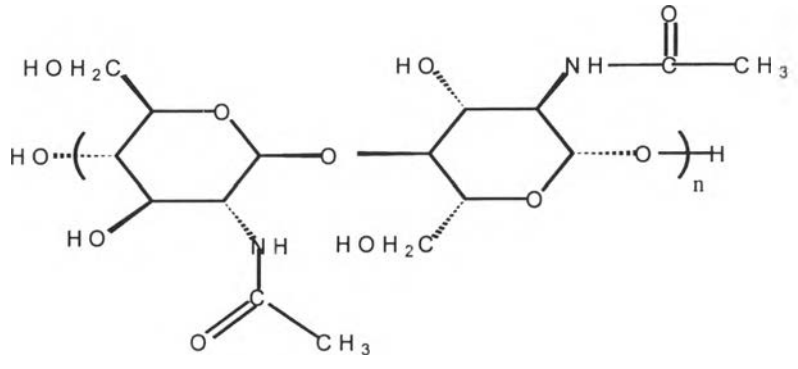

Figure 2.3 Chemical structure of chitin.

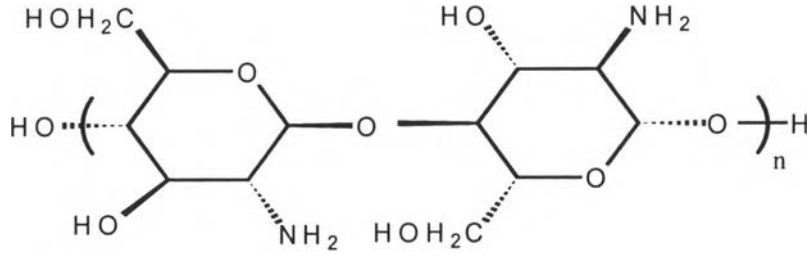

Figure 2.4 Chemical structure of chitosan.

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 marked effect on the solubility and solution properties of chitin and chitosan (Rathke and Hudson, 1994). The term chitosan is usually refered to chitin when it is highly deacetylated enough to become soluble in dilute aqueous acidic system (e.g., 1-10% by volume aqueous acetic acid) (Knaul *et*

al., 1999b). Otherwise, most publications use the term chitosan when the degree of deacetylation is more than 70% (Li *et al.*, 1992).

Although chitosan is easily soluble in most aqueous organic acid solutions, e.g. formic, acetic, 10% citric, pyruvic, and lactic acids (Rathke and Hudson, 1994) as a result of the basicity of the primary amine groups in its molecule (East and Qin, 1993), it is insoluble in water, alkali, and organic solvents (Li *et al.*, 1992) such as methanol, absolute ethanol, and acetone.

Interestingly, for the reason that the amino group is protonated at low pH (Zhang *et al.*, 2001), chitosan is a cationic polyelectrolyte which can interact with negatively charged substances like proteins, lipids, dyes, and polymers. On the other hand, the nitrogen in the amino group of its molecule acts as an electron donor which can also interact with positively charged metal ions such as copper, lead, mercury, and uranium (Li *et al.*, 1992).

Due to the positive charges of amino groups in the chitosan molecule, it can bound to negatively charged bacterial surface and then inhibit the growth of bacteria (Sudarshan *et al.*, 1992). Sudarshan *et al.* (1992) reported that at lower concentration chitosan may have bound to the negatively charged bacterial surface to disturb the cell membrane and cause cell death due to leakage of intracellular components; at high concentration, chitosan may have additionally coated the bacterial surface to prevent leakage of intracellular components as well as to impede mass transfer across the cell barrier.

Chitosan inhibits the growth of a wide variety of bacteria as shown in Table 2.1 (Liu *et al.*, 2001 and Li *et al.*, 2002).

Table 2.1 Antimicrobial activities of chitosan (Liu *et al.*, 2001 and Li *et al.*, 2002)

Bacteria	MIC[a] (ppm)
Agrobacterium tumefaciens	100
Bacillus cereus	1000
Corinebacterium michiganence	10
Erwinia sp.	500
Erwinia carotovora subsp.	200
Escherichia coli	20
Klebsiella pneumoniae	700
Micrococcus luteus	20
Pseudomonas fluorescens	500
Staphylococcus aureus	20
Xanthomonas campestris	500

[a]MIC: minimum growth inhibitory concentration.

