หอสมุดกลาง ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER II

THEORY

2.1 Cellulose

Cellulose is the most abundant biological macromolecule on the planet Earth. It forms the basic structural matrix of the cell walls of nearly all plants, many fungi and some algae. Several bacteria are in conditions to produce cellulose (Jonas and Farah., 1998). The cellulose molecules are composed of longer slander bundles of long chains of β-D-glucopyranose residues linked by 1-4 glucosidic bonds, called 'elementary fibrils' (Petre *et al.*, 1999). Cellulose molecule forms a straight, almost fully extended chain as shown in Figure 2.1. The cellulose chains are organized in a crystalline or semi-crystalline lattice, thus giving rise to microfibrils with a high tensile strength. The chemical formula of cellulose normally is $(C_6H_{10}O_5)_n$. Cellulose is an insoluble structure. In general, the advantages of cellulose include high specific strength and good thermal stability.

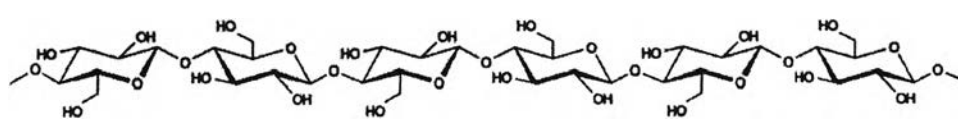

Figure 2.1 Structure of Cellulose (Gardner and Blackwell, 1974).

The cellulose's structure can be defined traditionally as two major types: cellulose I and cellulose II. Both of them have a difference in polarity of the cellulose

chains. The backbone conformations of the chains themselves are essentially identical. Cellulose I has parallel chains whereas cellulose II has alternating antiparallel chains (Gardner and Blackwell, 1974).

2.2 Bacterial Cellulose (BC)

Bacterial cellulose (BC) has generally been fermented by *Acetobacter xylinum* (Schramm *et al.*, 1957). *A. xylinum* is a simple Gram-negative bacterium which has an ability to synthesize a large quantity of high-quality cellulose organized as twisting ribbons of microfibrillar bundles (Czaja *et al.*, 2006). It can be produced from many different substrates such as *Nata de pina* and *Nata de coco*, synthesized by using *A. xylinum* with pineapple water and coconut water as medium, respectively. Morphologically, the reproducible pellicle is obtained by controlling parameters of bacterial growth, for instance, the composition of the culture media, pH, temperature, and oxygen tension.

Glucose is employed as a common substrate. Nonetheless, other simple carbohydrates, alcohols, or polyalcohols can be considered as carbon sources (Brown, 1991). During the process of actual biosynthesis, many carbon compounds of the nutrition medium are utilized by the bacteria, then polymerized into single, linear β-1, 4-glucan chains and finally secreted outside the cells through a linear row of pores located on their outer membrane. Bacteria build bacterial cellulose (BC) and confine themselves in it to protect themselves from enemies and heavy-metal ions while nutrients can be supplied by diffusion (Sangrungraungroj, 2003).

BC traditionally originates as a white gelatinous pellicle on the surface of the liquid medium in a static culture. These bacteria produce cellulose nanofibrils of 3-8 nm diameters. Together, the mesh of these fibrils forms a gelatinous membrane. The size of BC fibrils is about 100 times smaller than that of plant cellulose as shown in Figure 2.2 (Czaja *et al.*, 2006). This unique nano-structure results in a larger surface area. Consequently, BC has oustanding tensile strength, high crystallinity, pure fiber network structure, and especially remarkable water holding capacity. It is extremely hydrophilic, absorbing 60 to 700 times its weight in water (Suwanmajo, 2006).

