CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Alginate and chitosan-a co-biopolymer as a drug carriers

Alginate is a naturally occurring anionic polymer obtained from marine brown algae. Structure of alginate is linear copolymers containing blocks of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues joined by 1,4-glycosidic linkages (Fig. 2.1). Only the G-blocks of alginate are believed to participate in intermolecular cross-linking with divalent cation (e.g., Ca^{2+}) to form hydrogels. The water molecules are physically entrapped inside the alginate hydrogels but are still free to out from the matrix. This is useful property in many applications such as alginate gel for protein or small drug delivery. Alginate has also been widely used in many drug delivery systems in combination with chitosan, ionic complexes between alginate and chitosan [1].

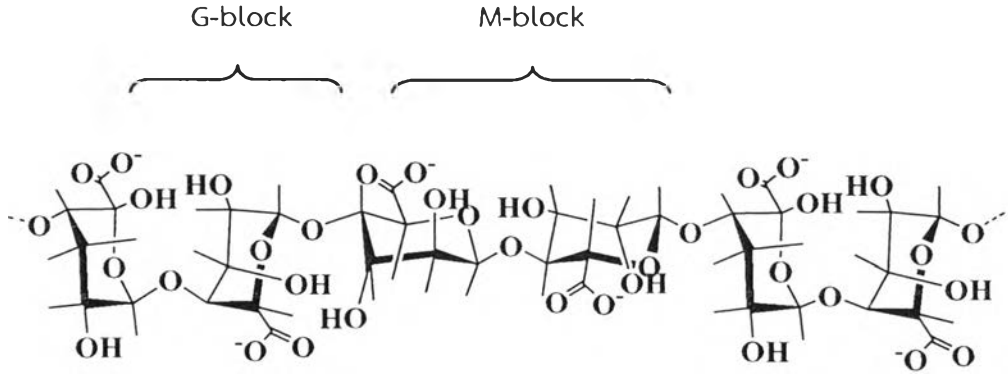

Figure 2.1 Copolymer of α-L-guluronic acid and β-D-mannuronic acid in alginate

Chitosan is the most abundant polysaccharide, a derivative of chitin. Chitosan is obtained by *N*-deacetylation of chitin, the removal of acetyl from the acetamide (-$COCH_3$) groups to amino groups (-NH_2). Chitin is a polymer found in crustacean shells such as a crabs, shrimps and lobsters. Chitosan is a linear copolymer that consists of D-glucosamine and *N*-acetyl-D-glucosamine units joined by β-(1-4)-glycosidic linkages (Fig. 2.2) [4].

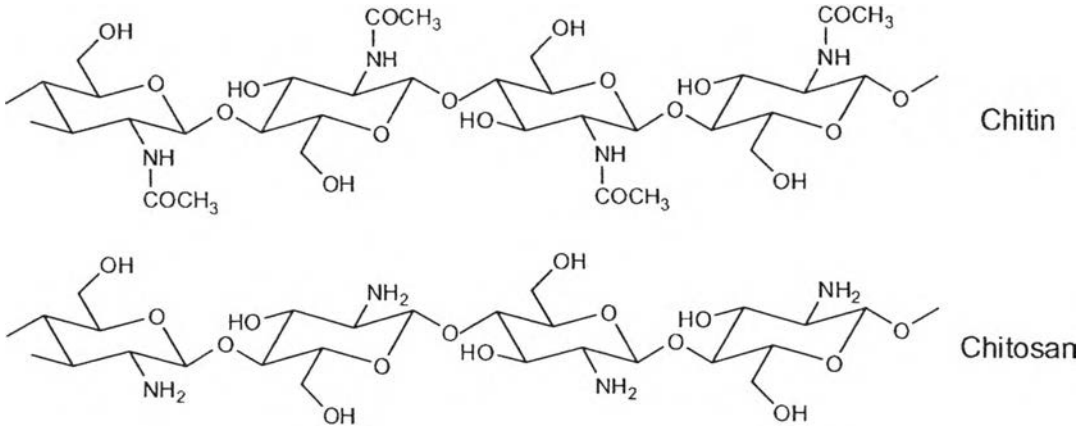

Figure 2.2 Structure of chitin and chitosan

Normal amino group in chitosan has a neutral charge but in acidic condition the amino group is changed to positively-charged ammonium ion ($-NH_4^+$). When mixed with calcium alginate, a polycation chitosan and a polyanion of alginate are attracted each other to form stable particles. These processes are called "ionotropic gelation".