Besides chitosan, O-carboxymethylated chitosan (O-CM-chitosan), a water-soluble chitosan derivative, 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 $-NH_2$ does not change (Kim *et al.*, 2002). Kim *et al.* (2002) have identified the antimicrobial activity of native chitosan (MW: 120 kDa, DDA: 95%), degraded chitosan and O-CM-chitosan against *Escherichia coli*, *Staphylococus aureus*, *Bacillus subtilis* and *Salmonella typhimunum*.

2.4 *Escherichia coli* and *Staphylococcus aureus* Bacteria

Bacteria today are recognized not just as one of many generally similar types of microorganisms, but rather as a fundamentally different type of cell called the procaryotic cell (in the kingdom *Procaryotae*). This definition of a bacterium crystallized in the 1950s when electron microscopy began to reveal its unique structural details.

The kingdom *Procaryotae* is separated into four categories: (I) Gram-negative eubacteria that have cell walls, (II) Gram-positive eubacteria that have cell walls, (III) eubacteria lacking cell walls, and (IV) the archaeobacteria. Ultimately, all bacteria are assigned to a genus and a species. The categories are based on the nature of the bacterial cell wall, and specifically its content of the unique procaryotic macromolecule, peptidoglycan (Batzing, 2001).

Category I: Gram-negative eubacteria that have cell walls contain all bacteria with the multilayered type of cell wall. The multilayered wall has a thin layer of peptidoglycan sandwiched between the cytoplasmic membrane and the outer membrane of the cell wall.

Category II: Gram-positive eubacteria that have cell walls contain bacteria with the thick layer type of wall composed mainly of peptidoglycan.

Category III: Eubacteria lacking cell walls contain all bacteria that do not have a cell wall. These bacteria typically are pliable and lack a definite shape. Bacteria in this category are commonly called mycoplasmas.

Category IV: The archaeobacteria contain bacteria that do not have typical peptidoglycan because it lacks muramic acid. Bacteria in this category are called archaeobacteria because when they were discovered, it was thought that they might be the primitive ancestors of bacteria.

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are Gram-negative and Gram-positive bacteria, respectively. They have similar cell structures but different in peptidoglycan content. Figure 2.5 shows common cell structure of Gram-negative and Gram-positive bacteria.

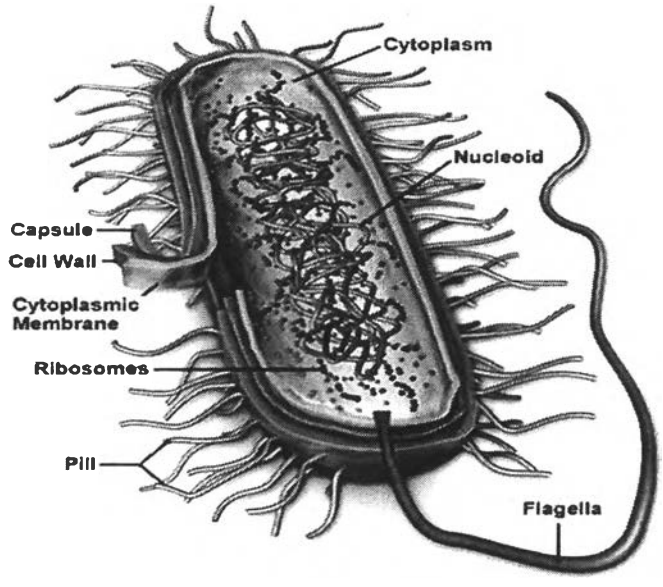

Figure 2.5 Common cell structure of Gram-negative and Gram-positive bacteria.

Cytoplasmic membrane, a selectively permeable barrier that allows certain substances to enter and leave the cell but prohibits the movement of others, is a complex of lipids and proteins (Figure 2.6).

