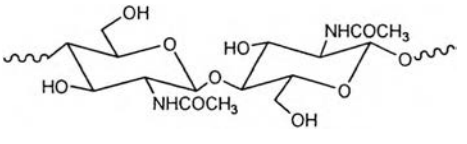
สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

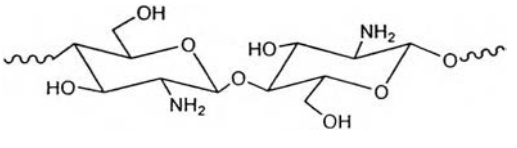
CHAPTER II
LITERATURE REVIEW

2.1 Chitin-Chitosan

Chitin-chitosan is the second-most naturally occurring polysaccharide biopolymer in nature next to cellulose. Chiton-chitosan is obtained from the shells of crustasceans, culticles of all the arthopods, including cell walls of fungi and yeast. In commercial process, chitin-chitosan is produced from shells of shrimp and crabs. The difference between chitin and chitosan is related to the possibility to solubilize in dilute acidic media. Chitin shows low chemical reactivity and insoluble either in water or most organic solvents. Chitin can be converted into chitosan, which is the most importance chitin derivative, by alkali deacetylation (Abdou *et al.*, 2007). When the degree of deacetylation of chitin achieves above 80%, chitin becomes soluble in aqueous acidic media and is called chitosan. In general chitosan is in a copolymer of *β*-(1-4)-2-acetamido-2-deoxy-*β*-D-glucose and *β*-(1-4)-2-amino-deoxy-*β*-D-glucose of which the molecular weight is about 10^5~10^7 Dalton (Figure1). Due to its structure, the copolymer shows the specific properties, such as non-toxicity (Kim *et al.*, 2003), antibacterial properties, biocompatibility and biodegradability (Sashiva *et al.*, 1990).



Chitin



Chitosan

Figure 2.1 Structure of chitin-chitosan copolymer.

2.1.1 Chemical Structure of Chitin-chitosan Copolymer

Chitin-chitosan has a primary hydroxyl group at C-6 position and secondary hydroxyl group at C-3 position. In general, primary hydroxyl group is more reactive than secondary hydroxyl. Consequently, chemical reactions are occurred at C-6 position. The primary amino group at C-2 is readily to be chemically modified or formed a complex with metal. Consequently, chitin-chitosan is used in wastewater treatment. For example, chitin-humic acid was applied as a sorbent to treat Ni (II) in wastewater (Santona *et al.*, 2007). The modified chitin was used for removing of azo dye (Dolphen *et al.*, 2007). The function of acetamide group is similar to amino group but it is less reactivity than amino and can be form a strong hydrogen bond. Pyranose ring backbone of copolymer is reported for its bioactivity, such as detoxification ability and the cholesterol or fatty acid interaction (Muzzarelli, 1996). Moreover, Glycoside linkages (C-O-C) are naturally biodegradable. Hirano *et al.*, (1989) reported the hen egg white lysozome shows hydrolysis activity to chitin.

2.1.2 Types of Chitin-chitosan

Chitosan can be prepared in various forms such as films, fibers, sponges and microparticles. As a consequent, the properties are depending on not only the chemical structure i.e. degree of acetylation (DA) and the molecular weight distribution, but also the morphology.

a) Films. Chtin-chitosan shows good film-forming abilities which can be simply obtained from a solution in the salt form and cast on a surface followed by evaporating the solvent. The thickness of films can be controlled by polymer concentration or evaporation. For example, chitosan film-forming solution with the presence of sucrose, raffinose and phosphate salts gave smooth, transparent and highly hydrophilic which is a good candidacy for tissue engineering due to the high cell affinity (Bettini *et al.*, 2007).

b) Fibers. The spinning of chitin-chitosan can be obtained by wet spinning process. The fiber is extruded from spinneret and coagulated in an alkaline bath to obtain chitin-chitosan fibers. Recently, the electrospinning is used to spin the finer in nano-scale by electrostatic force. This technique is in the expectation to