One of many methods to modify the chitosan structure is by connecting hydrocarbon chain on the amino side groups of chitosan. This can be done by reacting chitosan with an aldehyde, e.g. butyraldehyde, in a process called reductive alkylation with the use of sodium cyanoborohydride as a reducing agent [5, 6]. (Fig. 2.3). In this reaction, the amino group of chitosan reacts with an aldehyde to form a Schiff's base intermediate that is later reduced to a secondary amine.

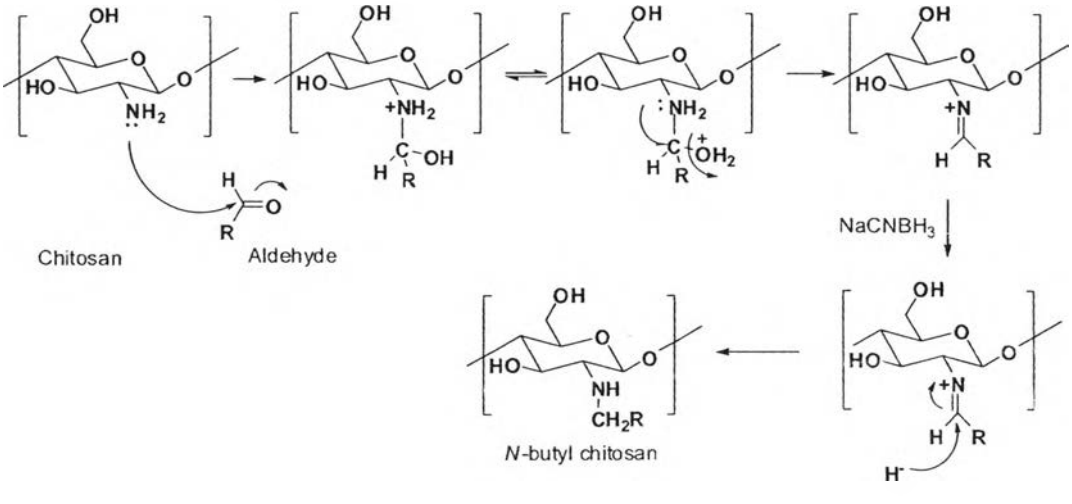

Figure 2.3 Reductive alkylation mechanism of chitosan

A number of reports on the preparation the nanoparticle of alginate and chitosan by ionic gelation method as the carrier for drug delivery focused on using calcium ion (Ca^{2+}) from $CaCl_2$ to partially crosslink the alginate polymers first. This was followed by the addition of chitosan solution to attract with the remaining carboxylate groups on alginate to form a complete polyelectrolyte complex. Also the solution for this method needed to be rather low to achieve nano sized particles [7, 8].

Preparation factors affect the size of particles include:

1. *Order of mixing*- the mixing alginate and calcium ion in order to partial crosslinking of alginate chain, making egg-box structure, in which alginate chain were compacted. Next polycation polymer e.g. poly-L-lysine was added, interacted with alginate to obtain a small and strengthen particle. In contrast, the first order if mixing of alginate and poly-L-lysine before Ca^{2+}, resulting in the large particle size because egg-box structure was less occurred (Fig. 2.4) [7].
2. *Mass ratio of calcium:alginate*- At Ca^{2+}:alginate ratio of 0.17, high density particle and narrow size distribution were obtained [9]. The micro particle was occurred when a Ca^{2+}: alginate ratio of poly-L-lysine and chitosan was over 0.25 and 0.33 respectively (Fig. 2.5).