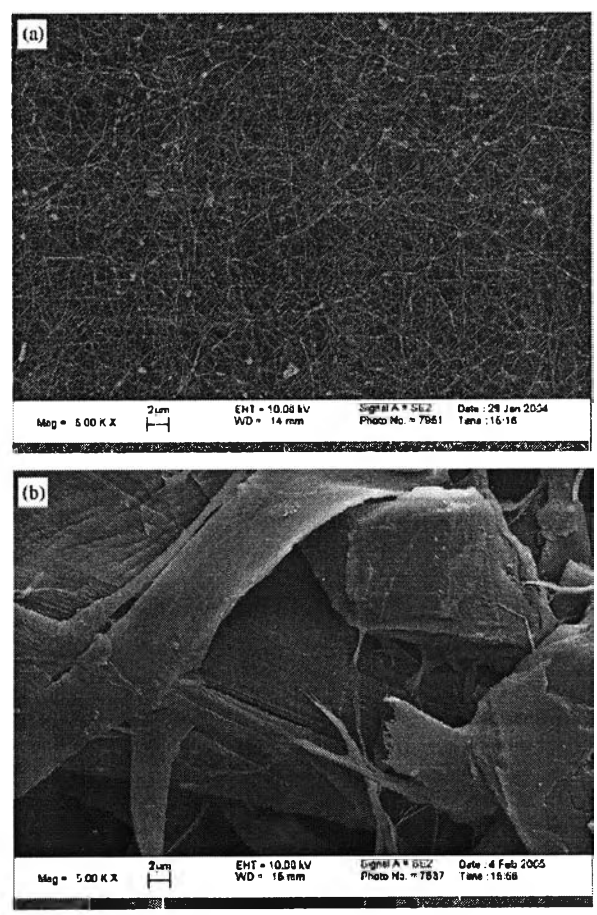

Figure 2.2 A comparison of microfibrillar organization between BC (a) and wood pulp (b).

Many kind of BC producers is presented in Table 2.1 The polymer structure depends on the organism. *Acetobacter xylinum* is the most representative BC producer.

Table 2.1 BC producers (Jonas and Farah, 1998).

Genus	Cellulose structure
Acetobacter	extracellular pellicle composed of ribbons
Achromobacter	fibrils
Aerobacter	fibrils
Agrobacterium	short fibrils
Alcaligenes	fibrils
Pseudomonas	no distinct fibrils
Rhizobium	short fibrils
Sarcina	amorphous cellulose
Zoogloea	not well defined

All strains of *Acetobacter xylinum* produce cellulose extracellularly in the form of flat, twisting ribbons. They have been used ordinarily for the production of vinegar from wine, and utilized for the production of gluconic acid, ketogluconic acids and sorbose as well.

Nata de coco is one of the commercially well known products of BC. There have been a wide range of applications in numerous areas. For example,

in the acoustic speaker diaphragms, BC is used to create a sound transducing membrane for meeting the strict requirements. In the field of paper, adding disintegrated BC to paper pulp was able to create a stronger paper. In the food industry, BC is used as a food additive for a chocolate drink in place of xanthan gum. In dialysis membrane, BC shows a significantly higher permeation rate and a greater molecular weight cut-off relative to a commercial dialysis membrane (regenerated cellulose membrane) (Brown, 1991; Yamanaka *et al.*, 1994).

Svensson *et al.* (2005) demonstrates bacterial cellulose is a promising material for tissue engineering of cartilage and is looked up on as the biomedical applications. Biomedical applications include a bacterial cellulose skin substitute, the replacement of blood vessels, gingiva, and the dura mater during *in vivo* animal testing. Moreover, the BC composite material may be used as a biomaterial in orthopedic applications. For example, osteoblasts were used for the *in vitro* evaluation of the compatibility of the calcium-deficient hydroxyapatite-BC matrix (Fontana *et al.*, 1990).

