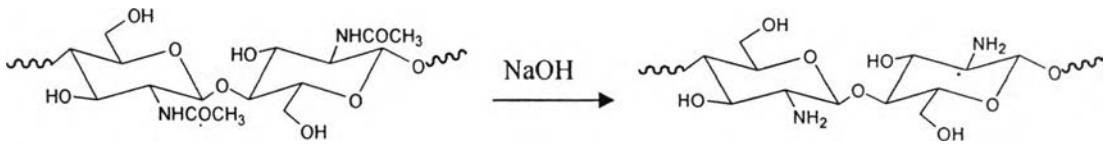
CHAPTER II
LITERATURE REVIEW

2.1 Chitin-Chitosan

Chitin-chitosan is the second-most abundant biopolymer in nature next to cellulose and obtained from the crustacean shells, insect cuticle, some fungi and microorganisms cell wall. Chitin-chitosan is a copolymer consisting of β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units. Chitosan is achieved from chitin by deacetylation in the presence of alkali (Bodmeier *et al.*, 1989).



Chitin Chitosan

Scheme 1. Deacetylation of chitin.

Chitin-chitosan shows the specific properties, such as biocompatibility (Richardson *et al.*, 1999), biodegradability (Yamamoto *et al.*, 1997), bioactivity (Dumitriu *et al.*, 1989), and non-toxicity (Kim *et al.*, 2003) which are suitable for the uses in environmental, health science, biomedical (Mao *et al.*, 2001), and pharmaceutical (Martinac *et al.*, 2005) fields.

It is important to note the most limitation for chitin-chitosan, including chitin-based (%DA > 70) or chitosan-based (%DD > 70) material is about its insolubility in almost all solvents and non-thermal plasticity. Chitosan dissolves in some specific organic acids, such as formic acid, acetic acid, citric acid, and succinic acid. Chitin-chitosan is hard to dissolve in neutral system and water because of its strong inter- and intramolecular hydrogen bonds network (Wang *et al.*, 2004). Although chitosan is a linear polymer, the consequence of the strong hydrogen bond networks also brings the non-thermal plasticity. These two main points obstruct the use of general procedures to produce chitin-chitosan based products.

In terms of materialization, chitosan has been received much more attention than chitin since (i) it is soluble in acids and (ii) it has not only hydroxyl but also amino groups for functionalization. Thus, the materialization of chitosan has to rely on the solution process. Up to present, chitosan-acetic acid solution is the most simplest condition to prepare chitosan products such as the cross-linked gel or membranes (Zeng *et al.*, 1996), the base-solidified beads (Hoagland *et al.*, 1997) or fiber (Tokura *et al.*, 1980), etc. The functionalizations of chitosan either in organic solvent of heterogeneous conditions or the chitosan-acid solution of homogenous conditions are also proposed, such as *N*-carboxybenzyl chitosan (Lin *et al.*, 2006), *N*-succinyl-N′-octyl chitosan (Xiangyang *et al.*, 2007), and *N*-alkyl-*N*-trimethyl chitosan (Zhang *et al.*, 2006). In most cases of functionalization, the ultimate goal is to introduce specific molecules and establish novel and unique properties onto chitosan.

For more than three decades, chitosan have been proposed for numorous applications. For example, in agricultural field, chitosan diluted acid solution (Lin *et al.*, 2006) or water soluble oligochitosan (Zhang *et al.*, 2006) were reported as the plant growth promoter (Bautista-Baños *et al.*, 2003), anti-bacteria and activity inducer (Lifeng *et al.*, 2004), including the post-harvest reagent (Reddy *et al.*, 2000). Chitosans for waste water treatment (Lalov *et al.*, 2000), ion extraction resin (Nagib *et al.*, 1999), hollow fibers (Liu *et al.*, 2006), etc. are examples for the uses in environment. Other applications such as chitosans for paper finishing (Allan *et al.*, 1982), cosmetics (Yannas *et al.*, 1982), and antimicrobial agent (Shahidi *et al.*, 1999) were also reported.