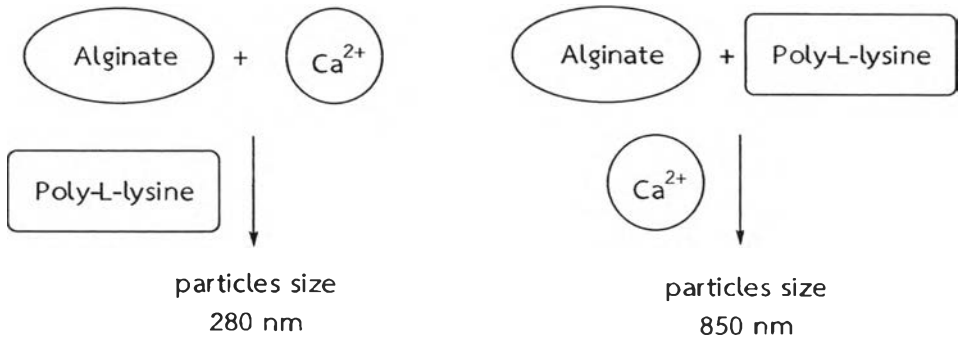

Figure 2.4 The effect of order of mixing on the particle size

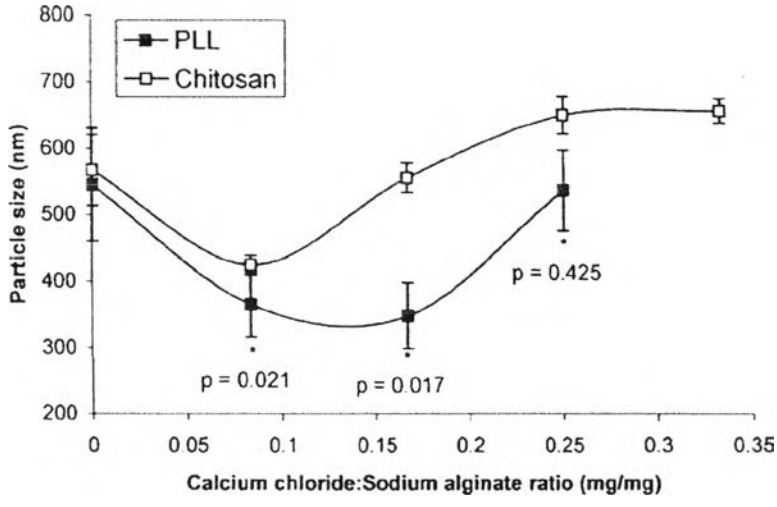

Figure 2.5 The effect of Ca^{2+}: alginate mass ratio on the particle size of calcium-alginate-poly-L-lysine (■) and calcium-alginate-chitosan (□), 0.6 mg/ml of alginate concentration and 0.1 of cationicpolymer ratio

3. *Cationic polymer to alginate mass ratio-* Addition of cationic polymer that cationic polymer to alginate mass ratio over 0.133, was obtained a large sized particle (Fig. 2.6). Since bridging between particles was agglomerated [10-12].
4. *pH in preparation-* the preparation of particle from alginate and chitosan, pH was important factor. The decrease of pH from 5.2 to 4.7 reduced the particle size of obtained particles. If the pH was decreased to lower than 4.2, the particle size was increased because alginate was precipitated at pH~3.3-3.6. The ionic interaction between alginate and chitosan depended on pH. A strong complex was derived at pH 4.5-5.0 [10, 13].
5. *Drug concentration-* The particle size was increased if addition of drug e.g. doxorubicin, amino group in molecule of drug can interact with carboxylic group of alginate before adding Ca^{2-}, therefore a probability of Ca^{2+} to produce egg-box structure was occupied by drug molecule. Steric hindrance was occurred when used high concentration of drug [7].

