CHAPTER II
LITERATURE SURVEYS

2.1 Chitin-Chitosan and their applications

Chitin is the second most abundant natural occurring polysaccharide. It acts as the structural polysaccharide in many lower organisms such as fungi. It is also found in bacterial cell walls, cuticles of insects, and the shells of crustaceans such as lobster, crabs or squids (Muzzarelli, 1976). The structure of chitin can be thought as a cellulose derivative which consists of β-(1→4)-D-glucopyranose units (Rathke and Hudson, 1994). Unlike cellulose, chitin has an acetamide group substituted at the C-2 carbon position instead of the hydroxyl group, resulting in mainly β-(1→4)-2-acetamido-2-deoxy-D-glucopyranose structural units.

Chitosan is the *N*-deacetylated derivative of chitin. Since this *N*-deacetylation is hardly complete, the nomenclature border between chitin and chitosan can not be defined exactly based on the degree of *N*-acetylation. Experimentally, chitosan can be distinguished from chitin by the solubility in aqueous diluted acetic or formic acid (Muzzarelli, 1976).

The structures of both chitin and chitosan compared to cellulose are shown in Figure 2.1.

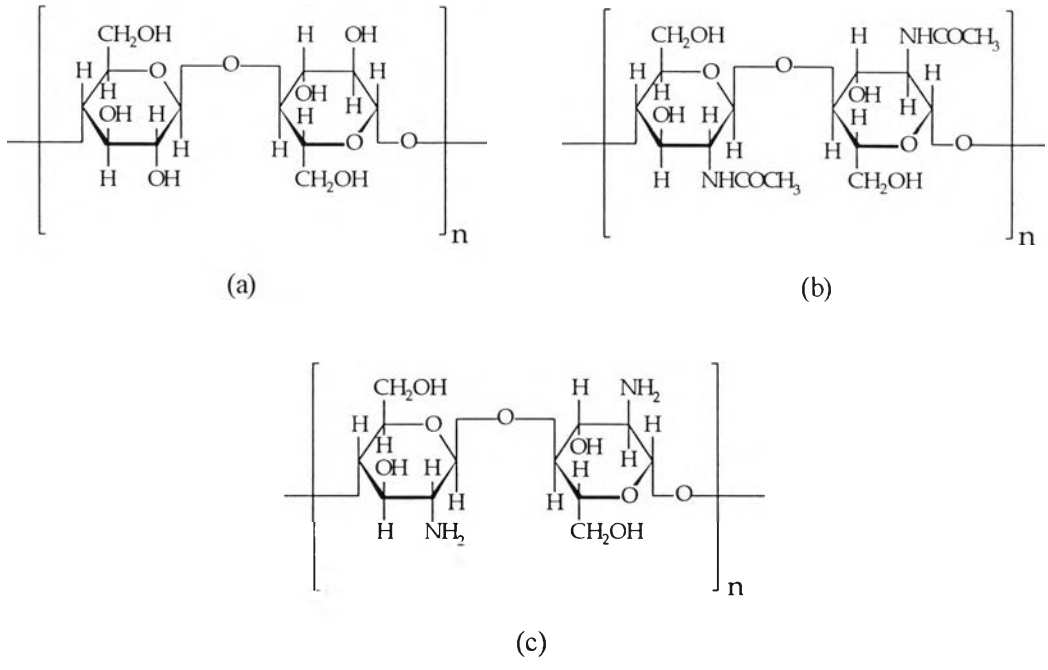

Figure 2.1 Chemical Structure of (a) cellulose; (b) chitin; (c) chitosan.

Owing to the structures of chitin and chitosan, they can be expected to have some specific properties as follows :-

1. Due to the lone pair electrons at hydroxyl, acetamide and/or amino groups, chitin-chitosan is found to chelate metal ions such as chromium, lead (Eiden, 1980), copper (Kurita, 1986), etc.
2. A well-oriented of hydrophobic and hydrophilic parts in the structure results in the inclusion property ; for example, dyes (Shimizu, 1995).
3. Both the hydroxyl and acetamide or amino groups in the pyranose rings show antimicrobial property (Suzuki, 1986; Seo, 1991).
4. Chitin and chitosan are biopolymer, which make them to be degraded by the action of enzymes from microorganisms and compatible to living cells.

As a result, chitin and chitosan are attractive for many applications such as a metal ions chelating agent or a molecular separating agent for waste water treatment and chromatographic separation or as a biocompatible material for

biomedical applications and a carrier for polymeric drug delivery system. However, the application of using chitin-chitosan is limited by their low solubility in most organic solvents due to its rigid structure. This makes most studies of chitin-chitosan are mainly focused on physical modification such as beads, gels, membranes or films preparation.