หอสมุดกลาง สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

produce wound dressing, tissue scaffolds and drug carrier vehicles (Chen *et al.*, 2008).

c) Sponges. Chitin-chitosan can be easily formed a sponge-like material from solution or hydrogel after water was removed. These materials have very low density close to 0.016 g/cm^{3}with highly porosity. Oungbho *et al.* (1997) proposed that chitosan sponges for drug delivery systems or scaffolds.

d) Microparticles. Microparticle of chitin-chitosan is one of most promising forms as drug delivery system because it can be injected or cooperated into human body easily. It can be processed from the emulsification method and followed by solvent evaporation (Ta *et al.*, 2008).

2.1.3 Development of Chitin-chitosan for Biomedical Application

Chitin-chitosan is used to prepare in many forms as previously mentioned, which is used in the biomedical field due to its biocompatibility. Chitosan is much easier to process than chitin, but the stability of chitosan materials is lower than that of chitin because of its hydrophilicity. Chitosan can be crosslinked by various reagents such as glutaraldehyde (Webster *et al.*, 2007). Chtiosan is positively charged to show the interaction with variety of proteins because the majority of soluble proteins carry a negative charge at low pH.

a) Tissue engineering applications. Tissue engineering is an approach to repair and regeneration of tissue by the use of polymer i.e. scaffolds to support, reinforce and offer the regenerating tissue. As the scaffold needs high porosity to permit an adequate cell distribution for cell seeding. The scaffold produced either from synthetic or natural polymer has to meet the requirement on biodegradability and non-toxicity. Furthermore, the degradation during the cell formation is an ideal. Natural polymer scaffolds have advantages over synthetic polymers in which they facilitate cell attachment and maintain differentiation of cells (Mao *et al.*, 2003).

One of the most promising applications related to the porous structure is the use in cell transplantation and tissue regeneration. Porous chitosan structures can be obtained by freezing and lyophilizing chitosan solutions. In the freezing process, ice crystals are formed in the solution. Exclusion of the ice crystal phase lyophilizating generates a porous material. Pore size can be controlled by varying the freezing rate (Madihally *et al.*, 1999). If chitosan is in salt form, the acid salt must be neutralized or otherwise removed prior to rehydrating the scaffold. The direct neutralization and rehydration in basic solution such as NaOH is also the choice. Madihally *et al.* (1999) prepared porous chitosan scaffolds by dissolution chitosan in acetic acid and lyophilization following rehydrated in ethanol to avoid the stiffness. Chtiosan can be cooperated with bioceramics such as, hydroxyapatile (HA) into chitosan scaffold to achieve mechanical properties (Yamaguchi *et al.*, 2000).

b) Drug delivery applications. Drug delivery system is a system that the drugs are controlled and released by phagocytosis and endocytosis mechanism into the cell or organ all of the body. It offers numerous advantages as compared to the conventional dosage form, such as increasing therapeutic activity, reducing toxicity, and reducing the number of drug administration's required during treatment (Sunil *et al.*, 2004). Other advantages of using controlled delivery systems include the maintenance of drug levels within a desired range. Ideal drug delivery system should be inert, biocompatible, mechanically strong, capable of high drug loading and safe from accident release. Before making use of polymer as the drug carriers, drug will be attached on to polymer chains via either covalent or non-covalent bond. As a consequent, the polymer protects the drug from degradation or premature metabolism. Release of the therapeutic agent is sustained over days to months, thereby maintaining plasma drug concentrations at therapeutic levels for longer periods of time (Champion *et al.*, 2007). Polymer drug carriers have been made from a variety of biodegradable polymers which focused on naturally occurring polymer for example, collagen, cellulose, etc (Pillia *et al.*, 2001). Many recent reports discussed the use of chitosan and modified chitosan in sustained release formulations and colon-targeting delivery systems (Chandy *et al.*, 2003). For example, (Ouchi *et al.* (1992) prepared 6-O-carboxymethyl chitin (CM-chitin) conjugated 5-fluorouracil

