CHAPTER II
LITERATURE REVIEW

2.1 Chitin

Chitin, poly(β-(1→4)-N-acetyl-D-glucosamine), is the second most abundant natural polysaccharide in the world after cellulose. The major sources are discovered in the shells of crustaceans such as crabs, shrimps and squid pens, the cuticles of insects, and the cell walls of fungi (Jayakumar *et* al., 2010). Chitin molecules consist of 2-acetamido-2-deoxy-D-glucopyranose while chitosan is an *N*-deacetylated derivative of chitin. Chitin may be noticed as cellulose with hydroxyl at position C-2 replaced by an acetamido group. The structure of cellulose ,chitin and chitosan are shown in Figure 2.1.

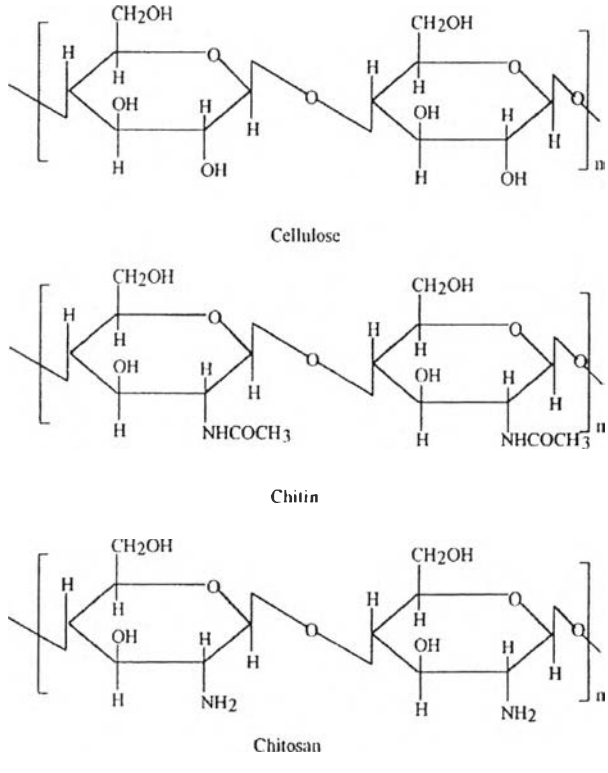

Figure 2.1 Structure of cellulose, chitin and chitosan (Kumar, 2000).

Chitin can be found in one of the three crystalline forms: α-chitin, β-chitin and γ-chitin, respectively. The molecules of α-chitin are arranged very tightly in an anti-parallel form. α-chitin is mainly present in shells of crabs, lobsters and shrimps. β-chitin obtain from squid pens, which the chains are arranged in a parallel form, while γ-chitin which the molecules are arranged in both parallel and anti-parallel form. As a result of the molecular packing, intermolecular interactions in β-chitin are weaker than α-chitin, making β-chitin being more liable to dissolution in a number of solvents. Thus β-chitin being more reactive and versatile (Peesan *et* al., 2003).

In the case of crabs or shrimp shells, the chitin manufacture is involved with food industries such as shrimp canning. The typical procedure for the processing of chitin from the shells as follows: the shells of crab or shrimp were cleaned, cut it into small flakes and treated with diluted hydrochloric acid at room temperature to remove calcium carbonate. After decalcified shells, heated in sodium hydroxide at 100 °C to decompose proteins and pigments. This α-Chitin extracted from these shells was obtained as colorless to off-white powdery materials (Shimahara and Takigushi, 1988).

Chitin is in commercial interest because of their high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%). This makes chitin become new functional material of high capability in various fields and recent progress in chitin chemistry is quite important. Most of natural sources were occurred in polysaccharides, e.g. cellulose, dextran, pectin, alginic acid, agar, agarose and carragenans, are neutral or acidic in nature, whereas chitin and chitosan are examples of basic polysaccharides. Moreover, chitin and chitosan have many unique properties include polyoxysalt formation, ability to form films, chelate metal ion and optical structural characteristics (Larry, 1998).

Chitin possess excellent biological properties, such as antiviral activity, low-toxicity, low-allergy, high radiation resistance, biodegradability, and etc. (Kumar, 2000) Furthermore, Chitin has an advantage in biocompatibility, which better than chitosan, because an acetamide group of chitin is similar to an amide group of protein in living body (Muzzareli, 1985) which made it attractive to use as biomedical applications. Many studies have reported the use of chitin and its

derivatives for pharmaceutical purposes, such as the drug delivery system (Nsereko and Amiji, 2002). However, the existence of hydroxyl and amino groups in the monomer unit of chitin produce strong hydrogen bonds provided highly crystalline structure, resulting in chitin presents a problem of difficult process-ability and limited in organic solvent. The solubility problem of chitin limits its application, therefore, chemical modification of chitin to enlarge its solubility in common solvents is necessary to extend its utilization (Jeong *et* al., 1999).