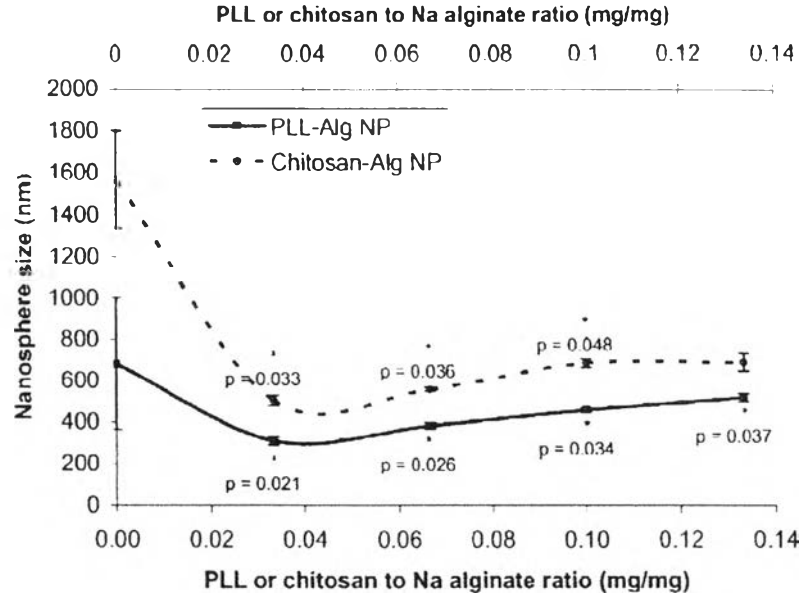

Figure 2.6 The effect of cationic polymer to alginate mass ratio on the particle size of calcium-alginate-poly-L-lysine (■) and calcium-alginate-chitosan (●), 0.6 mg/ml of alginate concentration and 0.17 of Ca^{2+}: alginate mass ratio

2.2 Controlled release system

The means by which a drug is introduced into the body is almost as important as the drug itself. Drug concentration at the site of action must be maintained at a level that provides maximum therapeutic benefit and minimum toxicity. The pharmaceutical developer must also consider how to transport the drug to the appropriate part of the body and, once there, make it available for use [14].

Controlled drug delivery occurs when a polymer is combined with the drug or other active agents in such a way that the active agent is released from the material in a predesigned manner. The drug can be released from the system by 3 mechanisms.

2.2.1 Diffusion controlled release

Diffusion occurs when drug molecules pass from the polymer matrix to the external environment. As the release continues, its rate normally decreases with this type of system, since drug has progressively longer distance to travel and therefore requires a longer diffusion time to release.

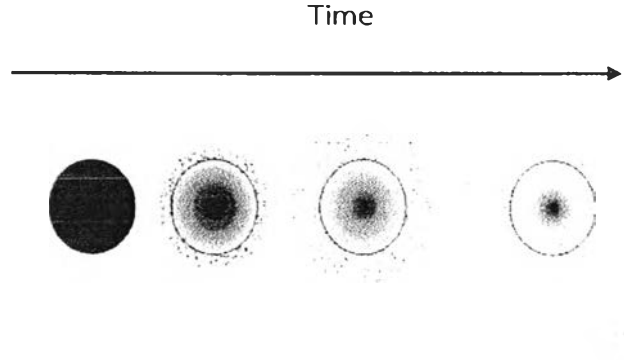

Figure 2.7 Schematic of diffusion-controlled release [15]

2.2.2 Swelling controlled release

The swelling of the carrier increases the aqueous solvent content within the polymer matrix, enabling the drug to diffuse through the swollen network into the external environment. Most of materials used are based on hydrogel. The swelling can be triggered by a change in the environment surrounding such as pH, temperature, ionic strength, etc.