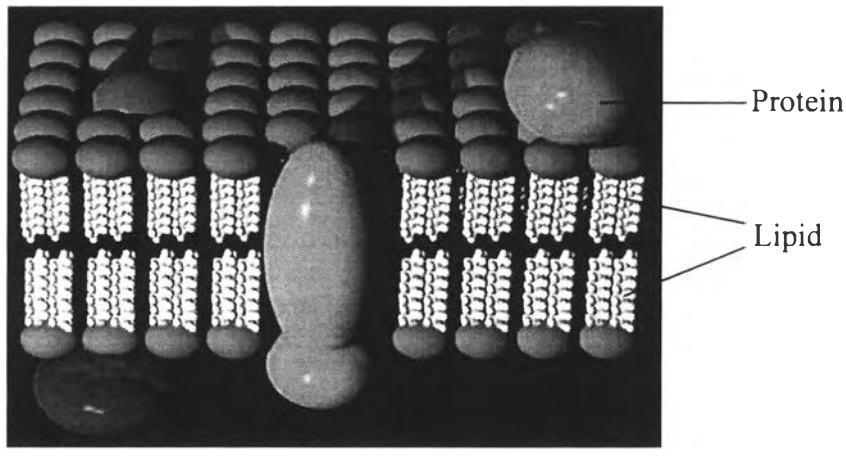

Figure 2.6 Cytoplasmic membrane.

The most abundant lipids in the cytoplasmic membrane are phospholipids, which form double layer in which the proteins are embedded.

The polar, hydrophilic heads of the phospholipid align to form the inner and outer surfaces of the cytoplasmic membrane whereas the nonpolar, hydrophobic tails of the phospholipid align to form the center of the membrane.

Hydrophilic heads of the phospholipid contain negative charges (see Figure 2.7) that can bound with positive charges of some antibacterial agents causing cell death.

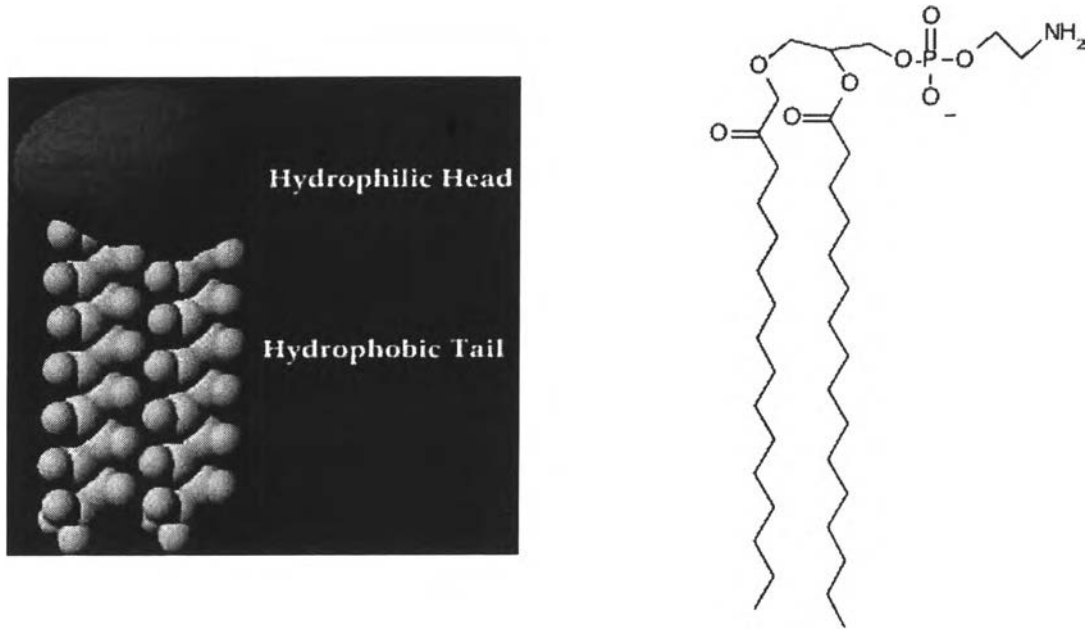

Figure 2.7 Negative charge on hydrophilic head of the phospholipid.