2.3 Gelatin

Gelatin is a protein obtained by partial hydrolysis of collagen, the chief protein component in skin, bones, hides, and white connective tissues of the animal body. Type A gelatin is produced by acid processing of collagenous raw material, type B is produced by alkaline or lime processing. Because it is obtained from collagen by a controlled partial hydrolysis and does not exist in nature, gelatin is classified as a derived protein. Animal glue and gelatin hydrolysate, sometimes referred to as liquid protein, are products obtained by a more complete hydrolysis of collagen and

can thus be considered as containing lower molecular-weight fractions of gelatin (Keenan, 2003).

The first step in manufacture is separation of the collagen as completely as possible from of the other substances and with the least possible injury to the collagen itself. Properly conditioned collagen when heat with water slowly changes to gelatin, but unless this process is carry out carefully, the gelatin will be further hydrolyzed in the hot solution, as shown below (Tientanacom, 1979):

$$\underset{\text{Collagen}}{C_{102}H_{149}N_{31}O_{38}} + \underset{\text{water}}{H_2O} \text{ -----> } \underset{\text{gelatin}}{C_{102}H_{151}N_{31}O_{39}}$$

$$\underset{\text{Gelatin}}{C_{102}H_{151}N_{31}O_{39}} + \underset{\text{water}}{2\,H_2O} \text{ -----> } \underset{\text{Semiglutin}}{C_{55}H_{85}N_{17}O_{22}} + \underset{\text{Hemicollin}}{C_{47}H_{70}N_{14}O_{19}}$$

Uses of gelatin are based on its combination of properties: reversible gel-to-sol transition of aqueous solution, viscosity of warm aqueous solutions, ability to act as a protective colloid, water permeability, and insolubility in cold water, but complete solubility in hot water. It is also nutritious. These properties are utilized in the food, pharmaceutical, and photographic industries. In addition, gelatin forms strong, uniform, clear, moderately flexible coatings which readily swell and absorb water and are ideal for the manufacture of photographic films and pharmaceutical capsules (Keenan, 2003).

2.3.1 Chemical Composition and Structure

Gelatin is not a single chemical substance. The main constituents of gelatin are large and complex polypeptide molecules of the same amino acid composition as

the parent collagen (Bailey and Light, 1989; Bailey and Paul, 1998; Keenan, 2003; Basavaraju *et al.*, 2006), covering a broad molecular weight distribution range. In the parent collagen, the 18 different amino acids are arranged in ordered, long chains, each having ~95,000 mol wt. These chains are arranged in a rod-like, triple-helix structure consisting of two identical chains, called α1, and one slightly different chain called α2. These chains are partially separated and broken, i.e., hydrolyzed, in the gelatin manufacturing process. Different grades of gelatin have average molecular weight ranging from ~20,000 to 250,000. Molecular weight distribution studies have been carried out by fractional precipitation with ethanol or 2-propanol and by complexing with anionic detergent molecules. The coacervates are isolated and recovered as gelatin fractions. Analysis shows the presence of amino acids from 0.2% tyrosine to 30.5% glycine. The five most common amino acids are glycine, 26.4–30.5%, proline , 14.8-18%; hydroxyproline, 13.3–14.5%, glutamic acid, 11.1–11.7% and alanine, 8.6–11.3%. The remaining amino acids in decreasing order are arginine, aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, hydroxylysine, histidine, methionine, and tyrosine.

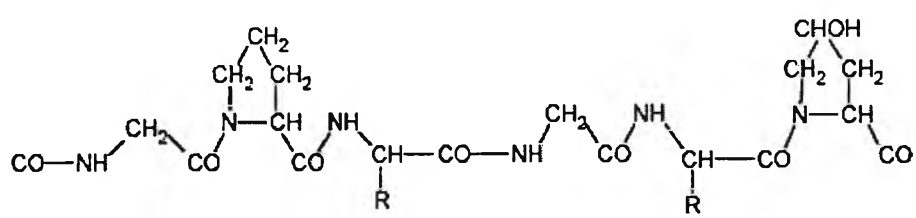

Figure 2.3 Structure of gelatin. (Tomihata *et al.*, 1994; Lee *et al.*, 2003)

2.3.2 Physical and Chemical Properties (Keenan, 2003)

Most physical and chemical properties of gelatin are measured on aqueous solutions and are functions of the source of collagen, method of manufacture, conditions during extraction and concentration, thermal history, pH, and chemical nature of impurities or additives.