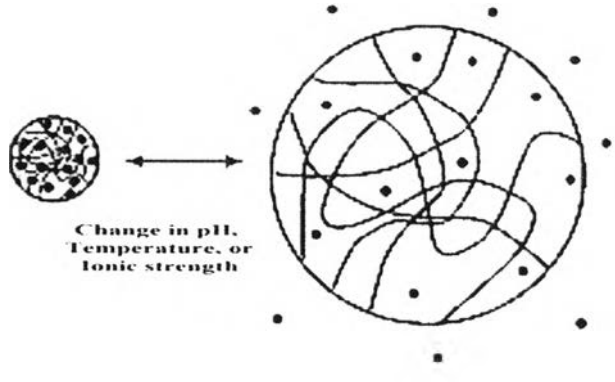

Figure 2.8 Schematic of swelling-controlled release [15]

2.2.3 Erosion controlled release

The drug can be released from the matrix due to erosion of polymers, which can be classified into 2 types.

Bulk erosion: The polymer degrades in a fairly uniform manner throughout the polymer matrix.

Surface erosion: The degradation occurs only at the surface of the polymer device.

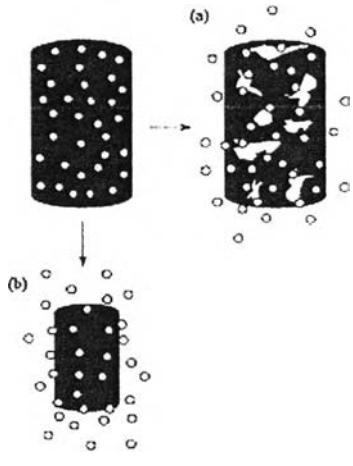

Figure 2.9 Schematic of erosion-controlled release [15]

2.3 Glucosamine

Glucosamine, an amino monosaccharide, is a natural component of glycoproteins found in connective tissues derived from chitin. It is a precursor of glycosaminoglycans (GAGs), disaccharide unit, which are the building blocks of the articular cartilage [16]. Recently, glucosamine was used for the treatment of osteoarthritis in humans. It can be injected or eaten as dietary supplement at a regular interval. Accordingly, glucosamine is commercially available as a nutritional supplement in the form of glucosamine hydrochloride (GH), glucosamine sulphate, and *N*-acetyl-glucosamine [2]. Most of the clinical studies examined the effect of glucosamine in the forms of hydrochloride or sulfate on osteoarthritis.

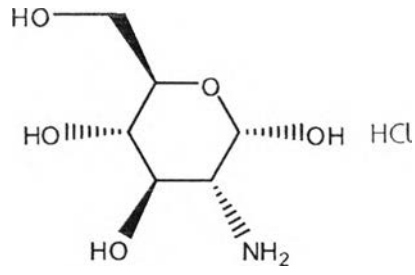

Figure 2.10 Chemical structure of glucosamine hydrochloride

Specification of glucosamine hydrochloride is as follows:

- IUPAC name: (3R,4R,5S,6R)-3-Amino-6-(hydroxymethyl)oxane-2,4,5-triol
- Other name: 2-Amino-2deoxy-D-glucose chitosamine
- Formula: $C_6H_{13}NO_5$
- Physical state and appearance: Solid (Powdered solid)
- Odor: Odorless
- Molecular Weight: 179.17 g/mol
- Color: White
- Solubility: very soluble in water, insoluble in alcohol
- Melting Point: 105°C, 423 K, 302°F

Pharmacokinetic data:

- Bioavailability: 1,500 mg/day
- Route: Oral, topical
- Side Effects: heartburn, drowsiness, skin rashes, headache, insomnia, and mild and temporary digestive complaints such as abdominal pain, poor appetite, nausea, heartburn, constipation, diarrhea, and vomiting [17].

2.4 Transdermal delivery systems

Transdermal drug delivery systems are an alternative to solve a problem of oral administration of drug. Many drugs have the limitations due to drugs was eliminated in stomach, colon and liver. The drug was delivered through skin and into the systematic circulation thus avoiding the hepatic first pass metabolism during oral administration and convenience to the patient. However, the penetration of drug through the skin was limited by the structure of stratum corneum as a barrier. The skin has a most surface area of the body. Many researchers have investigated its use in transdermal delivery [18]. Products of transdermal delivery devices include cream, gel, wax, lotion, and transdermal patch.