2.1.1. Chitin whiskers

Whiskers are very hopeful reinforcing materials for composites, because of their high stiffness and strength (Tjong *et* al., 1999). Besides, whiskers have a small diameter and nearly free of internal defects, thereby yielding strength near to the maximum theoretical value predicted by the theory of elasticity (Courtney, 1990). It was found that the enhancement of their reinforcement depends on many factors (Chazeau *et* al., 2000) such as the nature of the matrix, the aspect ratio, the generation of a strong fiber-matrix interface through physicochemical bonding and dispersion of the whiskers in the matrix. Moreover, whiskers from renewable resources have many advantages such as renewability, low cost, easy availability, good biocompatibility and easy chemically and mechanically modification, compared with inorganic whiskers (Zinai *et* al., 1996).

Figure 2.2 shows the hierarchical structure of chitin microfibrils in the shell of a lobster (Raabe *et* al., 2005). The epicuticle (outer layer) is characterized by a very fine woven structure of the fibrous chitin–protein matrix ('twisted plywood' structure) and by a high stiffness. There is possibility of the improving the mechanical properties of composites was occurred by a parallel array of microfibrils of chitin fibers (Revol and Marchessault, 1993).

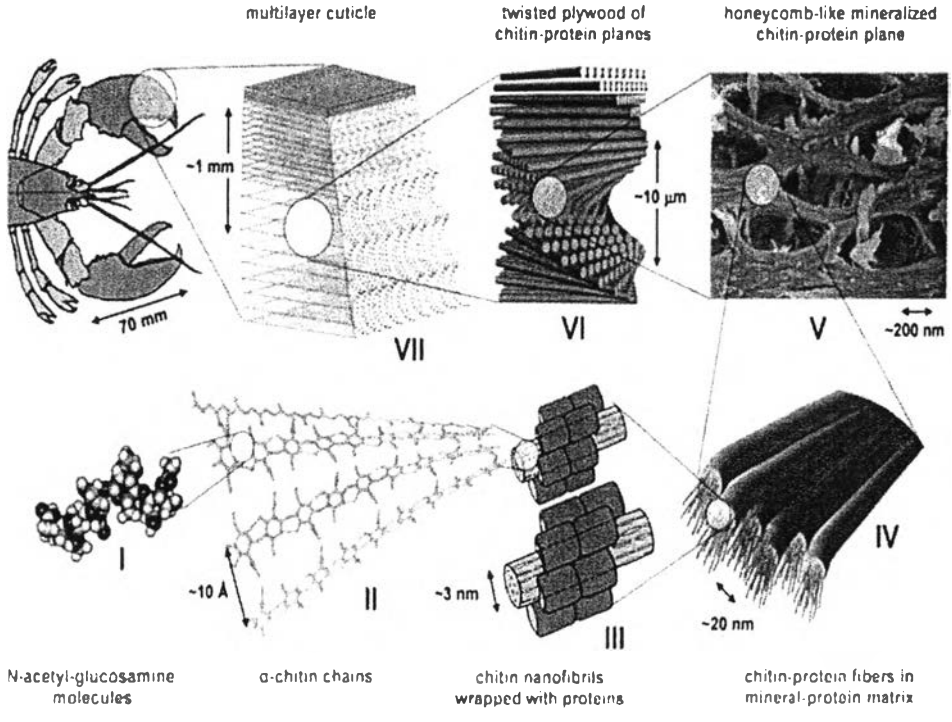

Figure 2.2 The hierarchical structure of cuticles showing the ordered structure of chitin.

Chitin whisker is ordered nanocrystallites embedded into low-ordered nano-domains, as shown in Figure 2.3a, which mostly found in the exoskeleton of crustacean. Chitin whisker is prepared by acid hydrolysis to remove low-ordered region, then high crystalline chitin is obtained as shown in Figure 2.3b. The vigorous mechanical shearing will generate individual chitin fibril or called chitin whisker as illustrate in Figure 2.3c (Nair and Dufresne, 2003). The nanocrystallites of chitin are obtained which having aspect ratio ranges between 10-120, depending on the sources (Wongpanit *et* al., 2007).