To overcome the limited application, a chemical modification is expected to solve their weak points since many functional groups for further chemical modification are available in chitin and chitosan main chain.

2.2 Chemical Modification of Chitin-Chitosan

Recently, the chemical modification of chitin-chitosan has received much attention not only for improving the low solubility but also for achieving some expected novel properties which cannot be found in the natural chitin-chitosan. The approach is, such as, the addition of functional groups or highly reactive group to the main chain in the structure as a concept of functional polymer.

For example, different *N*-acylated derivatives of chitosan, including octanonyl, dodecanoyl, octadecanonyl, and benzoyl chitosan, were prepared and studied on the sorption behavior of some organic dyes (Seo, 1991). Those derivatives were further studied on the separation of racemic mixture of amino acids. The study was concluded that chitosan containing hydrophobic groups by acylation could be used effectively in the hydrophobic separation and the resolution of optical isomers of D,L-amino acids (Seo, 1991).

Another application for chitin that is interesting is as a carrier for controlled release system or a prodrug for medical treatments such as 6-*O*-carboxymethyl chitin (CM-chitin). Ohya *et al.* proposed the preparation of

CM-chitin conjugated with doxorubicins *via* tetrapeptide spacer groups. The *in vivo* and *in vitro* antitumor activities of this conjugates were investigated compared to the one having simple hydrophobic pentamethylene spacer group. The results showed that conjugates having peptide spacer groups gave higher cytotoxic activities than the one having pentamethylene spacer groups (Ohya, 1995).

2.3 Controlled Release System

In medical treatment, drugs are introduced at intervals by the ingestion of pills or liquids or the injection. The drugs then circulate throughout much of the body and the concentration of the active agents rises to high levels at least initially and then declines gradually due to the excretion and/or metabolic conversion as shown in Figure 2.2.

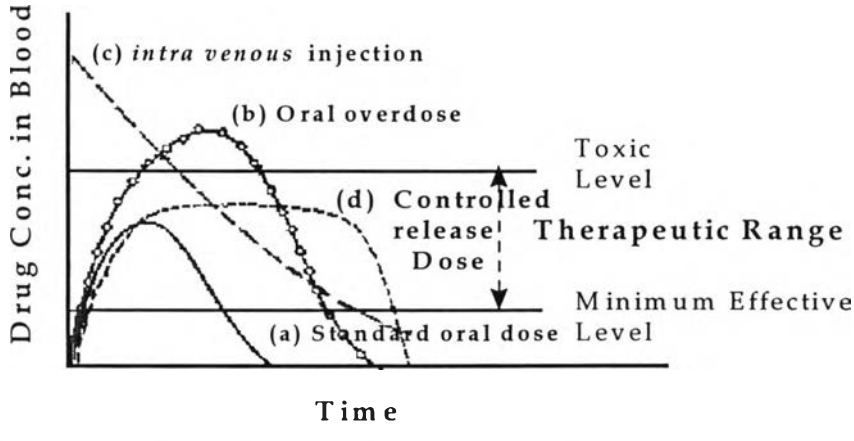

Figure 2.2 Schematic of typical drug concentration as a function of time for various types of drug administration.

Conventionally, many biologically active agents are often administrated to a system by non-specific and periodic application.

To get the most effective drug therapy, compared to conventional systems, controlled release systems have been proposed. In this case, the constant concentration of drug, which is effective but non-toxic, is maintained for a desired time.

The concepts of drug controlled release system have been proposed in terms of time-controlled systems, site-controlled systems or targeting, and controlled systems through feedback. Up to now, at least two methods have been used to prepare the controlled release system. One is the concept of prodrug and another is the polymeric controlled release system. A prodrug may be considered as a pharmaceutically inactive derivative which requires a chemical and/or enzymatic transformation in the body to release the active parent drug. The prodrug approach may be applied to achieve prolonged drug action. However, the other way to prepare a controlled release system which is the preparation in a form of polymer, so called a polymeric drug delivery system or polymeric controlled release system, has received more attention. Controlled release systems can be applied not only for pharmaceutical application but also agriculture applications. For example, Lee (Lee, 1993) prepared a controlled release system using poly(vinyl alcohol) as membranes and studied the release of chlorinated isocyanurates which showed bactercidal and algicidal properties and have been employed as topical antiinfectives and disinfectants for water cooling system or swimming pools. This system gave a prolonged constant rate of release and also exhibited good stability in the presence of saturated solution of chlorinated isocyanurates.