Nowadays, nanoparticles have shown a great potential as novel drug carriers for transdermal drug delivery. The smaller size of particles could ensure close contact with the stratum corneum and increases the encapsulated amount of drug penetrating into the skin [19]. The advantages of nanoparticle carriers are protection of unstable drugs from degradation and control of drug release rate from the particles.

Water-soluble molecules and drugs are normally not able to cross the skin as the skin is a natural barrier to water. Lynk Biotech has developed TGC (Transdermal Glucosamine Cream), which contains a high concentration of the water-soluble compound glucosamine (10% w/w) encapsulated within a lipovesicular system in a stable cream dosage form [20]. TGC has the capability of being able to deliver glucosamine across the skin in a sustained manner for up to six hours. In this trial, excellent transdermal glucosamine profiles were observed with the human volunteers. Plasma glucosamine concentration remained high for up to 8 hours post treatment (Fig. 2.11 and 2.12). This means that the lipovesicular system can provide a high and constant source of glucosamine that is beneficial to patients suffering from osteoarthritis.

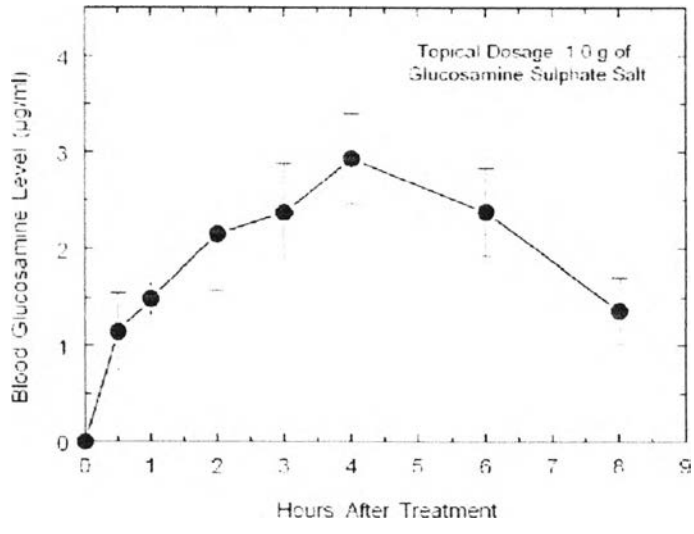

Figure 2.11 Transdermal profile of Mediflex™ glucosamine cream in human volunteers during the course of 8-hr study [20]

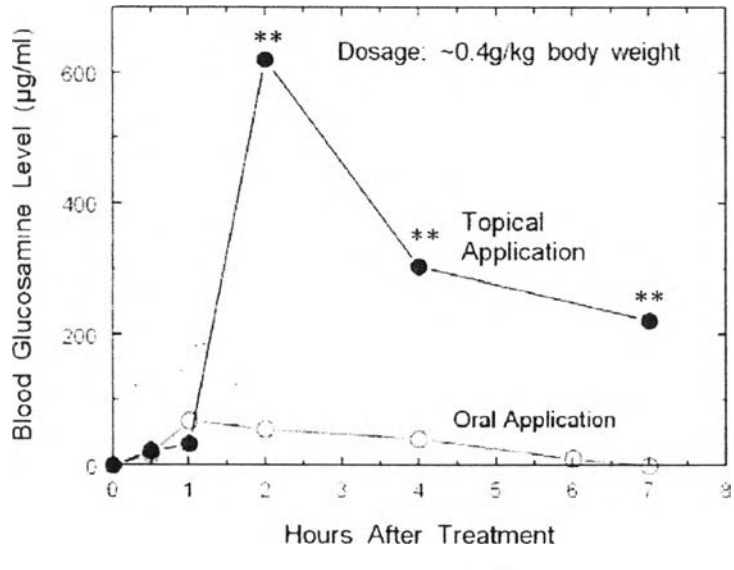

Figure 2.12 Comparing the efficiency of Mediflex™ glucosamine cream and oral delivery of glucosamine into blood of adult mice [20]