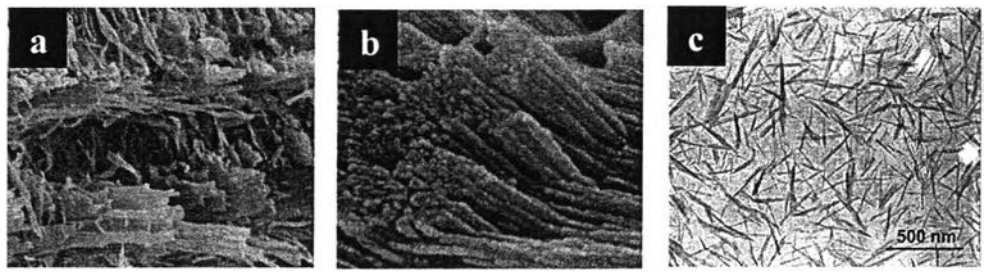

Figure 2.3 Illustration of (a) chitin, (b) crystallite chitin, and (c) chitin whisker.

Chitin whisker have been successfully prepared from squid pens (Paillet and Dufresne, 2001), tube of Riftia pachyptila tubeworms (Morin and Dufresne, 2002), crab shells (Nair and Dufresne, 2003) and shrimp shells of Penaeus merguiensis shrimps (Sriupayo *et* al., 2005).

In 2001, Paillet and Dufresne prepared chitin whisker suspensions were extracted from squid pen by acid hydrolysis of chitin with the average aspect ratio closed to 15, to reinforcing the copolymer of styrene and butyl acrylate. The propose of this treatment was to eliminate regions of low order so that the water-insoluble of highly crystalline residue may be converted into a stable suspension by vigorous mechanical shearing action. Samples were firstly boiled and then stirred in a KOH solution for 6 hours to remove of the proteins. This suspension was subsequently kept at room temperature overnight under stirring, filtered, and washed several times with distilled water. Chitin samples were then bleached with $NaClO_2$ solution which containing sodium acetate buffer for 6 hours at 80 °C. The bleaching solution was changed every 2 hours followed by abundant rinsing the sample with distilled water. After bleaching, the suspension was kept in KOH solution for 72 hours to remove residual protein. The resulting suspension was centrifuged to separate the product (Paillet and Dufresne, 2001).

Chitin whisker suspensions were prepared by hydrolyzing the purified chitin sample with 3 N HCl at the boil for 1.5 hours under stirring. The ratio of 3 N HCl to chitin was 30 ml/g. After acid hydrolysis, the suspensions were diluted with distilled water followed by centrifugation at 10,000 rpm for 5 minutes. This process was repeated for three times. And then, the suspensions were transferred to a dialysis bag and dialyzed for 24 hours against distilled water until neutral. The dispersion of

whiskers was completed by a further 2.5 minutes ultrasonic treatment and kept in a refrigerator until used. The adding sodium azide in chitin whisker suspensions acts as protectant for against microorganisms (Paillet and Dufresne, 2001). These suspensions display a colloidal behavior, which stability was attributed to the presence of positive charge (NH_3^+) at the surface of the crystallites, resulting from the protonation of amino groups (Marchessault *et* al., 1959).

In 2002, Morin and Dufresne prepared nanocomposite materials from a colloidal suspension of high aspect ratio β-chitin whiskers to reinforce with poly(caprolactone) as the matrix. The chitin whiskers prepared by acid hydrolysis of Riftia tubes (tubes secreted by a vestimentiferan worm called Riftia.), consisted of slender parallel rods with the aspect ratio close to 120. The chemical and mechanical treatments of Riftia tubes for the preparation of chitin whiskers can be seen in Figure 2.4