2.2 Development of Chitosan for Biomedical Drug Administration Products: From Drug Delivery System to Drug Targeting System

The uses of chitosan in pharmaceutical and biomedical fields (Sandford *et al.*, 1991) are the way to develop the most value-added chitosan products. The appications in the area requires many conditions such as (i) the safety of the product, (ii) the exact reproducible product preparation, and (iii) the products should be compatibility in bio-system, including the solubility in water-based system. In the

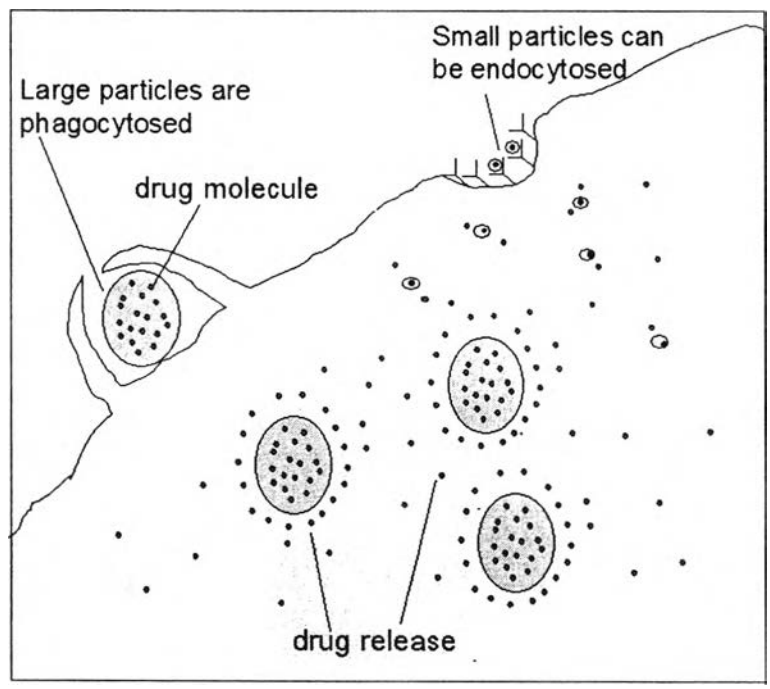
past, various biomedical and pharmaceutical chitosan applications hardly succeeded. Most cases end up as the research works or models which can be overviewed as follows.

2.2.1 Applications in Drug Delivery System Drug delivery system, or in other words, controlled release system, is defined by Flynn as "the use of whatever means possible, be it chemical, physiochemical, or mechanical, to regulate a drug's access rate to the body's central compartment, or in some cases, directly to the involved tissues" (Flynn *et al.*, 1994).

Drug delivery system is a system that the drugs are controlled and released by phagocytosis and endocytosis mechanisms from the drug carriers into the cell or organ (Scheme 2). It offers the advantages as compared to the conventional dosage form, such as increasing therapeutic activity, reducing toxicity, and reducing the number of drug administrations required during treatment (Sunil *et al.*, 2004). On the viewpoint of material, drug carriers, in most cases are polymers. The drugs are attached on polymer chain either via covalent or non-covalent bond. The conjugation of the drug onto the polymer can be carried out under two main methods, physical modification and chemical modification. In the case of the physical modification, the drugs are introduced to those drug carrier solutions by mixing or blending the drugs before forming films, gels, membranes, and beads (Kande *et al.*, 1998). For example, Bodeier *et al.* (1989) prepared sulfadiazine beads by dropping drug-containing solutions of chitosan into a tripolyphosphate (TPP) solution. The drug was entrapped within a three-dimensional network of the ionotropic gel-like spheres and the release was involved with pH. The beads were swelled and dissolved at low pH and remained intact at high pH. The release decreased with an increasing of TPP concentration. Although, up to now, a large number of drug carriers prepared by physical modification method have been reported, the uses of the acidic solvents and the toxicity of crosslinking agents are the points to be considered.

For chemical modification, the drugs are covalently bonded to polymer via spacer molecules. The introduction of drug requires chemical reaction with the functional groups of polymers and drugs. The possible reactions are also the points

to be considered. At present, various polymers that are used for drug carriers, such as cellulose derivatives (Rosalia *et al.*, 2002) and poly(vinyl alcohol) (Constantin et al., 2006) have been reported. For chitosan, Ohya *et al.* (1992) prepared 6-O-carboxymethyl chitin (CM-chitin) conjugated with 5-fluorouracil via pentamethylene and monomethylene spacers. The CM-chitin/5-fluorouracil prodrug obtained showed a slow release of 5-fluorouracil and exhibited antitumor activity against leukemia. In general, the chemical modification for drug conjugating on polymer shows more regular controlled release than that of physical modification. The drug carriers prepared by chemical modification are risky due to the loss of drug active sites during the chemical reaction.