Isabelle (Isabelle *et.al.*, 1991) proposed a model to describe drug release kinetics from pellets coated with acrylic resins and various plasticizers. It was concluded that the optimized parameters of the model are dependent on

manufacturing conditions, particularly the film-coating conditions which affect the film structure.

Generally, two main types of polymeric controlled release system are known.

1. Physical insertion in polymer matrix system :- The drug molecule is dispersed or entrapped in the polymer matrix. Controlled release systems are formed by physical modification, such as the forming of beads, gel, membrane or film.
2. Polymer-drug conjugation system :- The drug molecule is chemically bound to the polymer main chain.

In controlled release process, drug delivery system responds to changes in environmental conditions , e.g., temperature, pH, light, etc. Those conditions can cause the bond breaking between drug molecules and polymer main chain or /and changing the structure of polymer network and cause the release of drug molecules from the matrix.

Historically, controlled release systems were firstly developed in the 1950s and were originally applied to introduce non-medical agents such as antifouling agents and pesticides. In 1970s, controlled release systems were developed for the slow release of large molecular weight molecules ; for example, polypeptides, polyesters, etc. By 1980s, several polymer-drug systems were studied and some are prepared for clinical use.

For pharmaceutical aspect, G.D. Searle & Co. developed and then licensed a controlled release product that acts as an estrus-synchronization treatment for cattle by using a matrix of the copolymer of hydroxyethyl methacrylate and ethylene glycol dimethacrylate having norgestomet hormone. Other example is Uekama (Uekama *et.al.*, 1993) who used *O*-carboxymethyl-*O*-ethyl-β-cyclodextrin (CME-β-CyD) as a controlled release carrier for diltiazem. The release characteristics of the prepared system were further

studied in vitro and in vivo. The in vivo result showed that the system served as a delayed-release type carrier for diltiazem.

Dumitriu (Dumitriu, 1986) synthesized a bioactive polymer from xanthan. The coupling reaction between xanthan and some antibiotic agents, which are chloramphenicol and ampicillin, was done successfully through the dicyclohexylcarbodiimide (DCC) spacer group. The synthesized conditions were studied and the obtained product was characterized to show that the drug retardation could be achieved by hydrolysis mechanism of the spacer group.

2.4 Chitin-chitosan in Polymeric Drug Delivery system

2.4.1 Polymer Matrix Insertion System

Since chitin shows a low solubility in various types of organic solvents, the most common used chitin derivative is chitosan which shows a better solubility in organic solvents, especially in aqueous formic acid or acetic acid. Sawayanagi (Sawayanagi *et al.*, 1982) developed a sustained release system of a water soluble drug using lactose/chitosan as a carrier in the form of a compressed tablet. The prepared systems showed different hardness and release patterns depending on chitosan content. Bodmeier (Bodmeier *et al.*, 1989) prepared drug-containing chitosan beads and studied the release profiles from obtained beads at various conditions. Lin (Lin *et al.*, 1992) studied the adsorption and desorption of indomethacin on chitin-chitosan compared to cellulose. The results proposed an application for the formulation of controlled release drug dosages.

2.4.2 Polymeric Drug Conjugate

One of the interesting chitosan conjugates can be raised by the work of Ohya (Ohya *et al.*, 1992). 6-*O*-Carboxymethyl chitin (CM-chitin) was coupled with the anticancer drug 5-fluorouracil (5-FU) through pentamethylene and monomethylene spacer groups *via* amide and ester bonds. The obtained product was water soluble and gave slow release of 5-FU depending on the stability of the spacer groups. Onishi (Onishi, 1995) synthesized the monoester of 5-fluorouridine with 4-carboxybutylic acid. This prodrug was further conjugated with chitosan. The release of 5-fluorouridine from this conjugate was found to be related to the pH of the system.

In the present work, a controlled release system was prepared by both physical modification and chemical conjugation. The bead type of chitosan/drug mixture was focused on as a physical modification approach, while the powder of chitosan/drug conjugate was considered as a chemical modification one. In order to satisfy the preparation of chitosan beads or powder, the model drug applied in this study has to be a solution, stable under basic or acidic condition for controlled release study, easily detected by analytical methods, and have functional groups available to couple with chitosan. The present work is concerned on a chitosan conjugate via a spacer group of *N*,*N*'-carbonyl diimidazole (CDI) with chloramphenicol as a model drug. Here, the release system and the mechanism of release were also considered.