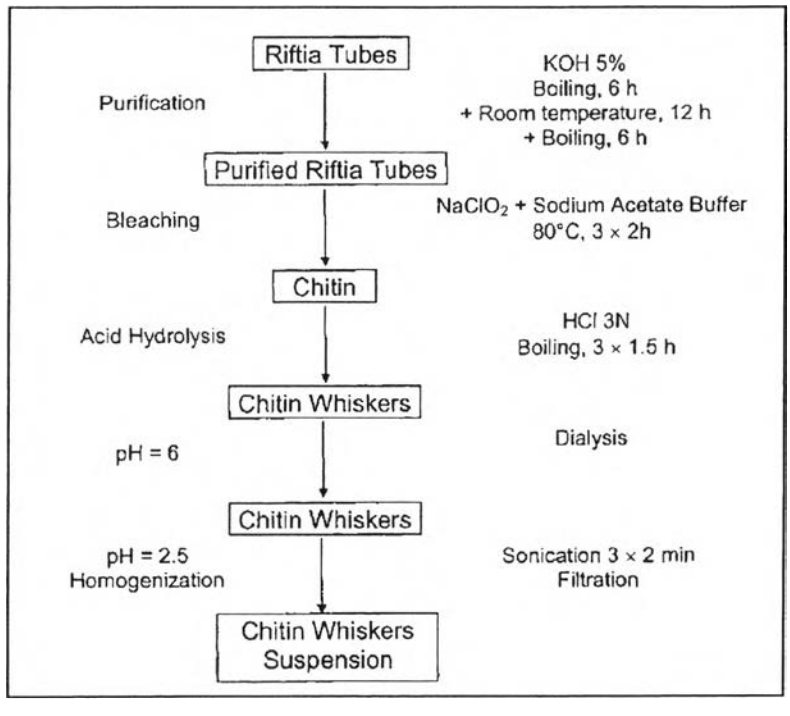

Figure 2.4 Chemical and mechanical treatments of Riftia tubes for the preparation of chitin whiskers (Morin and Dufresne, 2002).

In the Figure 2.5, transmission electron micrograph (TEM) represents chitin whiskers which obtained from a dilute suspension. The suspension was consist of individual chitin fragments which had a slender parallel rods that had a broad distribution in size. These fragments had a length ranging from 500 nm up to 10 μm, and they had a width around 18 nm. The dimensions of the whiskers were averaged on 240 representative items.

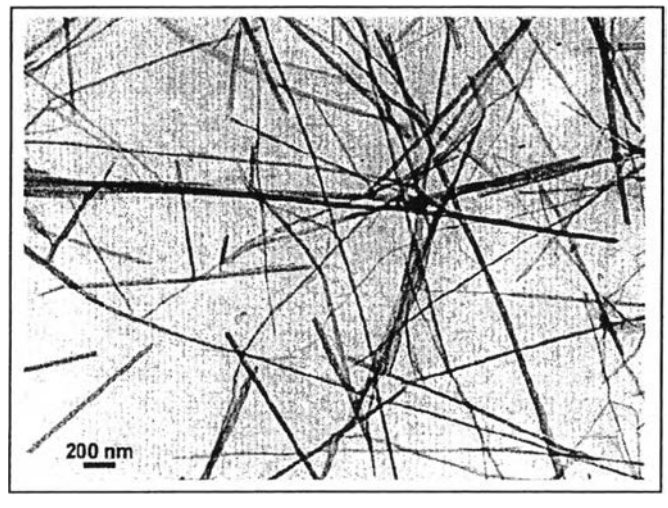

Figure 2.5 Transmission electron micrograph from a dilute suspension of chitin whiskers from Riftia tubes (Morin and Dufresne, 2002).

Because of the great mechanical and biological properties of chitin whiskers. therefore, they has been extensively incorporated in various interesting biomaterials such as soy protein (Lu *et* al., 2004), chitosan (Sriupayo *et* al., 2005) and silk fibroin (Wongpanit *et* al., 2007).

Environmentally friendly thermoplastic nanocomposites were successfully developed using of chitin whiskers as a filler to reinforce soy protein isolate (SPI) plastics (Lu *et* al., 2004). The chitin whiskers had a lengths from 50 to 500 nm and an average diameters of 10 to 50 nm and they were prepared from chitin by acid hydrolysis. The results indicated that the strong interactions between SPI matrix and fillers play an important role in reinforcing the composites without interfering with their biodegradability. The SPI/chitin whisker nanocomposites increased in both tensile strength and Young's modulus. Furthermore, impletion of

chitin whisker into the matrix leads to improve water resistance of composites. In Figure 2.4 shows the FT-IR spectrum of chitin whiskers. The characteristic absorption bands of R-chitin were at 1662, 1625 and 1580 cm^{-1} in the carbonyl region. The absence of the absorption peaks at 1540 cm^{-1} corresponding to the proteins proves that the successive treatments were strong enough to eliminate all the proteins and to obtain pure chitin (Brugnerotto, 2003).