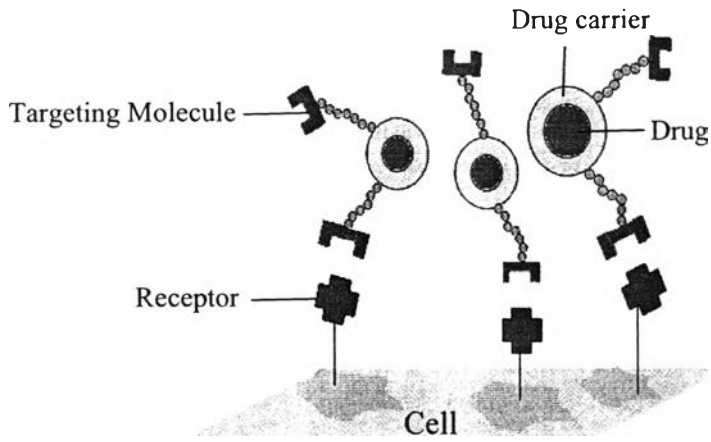
Scheme 2. Representative scheme of drug delivery system. (www.rsc.org)

2.2.2 Applications in Drug Targeting As the drug has to come across the biological barriers, such as organs, cells and intercellular compartment, the drug might be deactivated and express undesirable influences on organs and tissues including the high therapeutic concentration of drug in the body to compensate the

loss during the absorption onto specific tissues is required. In addition, under these circumstances, cytotoxic and/or antigenic drugs become the negative effects. In recent years, the attention about the direct drug administration in the target organ or tissue in a selective manner with a quantitative amount, including the independent of the site and methods of its administration has been established. Ideally, under such conditions, the local concentration of the drug at specific cells or organs should be high, while its concentration in other non-target organs and tissues should be below the minimal level to prevent any of drug from the negative side-effects. The advantages of drug targeting are, for example, (a) the simplification of drug administration protocols, (b) the reduction of drug at a therapeutic effective amount, and (c) the low drug concentration in the required sites including on non-target compartments (Vasant *et al.*, 1998).

In the past, The drug targeting concept as suggested by Paul Ehrlich (1999) was a hypothetical 'magic bullet' as an entity consisting of two conditions — the first is the the target binding, while the latter is the therapeutic action in this target. However, in the recent years, the concept of magic bullet includes the three components, which are; drug, targeting moiety, and pharmaceutical carrier (Scheme 3). Pharmaceutical carriers include micelles, microcapsules, microparticles, cells, cell ghosts, lipoproteins, liposomes, and soluble polymers (Ottenbrite, 2001).

It should be noted that the pharmaceutical carriers for drug targeting should be biocompatible, non-toxic, inert (not react with immune response or other substance), and capable for achieving high drug loading. Polymer-based systems intended for medicinal applications have to be designed with the consideration of number of factors, such as the nature of disease, drug properties, type of therapy, physiology of the patient, administration route, therapy site, and characteristic of the polymeric materials used, including the mechanism of drug targeting. The proposed polymers are polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), polyanhydrides, polyorthoesters, poly(ethylene glycol) (PEG) and polysaccharide, including chitin-chitosan (Raphael M. Ottenbrite, 1996).



Scheme 3. Representative scheme of drug targeting.

2.2.3 Points to be considered for Chitosan Drug Targeting System It is known that the main problems of the systemic drug administration are the biodistribution of pharmaceuticals throughout the body, the lack of drug specific affinity toward a pathological site, the necessity of a large total dose of a drug to achieve high local concentration, and the non-specific toxicity and other adverse side-effects due to high drug doses. Conceptually, drug targeting has to overcome all these problems (Torchilin, 2000).

It is important to note that chitosan has the specific properties which are suitable for pharmaceutical or drug carriers. Park *et al.* (2003) and Li *et al.* (2003) demonstrated that the conjugation with bioactive molecules, such as galactose and fructose, onto chitosan increased the hepatocytes adhesion in the liver cell. Zhang *et al.* (2004) also reported the systhesis of galactosylated or lactosaminated chitosan and the preparation of microspheres. The material was proposed for hepatic targeting drug delivery and prolonged the residence in blood circulation.

2.2.4 Motivation of the Present Work on Drug Targeting System Chitosan faces the weak point about its solubility because chitosan is only soluble in acid. This leads to the problems about the toxicity related to the solvents used in preparing the materials. Up to present, the functionalization and/or modification in heterogeneous organic solvent system or homogeneous carboxylic acid system has

been variously reported. The derivatization in water is an ideal case to ensure the products for the biomedical-based applications. Recently, our group succeeded in developing chitosan conjugating reaction in water-based system.