Gelation Perhaps the most useful property of gelatin solution is its capability to form heat reversible gel–sols. When an aqueous solution of gelatin with a concentration greater than about 0.5% is cooled to about 35–40°C, it first increases in viscosity, and then forms a gel. The gelation process is thought to proceed through three stages:

(*1*) Rearrangement of individual molecular chains into ordered, helical arrangement, or collagen fold.

(*2*) Association of two or three ordered segments to create crystallites.

(*3*) Stabilization of the structure by lateral inter-chain hydrogen bonding within the helical regions.

The rigidity or jelly strength of the gel depends on the concentration, the intrinsic strength of the gelatin sample, pH, temperature, and additives. Because the economic value of gelatin is commonly determined by jelly strength, the test procedure for its determination is of great importance. The conversion temperature for gelatin is determined as setting point, i.e., sol to gel, or melting point, i.e., gel to sol.

Commercial gelatins melt between 23 and 30°C, with the setting point being lower by 2–5°C.

Solubility In most commercial applications, gelatin is used as a solution. Gelatin is soluble in water and in aqueous solutions of polyhydric alcohols such as glycerol and propylene glycol. Examples of highly polar, hydrogen-bonding organic solvents in which gelatin dissolves are acetic acid, trifluoroethanol, and formamid. Gelatin is practically insoluble in less polar organic solvents such as acetone, carbon tetrachloride, ethanol, ether, benzene, dimethylformamide, and most other nonpolar organic solvents. Many water-soluble organic solvents are compatible with gelatin, but interfere with gelling properties. Dry gelatin absorbs water exothermally. The rate and degree of swelling is a characteristic of the particular gelatin. Swelled gelatin granules dissolve rapidly in water above 35°C. The cross-linking of gelatin matrix by chemical means is used extensively in photographic products, and this so-called hardening permanently reduces the solubility of gelatin.

Amphoteric Character The amphoteric character of gelatin is due to the functional groups of the amino acids and the terminal amino and carboxyl groups created during hydrolysis. In strongly acidic solution the gelatin is positively charged and migrates as a cation in an electric field. In strongly alkaline solution, it is negatively charged and migrates as an anion. The intermediate point, where net charge is zero and no migration occurs, is known as the isoelectric point and is designated in pH units. A related property, the isoionic point, can be determined by utilizing a mixed-bed ion-exchange resin to remove all nongelatin cations and anions. The resulting pH of the gelatin solution is the isoionic point and is expressed in pH units. The isoionic point is reproducible, whereas the isoelectric point depends on

the salts present. Type A gelatin has a broad isoionic region between pH 7 and pH 10, type B is in a lower, more reproducible region, reaching an isoionic point of 5.2 after 4 weeks of liming, which drops to 4.8 after prolonged or more vigorous liming processes. The isoelectric point can also be estimated by determining a pH value at which a gelatin solution exhibits maximum turbidity. Many isoionic point references are recorded as isoelectric points even though the latter is defined as a pH at which gelatin has net charge of zero and thus shows no movement in the electric field.

Viscosity The viscosity of gelatin solutions is affected by gelatin concentration, temperature, molecular weight of the gelatin sample, pH, additives, and impurities. In aqueous solution above 40°C, gelatin exhibits Newtonian behavior. Standard testing methods employ use of a capillary viscometer at 60°C and gelatin solutions at 6.67 or 12.5% solids. The viscosity of gelatin solutions increases with increasing gelatin concentration and with decreasing temperature. For a given gelatin, viscosity is at a minimum at the isoionic point and reaches maxima at pH values near 3 and 10.5. At temperatures between 30 and 40°C, non-Newtonian behavior is observed, probably due to linking together of gelatin molecules to form aggregates. Addition of salts decreases the viscosity of gelatin solutions. This effect is most evident for concentrated gelatin solutions.