via pentamethylene groups and monomethylene spacer. The obtained CM-chitin/5-fluorouracil prodrug showed the slow release of 5-fluorouracil and exhibited antitumor activity against leukemia. Hydrogel-type materials are also reported for various medications through the stomach and into the more alkaline intestine. Falk *et al.* (2004) prepared macroporous chitosan hydrogels drug release by using acetic acid to dissolve chitosan. In this contribution, the diffusion coefficient of paracetamol in chitosan hydrogel has been measured.

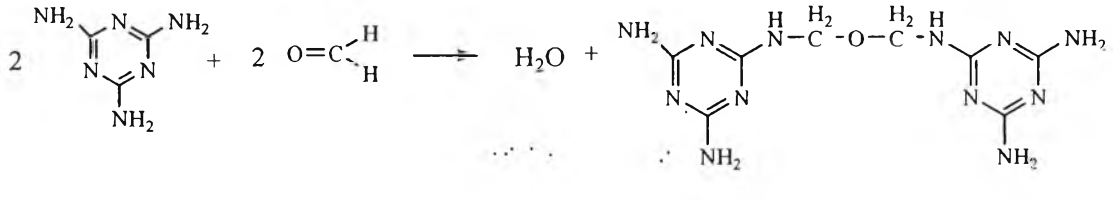
2.2. Aerogel

Aerogels are dried gels which are in fibrons morphology, low density , foams and spongelike with highly porosity and large specific area. The microstructure including nano-sized of pores can be obtained via sol-gel process method. The originality of aerogels comes from the technique used to evacuate the liquid under supercritical method. Now, other techniques are used to dried materials such as freeze-drying, to be called cryogels or aerogels. Materials that can be synthesized as wet gels by sol-gel process can be dried by the supercritical process to obtain aerogels.

2.2.1 Types of Aerogel

a) Inorganic aerogels. The precursors used in sol-gel processing are metallic salts MX_n in which a metal M is bonded to number n of anions X. In solution in aqueous solvents, these precursors are present as ionic species which the metal atoms exist as solvated cations $M\,[H_2O]_n^{z+}$. The reactions to form sol particles and gels comprise hydrolysis reactions which replace H_2O by OH group with loss of protons and condensation reactions leading to the construction of M-OH-M or M-O-M bridges with elimination of water molecules. For example, silica aerogels with titanium dioxide powder and inorganic binders were prepared via a sol–gel process with a TEOS–water–ethanol system (Deng *et al.*, 1998).

b) Organic Aerogels. Organic precursors is used to prepare organic polymers via sol-gel process on covalent bond, including aerogel partical particle with a size ranging from submicrometers to a few hundred micrometers. This type of aerogels can be polymerized of resorcinol with formaldehyde (Tamon *et al.*, 2000). Resorcinol-formaldehyde (RF) can be prepared by polycondensation of resorcinol with formaldehyde in a slightly basic solution with sodium hydroxide as the catalyst. Resorcinol-formaldehyde aerogels are even better thermal insulators than silica aerogels. A major advantage of organic aerogels is their low Z (atomic number) composition (Pekala *et al.*, 1995).



Scheme 2.1 Reaction of resorcinol-formaldehyde (RF).

For another example, dimethylformamide solution of poly(vinyl chloride) (PVC) can be prepared organic aerogels via dehydrochlorination and supercritical drying using carbon dioxide (Yamashita *et al.*, 2002). Polyurethane foams, new organic gels, were prepared by using dichloromethane (CH_2C_{12}) as solvent and solvent exchange occurs by flushing the gel with supercritical CO_2 (Biesmans *et al.*, 1998).

c) Clay Aerogels. Clays, naturally occurring minerals, are alumino-silicates which are in sheet-like structure and composed of silica (SiO_4) tetrahedral bonded to alumina (AlO_6) octahedral in a various ratios. A 2-to-1 layer type has 2

tetrahedral sheets fused to an ocatahedral sheet resulting in smectite clay, that most common of which is montmorillonite.