This work focuses on the use of water-based conjugating reaction to introduce amino acid molecules onto chitosan chain. The amino acids in the consideration are tryptophan and asparagine (Figure 1). The differences in hydrophilicity between two compounds give us guidelines about reactivity, degree of substitution, and the morphology in developing chitosan drug targeting.

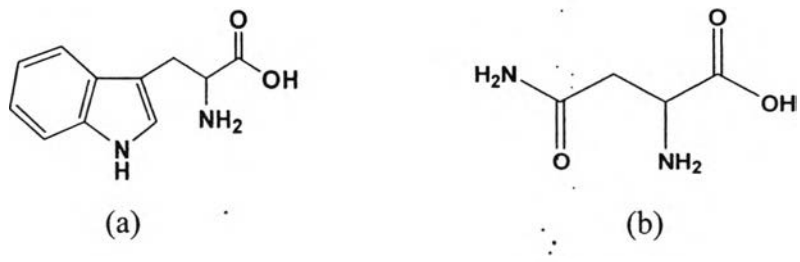


Figure 1. Chemical structures of; (a) tryptophan, and (b) asparagines.

2.3 Development of Chitosan for Biomedical Scaffold Material

2.3.1 Production of Scaffold Material In recent years, scaffolds are recognized as an alternative material with porous structure for advanced applications, such as bioactive molecules delivery (Mao *et al.*, 2003), cultured artificial organs (Davis *et al.*, 2001), and tissue engineering (Khor *et al.*, 2003). Until now, the techniques known for preparing scaffold are, for example, polymer assembly, phase separation, and electrospinning. The polymer assembly is a preformed structure based on the molecular interactions, such as hydrophilic, hydrophobic, and ionic interactions, etc. The phase separation technique deals with a three dimensional continuous fibrous network after lyophilizing the aqueous polymer solution (Smith *et al.*, 2004). Electrospinning is another technique practical to many types of polymers in the solution state. The precipitation of micro/nanofiber under electric potential gives an effective scaffold structure. For chitosan, phase separation (Madihally *et al.*, 1999) and electrospinning (Bahattrari *et al.*, 2005) techniques to

initiate the chitosan scaffold using chitosan solutions or the cross-linked chitosan gels have been reported.

2.3.2 Nanocomposites-based Aerogel Scaffold Aerogels are unique materials with many attractive properties, such as extremely high porosity (> 98%), large surface area (~ 10^6 m^2/kg), very low density (~3 kg/m^2), and low thermal conductivity (~ 0.05 W/mK) (Fricke and Tillotson, 1997). Sol-gel method followed by supercritical drying is a practical way to develop nanoporous aerogel network from polymer solution. It should be noted that aerogels have been received much attention in various fields, especially, adsorption (Hrubesh *et al.*, 2001) and drug delivery system (Smirnova *et al.*, 2004), etc. Even if a large majority of the aerogel studies are dealing with inorganics especially silica-based aerogel (Shi *et al.*, 2006), some classes of organic aerogels under the concept of nanocomposites-based aerogel has become a new challenge in the area of polymer nanocomposite.

The term "nanocomposite" implies that the physical arrangement of the different phases is on a scale of less than 100 nm (Roy *et al.*, 1986). Up to now, nanocomposites are recognized with advantages, such as high strength, long shelf life and lighter-weight (Pinnavaia *et al.*, 1996). Most of nanocomposites were prepared by clay or silicate layers reinforced with polymer matrix (Giannelis *et al.*, 1996). It is important to note that clay is a material appropriate for backbone layer composite because it provides the layer in nano-scale to improve some physical properties (Mascia *et al.*, 1995).

2.3.3 Points to be considered for Chitosan Aerogel Nanocomposite The formation of aerogel consists of three main parts which are polymer solution, clay, and crosslinker. For example, Chen *et al.* (2005) proposed the immobilization of chitosan-acetic acid solution onto poly(*N*-isopropylacrylamide) (PNIPAAm) gel/polypropylene (PP) nonwoven composites. The use of glutaraldehyde as the cross-linking agent followed by freeze-drying gave chitosan aerogels with antibacterial ability. The porous network exhibited good biocompatibility to fibroblast.

2.3.4 Motivation of the Present Work on Aerogel Nanocomposite Although Chen *et al.* (2005) showed a concrete work in preparing chitosan aerogel, the uses of acetic acid, and glutaraldehyde are the points to be overcome.

In this work, we applied the conjugation in water-based system and focus on the crosslink reaction with PEG derivatives to initiate the gel formation. The work also considers how clay (montmorillonite) plays an important role in aerogel formation of chitosan interpenetrating network with clay after the freeze-dried process.