Colloid and Emulsifying Properties Gelatin is an effective protective colloid that can prevent crystal, or particle, aggregation, thereby stabilizing a heterogeneous suspension. It acts as an emulsifying agent in cosmetics and pharmaceuticals involving oil-in-water dispersions. The anionic or cationic behavior of gelatin is important when used in conjunction with other ionic materials. The protective colloid property is important in photographic applications where it stabilizes and protects

silver halide crystals while still allowing for their normal growth and sensitization during physical and chemical ripening processes.

Coacervation A phenomenon associated with colloids wherein dispersed particles separate from solution to form a second liquid phase is termed coacervation. Gelatin solutions form coacervates with the addition of salt such as sodium sulfate, especially at pH below the isoionic point. In addition, gelatin solutions coacervate with solutions of oppositely charged polymers or macromolecules such as acacia. This property is useful for microencapsulation and photographic applications.

Swelling The swelling property of gelatin is not only important in its solvation but also in photographic film processing and the dissolution of pharmaceutical capsules. That pH and electrolyte content affect swelling has been explained by the simple Donnan equilibrium theory, treating gelatin as a semipermeable membrane. This explains why gelatin exhibits the lowest swelling at its isoelectric pH. At pH below the isoelectric point, proper choice of anions can control swelling, whereas above the isoelectric point, cations primarily affect swelling. These effects probably involve breaking hydrogen bonds, resulting in increased swelling. The rate of swelling follows approximately a second-order equation. In photographic products, the swelling of the gelatin layer is controlled by coating conditions, drying conditions, chemical cross-linking, and the composition of the processing solutions. Conditioning at 90% RH and 20°C for 24 h greatly reduces swelling of hot dried film coatings. The ratio of lateral to vertical swelling is of great concern in the photographic industry since it can cause curling of photographic papers or films when changes in humidity or general moisture content take place.

2.3.3 Uses

The industry recognized four different kinds of gelatin. (Tientanacom, 1979)

Edible Gelatin is used extensively in food formula as a gelling agent, as a wipping agent in foams, as a clearing agent in fruit juices, wines, and beer, to increase viscosity and to prevent ice-crystal growth in frozen dessert.

Technical Gelatin is quite an arbitrary name applied to small amount used for miscellaneous purpose such for sizing paper, textiles and straw hats.

Pharmaceutical Gelatin is used by pharmaceutical houses for making capsules and as an emulsifier.

Photographic Gelatin has played an important part in the rapid development of the motion-picture and photograph industries. It is coated on the film base, constituting the sensitized emulsion of the light-sensitive silver salts.

Gelatin has been widely also used in medicine as a wound dressing, and as an adhesive and absorbent pad for surgical use. Gelatin does not show any antigenicity, exhibits an activation of macrophages, and has a high hemostatic effect (Lee *et al.*, 2003). Moreover gelatin based biomaterials have been applied to artificial skin, bone grafts and scaffolds for tissue engineering (Lien *et al.*, 2009).

For arresting hemorrhage during surgery, a special sterile gelatin sponge known as absorbable gelatin sponge or gel foam is used. The gelatin is partially insolubilized by a cross-linking process. When moistened with a thrombin or sterile physiological salt solution, the gelatin sponge, left in place after bleeding stops, is slowly dissolved by tissue enzymes. Special fractionated and prepared type B gelatin can be used as a plasma expander (Keenan, 2003).

Gelatin can be a source of essential amino acids when used as a diet supplement and therapeutic agent. As such, it has been widely used in muscular disorders, peptic ulcers, and infant feeding, and to spur nail growth. Gelatin is not a complete protein for mammalian nutrition, since it is lacking in the essential amino acid tryptophan and is deficient in sulfur-containing amino acids (Keenan, 2003).