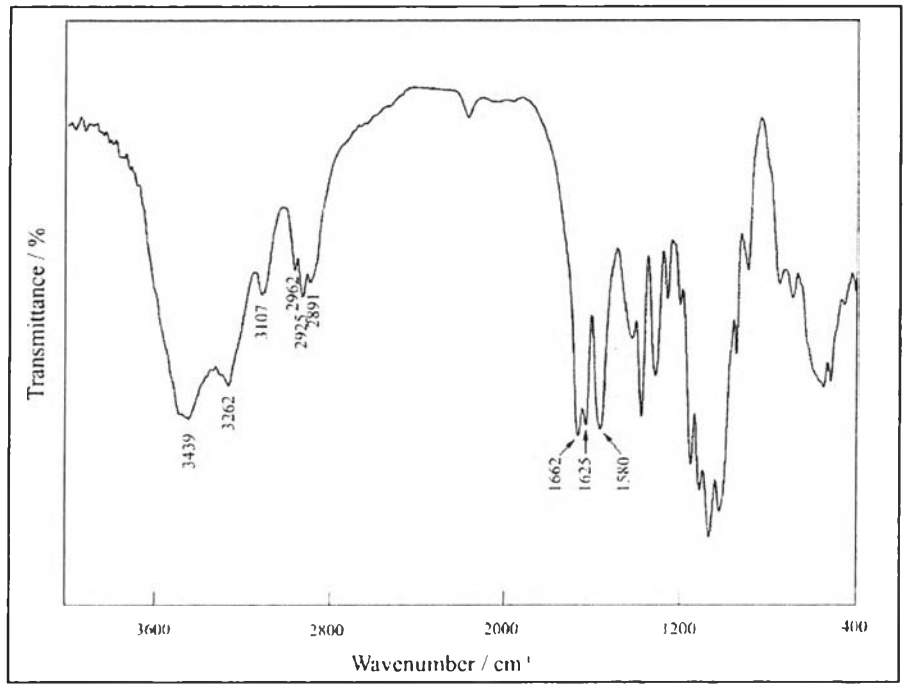

Figure 2.6 FT-IR spectrum of chitin whiskers (Lu *et* al., 2004).

α-chitin whiskers as reinforcing agent for the matrix of poly(vinyl alcohol) nanocomposite films was studied on with and without heat treatment by using solution-casting technique (Sriupayo *et* al., 2005a). The length of the as-prepared whiskers obtained from shrimp shells ranged between 150 and 800 nm, while the width ranged between 5 and 70 nm, with the average values being about 417 and 33 nm, respectively. The addition of chitin whiskers did not affect much the thermal stability and the apparent degree of crystallinity of the PVA matrix. The tensile strength and thermal stability increased from that of the pure PVA film with initial increase in the whisker content, while the percentage of elongation at break decreased from that of the pure PVA film with initial increase in the whisker content

and leveled off when the whisker content was greater than or equal to 2.96 wt%. The addition of α-chitin whiskers as well as heat treatment can improve the water resistance, leading to decrease the percentage of degree of swelling, of the nanocomposite films.

Furthermore, the same α-chitin whiskers were also reinforced in chitosan films by using solution-casting technique (Sriupayo *et* al., 2005b). The results indicated that the tendency of mechanical properties similar to α-chitin whiskers/PVA nanocomposite films. While the presence of chitin whiskers did not affect much on the thermal stability, like α-chitin whiskers/PVA nanocomposite films.

Nanocomposite sponges at various chitin whiskers as nanofiller to silk fibroin as a matrix were prepared by using a freeze-drying technique (Wongpanit *et* al., 2007). Chitin whiskers exhibited the average length and width of 427 and 43 nm, respectively. The presence of chitin whiskers embedded into silk fibroin sponge not only improved dimensional stability of the sponge due to the β- sheet formation of silk fibroin but also enhanced compression strength. It was found that the percent shrinkage decreased with an increasing of the whisker content.

Then, the chitin whiskers reinforced alginate nanocomposite fibers were investigated in the objective of biodegradability (Wattanaphanit *et* al., 2008). The chitin whiskers were prepared from shrimp shells which had the average aspect ratio about 7.5. The mechanical and the thermal properties of the nanocomposite fibers significantly increased at the low content of chitin whiskers. The investigation of biodegradability using lysozyme/Tris-HCl solution found that the chitin whiskers in the nanocomposite fibers accelerated the biodegradation process.

2.2 Pluronic F127

Temperature-responsive polymers have become increasingly attractive as carriers for the injectable drug delivery systems over the past decade. Pluronic or the trade name of Poloxamer, a tri-block ABA type copolymer composed of poly(oxyethylene)-block-poly(oxypropylene)-poly(oxyethylene), (PEO_x-PPO_y-PEO_z),