Other metals such as magnesium or sodium may replace the aluminium in the crystal structure. Naturally montmorillonite is hydrophilic while polymers are generally organophilic. As a result, monmorillonite is hardly to disperse in polymers. Surface modification is a practical way to make montmorillonite be compatible with organic polymers. It is known that surface modification of montmorillonite can be effectively done by silane coupling or surfactant (www.nanocor.com /nano_ struct .asp).

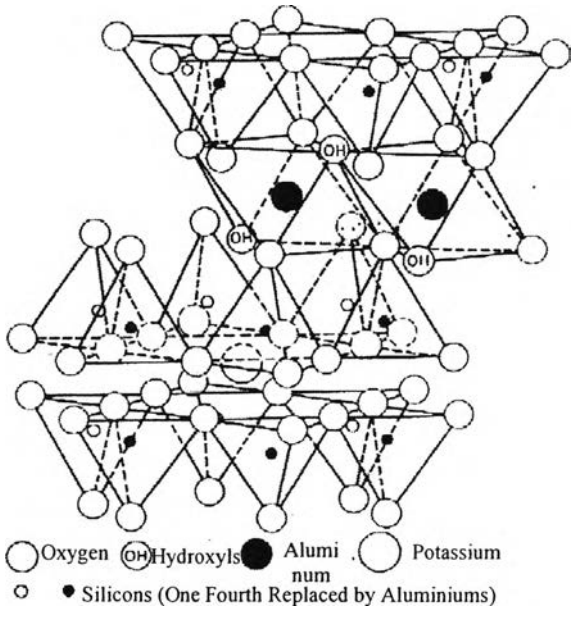


Figure 2.2 Structure of montmorillonite (http://www.up.ac.za).

The advantage of montmorillonite clays is well-known for their ability to reinforce polymers. Clay aerogels are produced by freeze-drying the clay hydrogels to obtain low-density aerogel structure. An alternative way to avoid collapse structure of clay aerogel is combining with polymer to produce composite materials. For example, polymer composites reinforced by clay aerogels were produced via in-situ polymerization of *N*-isopropylacrylamide within the clay aerogels as reported by Somlai *et al.*, (2005). Bandi et al (2005) reported that the organic polymer prevented

loss of aerogel structure in water by encapsulation, while the inorganic filler increased the structural integrity of the polymer. Somlai *et al.*, (2006) reported that the composites of clay aerogel with a poly(vinyl alcohol) (PVOH) matrix polymer could be accomplished by a freeze-drying process.

2.3 Drying Technique (Freeze-drying)

In generally after complete sol-gel process, these are many techniques to convert wet gels to aerogels such as, supercritical drying technique, drying control chemical additives and ambient-pressure drying technique (Fricke *et al.*, 1997). Recently, dehydration by lyophilizing is accepted as a practical technique .

This technique can be divided into three stages: freezing, primary drying and secondary drying.

a. Freezing

The freezing process is done by placing the material in a freeze-drying flask and rotating the flask in a bath, called a shell freezer. It is important to freeze the material at temperature below the eutectic point of material because this point ensures that sublimation rather than melting will occur in the following steps.

b. Primary Drying

In this phase, the pressure is lowered and enough heat is supplied to the material for the water to sublimate. In this initial drying phase, about 98% of the water in the material is sublimated. This phase may be slow, because if too much heat is added the material's structure could be altered.

c. Secondary Drying

In this phase, the secondary drying phase is possible when the water molecules that are absorbed during the freezing process are sublimed. In this drying process is governed by the material's adsorption isotherms. Temperature is raised even higher than in primary drying phase to break any physio-chemical interactions that have formed between the water molecules and the frozen material (http: //en. wiki- pedia.org/wiki/Freeze_drying).