was widely employed to chemically decorate biomedical materials with the macromolecular surfactant for bio-functionalization. These block copolymers shows hydrophilic property in the flank of poly(ethylene oxide) and hydrophobic property in the center of poly(propylene oxide) (PPO) blocks as shown in Figure 2.7. At concentrations above critical micelle concentration (CMC), the aqueous solutions of these copolymers form self-assemble into micelles. The diameters of Pluronic micelles usually vary from 10 nm to 100 nm. The core of the micelles consists of hydrophobic PPO blocks that are separated from the aqueous outside by the shell hydrated of hydrophilic PEO chains. The core of micelles represents a "cargo hold" for incorporation of various therapeutic or diagnostic reagents (Figure2.7b). The PEO shell confirms that the micelles remain in a dispersed state and reduces undesirable drug interactions with cells and proteins in the body. For the describing in term of critical solution temperature (CST). Pluronic is the temperature responsive triblock copolymer which shows structural transitions between relaxed and collapsed states according to temperature changes across their lower critical solution temperature (LCST). Above the LCST, these spherical micelles are closely packed together to physically crosslink individual micelles, the hydrophobic central of PPO blocks form into micelle cores, whereas the flank of PEO blocks form into the shells, which exhibits sol-gel transition behaviors with varying gelation temperature. For the primary data of Pluronic F-127, consists of ethylene oxide 70 % and propylene oxide 30 % by weight and it had an average molecular weight of 11500. The polymer solutions are fluid below room temperature but become a hydrogel at physiological temperature after injection into the body (Oh *et* al., 2012). These thermo-sensitive hydrogels have to biocompatible and biodegradable, thus they had been use for injectable drug delivery system. However, their use for biomedical applications is greatly limited due to poor chemical modification study without end-capped hydroxyl group of Pluronic, low mechanical strength, fast dissolution and rapid drug release under physiological condition, primarily caused by the immediate dilution of polymer concentration with the body fluid below a critical gelation concentration (Lee *et* al., 2011).

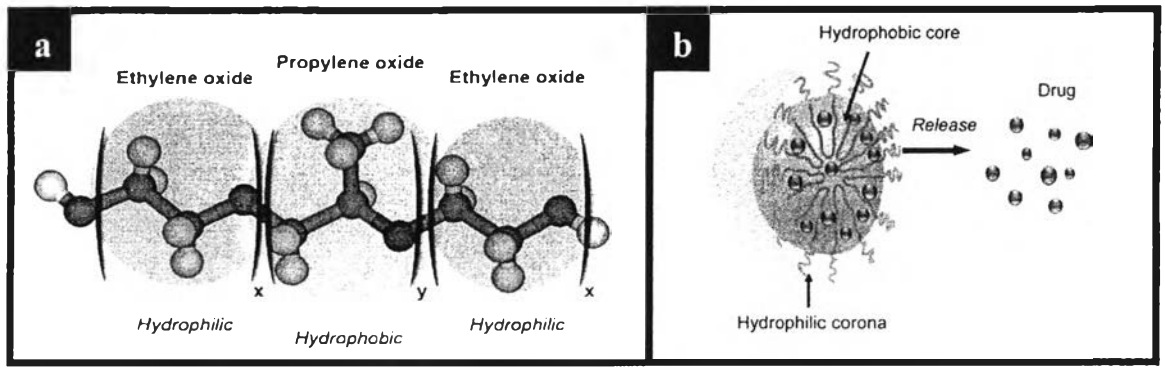

Figure 2.7 Pluronic block copolymer molecule (a) and micelle with a solubilized drug (b), (Batrakova and Kabanov, 2009).

To studied about the drug release behavior from its drug-loaded nanoparticles. Poly(ε-caprolactone), (PCL) is a well-known FDA-approved biodegradable and biocompatible material with hydrophobic properties, which has been widely used in biomedical fields. Due to the blended great advantages of Pluronic and PCL, PCL–Pluronic–PCL copolymer have great potential application in biomedical fields. Liu *et* al., (2007) was successfully prepared PCL–Pluronic–PCL block copolymer from ε-caprolactone and low molecular weight Pluronic (L35, BASF) by ring-opening polymerization method. Lee *et* al., (2011) prepared Pluronic F-127 copolymers which dimerized by introducing tyramine moieties at both terminal ends of Pluronic using tyrosinase. The enzyme-mediated cross-linking of Pluronic hydrogels showed controlled erosion with sustained release of a model macromolecular drug. They also showed bio-adhesive, thermo-sensitive, and injectable properties. In another study, temperature-sensitive hyaluronic acid (HA) hydrogels were synthesized by photopolymerization of vinyl group modified HA with di-acryloyl Pluronic F-127. HA/Pluronic hydrogels showed reversible cyclic swelling and de-swelling of the hydrogels were induced by cycling temperatures between 13°C and 40°C in a step-wise function (Kim and Park, 2002). Pluronic/heparin nanocapsules prepared by cross-linking between heparin and activated Pluronic F-127 with p-nitrophenylchloroformate. They

exhibited a 1000-fold volume transition and a reversible swelling and de-swelling behavior between 20°C and 37°C (Choi *et* al., 2006).

2.3 Drug delivery System (DDS)

Drug delivery system is the control of the drug into a body at a desired target with an appropriate amount of drug for the best treatment. A drug delivery system has two functions. The first function is the introducing of the drug to a particular part of the body and the second function is controlling the drug release. The control-released drug delivery is necessary because if the drug concentration is very high, it contributes to adverse side effects. And if the drug concentration is too low, it provides low therapeutic benefit (Nikam *et* al., 2011).

Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance.

Diffusion, swelling and erosion are the most important rate-controlling mechanisms of commercially controlled release mechanisms (Lange and Peppas, 1983).

For erosion system or degradable system loaded with the compound of interest, release will be controlled by the cleavage of the polymer bonds within the network, The most common reactions that occur within matrix delivery systems are cleavage of polymer chains via hydrolytic or enzymatic degradation even though the diffusion of the released therapeutic compound may be rate-limiting (Gupta *et* al., 2002). In this system with surface erosion (heterogeneous erosion) drug release is caused by degradation of the polymer surface (Figure 2.8a). Erosion occurs mostly in the outer layers of the polymer matrix. The degradation takes place only on the surface of the matrix. The system of drug release occurs only in enzymatic-degrading systems in which the rate of enzymatic degradation is much faster than the transport of enzyme into the inside of polymer (Grassi *et al.*, 2005; Siepmann and Gopferich, 2001).

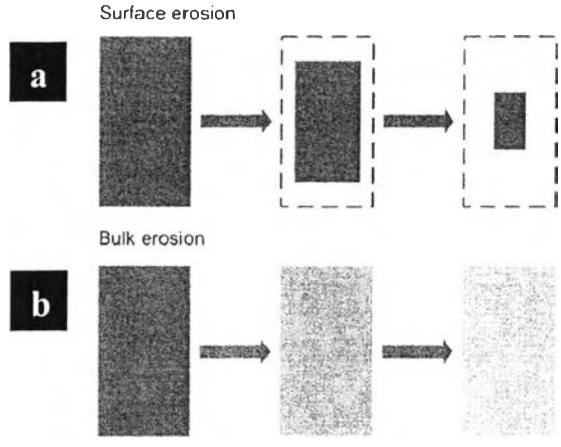

Figure 2.8 Schematic illustration of (a) surface and (b) bulk erosion.

In bulk erosion systems, the drug release is controlled by degradation of the network and molecule diffusion (Figure 2.8b). Bulk eroding polymers slowly degrade and water infuse into the system is much faster than the degradation of polymer (Grassi *et al.*, 2005; Lin and Metters, 2006; Siepmann and Gopferich, 2001). Thus, the whole drug delivery system is rapidly hydrated and polymer chains break off throughout the system. Erosion takes place in the entire system.

For non-biodegradable systems, release will be diffusion-controlled and will depend on the concentration gradient. However, release may also depend on osmotic pressure and matrix swelling (Leong and Langer, 1988; Markland *et* al., 1999).

Swelling system occurs when diffusion of drug is faster than hydrogel swelling. The modeling of this mechanism usually involves moving boundary conditions where molecules are released at the interface of rubbery and glassy phases of swollen hydrogels. The rate of drug release is controlled by the velocity and position of the front dividing the glassy (dry) and rubbery (swelled) portions of the polymer as shown in Figure 2.9 (Coviello *et* al., 2005). This transition occurs when the characteristic polymer transition temperature is lower than temperature of solution which encloses the drug delivery matrix. In the glassy state, entrapped molecules remain immobile. In the rubbery state dissolved drug molecules rapidly diffuse to the solution through the swollen layer of polymer. Released fluid molecules contact the outer layer of hydrogel. This forms a moving front that divides

hydrogel matrix into a glassy and swollen region. In these systems the rate of molecule release depends on the rate of gel swelling (Zarzycki *et* al., 2010). In the swelling-controlled delivery system following phenomena happens (Siepmann *et* al., 2008): 1) The length of drug diffusion way increases. This causes a decrease of drug concentration gradient (driving force of diffusion) and a decrease of drug release rates. And 2) The mobility of drug molecules increases. This causes an increase of drug release rates.

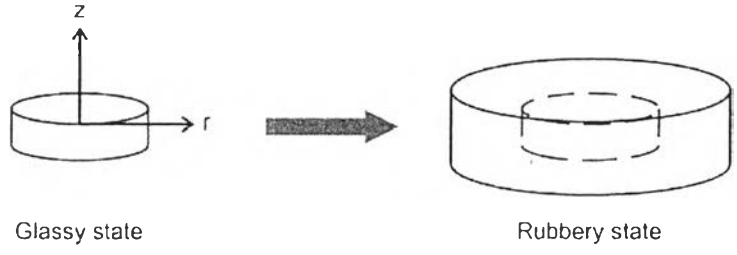

Figure 2.9 Schematic illustration of drug delivery device in glassy and rubbery state matrix.

For diffusion system, Figure 2.10 shows physically controlled release, which is the most widely applicable mechanism for describing drug release and is classified into two types, depending on its mode release: (a) reservoir-type diffusion or (b) matrix-type diffusion (He *et* al., 2004; Juntanon *et* al., 2008). Reservoir-type: In these hydrogels, therapeutic compounds (solid or liquid) are entrapped in a reservoir within a microporous or non-porous polymeric network. If the therapeutic agent is saturated, its transport will be constant (or follow zero-order release kinetics). Matrix-type: These hydrogels, where the drug is dissolved or dispersed within a polymer network, tend to be the most common. A decreased release rate over time due to the increased diffusion distance is a characteristic of these systems (Isiklan *et* al., 2008). Reservoir systems consist of a polymeric membrane surrounding a core containing the drug. In matrix devices, the drug is dispersed throughout the three-dimensional structure of the hydrogel. Drug release from each type of system occurs by diffusion through the macromolecular mesh or through the

water filled pores. Fick's law of diffusion is commonly used in modeling diffusion-controlled release systems (Lin and Metters, 2006).

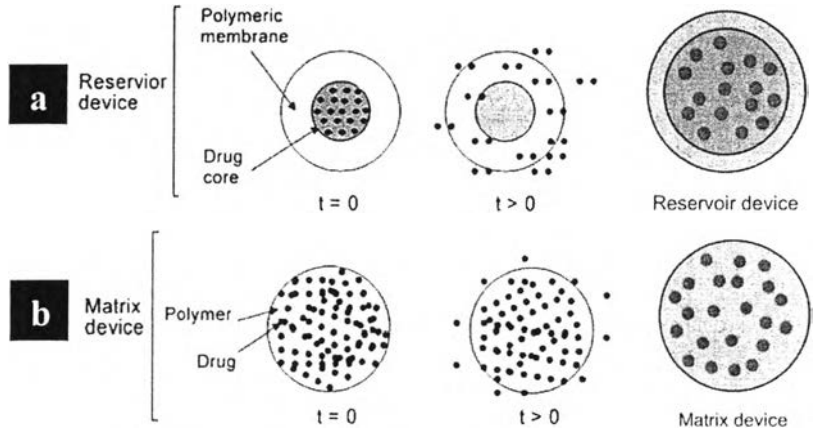

Figure 2.10 Schematic illustration of two types of diffusion system (a) resevoir type and (b) matrix type (Zarzycki *et* al., 2010).

Paavola *et* al., (1998) prepared injectable poloxamer gel in controlling the drug release. Poloxamer gel with the concentration of 25% blended with hydroxypropylmethylcellulose (HPMC), sodium carboxymethylcellulose (CMC) and dextran (DE) was studied in vitro drug release of lidocaine-HCl and ibuprofen-Na and found that those cellulose additives significantly prolonged ibuprofen release, whereas additives were found to have slight release-increasing effect as compared with the PO gel.

Kim *et* al., (2000) synthesized Pluronic/PCL by ring-opening polymerization of ε-caprolactone to study thermo-responsive drug release behaviors. They showed that Pluronic/PCL copolymeric nanospheres which loaded indomethacin (IMC) had significant sustained release characteristics of less than 30% for 100 hours.

Nsereko and Amiji, (2002) prepared Paclitaxel (Taxol)-containing chitin and chitin-Pluronic F-108 microparticles were formulated as biodegradable systems for localized administration in solid tumors. To studies swelling property in phosphate buffered saline (PBS, pH 7.4). Lysozyme-induced degradation and in vitro release of paclitaxel was examined in PBS at 37°C. After 48h,the Amount of

paclitaxel was released from chitin-Pluronic microparticles more than pure chitin microparticles.

Xiong *et* al., (2005) prepared poly(lactic acid),(PLA) grafted to both ends of Pluronic block copolymer (PLA-F87-PLA) to study drug release by using a hydrophilic model drug procain hydrochloride (PrHy). From preliminary results show a constant initial release rate and no burst release was observed.

Gou *et* al., (2008) prepared an injectable hydrophobic drug delivery system (honokiol) loaded with poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) as a nanoparticle by emulsion solvent evaporation method, and then were incorperated into thermo-sensitive F127 matrix. In this work obtained injectable drug delivery system which sustained release of honokiol.

Lee *et* al., (2010) prepared thermo-sensitive and injectable HA/Pluronic F127 composite hydrogels. HA conjugated with dopamine (HA-DN) and was mixed with thiol end-capped Pluronic F127 copolymer (Plu-SH) to produce a lightly crosslinked composite gel synthesis Michael-type catechol-thiol addition reaction and besides could be injected in vivo by using a syringe.