CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Cancer

Cancer is a generic term use for diseases caused by unregulated proliferation of cells and are able to invade other tissue (Figure 2.1). Cancer affects people at all ages with the risk for most types increasing with age. According to World Health Organization (WHO), it is causing 7.6 million deaths every year or 13% of deaths worldwide in 2007. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030. In addition, it is the second major cause of death following cardiovascular diseases [32]. The total number of cases of cancer is predicted to increase in between 2000 and 2020 by 73% in the developing countries and by 27% in the developed countries [33]. In Thailand, the rate of people dying from cancer is still increasing every year and it is the first leading cause of death [34]. There are various methods of treatment for cancer such as: surgery, radiation therapy, immunotherapy and biologic therapy, chemotherapy and etc.

Chemotherapy is a kind of cancer treatment that uses of drugs to eliminate cancer cells. It is most effective against cancers that divide rapidly and have a good blood supply. Chemotherapy is most commonly given by pill or intravenously, but can be given in other ways. However, the administered of drug will be depend on the most effective way to treat your cancer and on the chemical properties of the drug. Many of the currently effective anti cancer drugs are used in the clinical activity such as: etoposide, taxol, doxorubicin and etc.

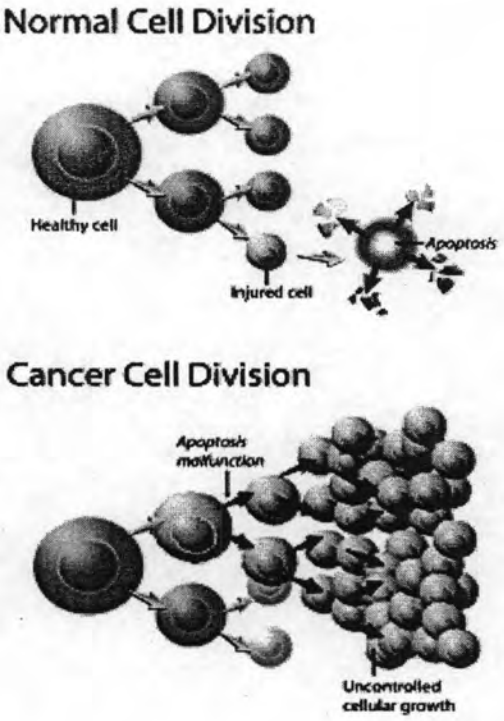

Figure 2.1 Cell division of normal and cancer cell [33]

2.2 Doxorubicin

Doxorubicin (also known as adriamycin) is a drug use in cancer chemotherapy. It is an antineoplastic in the anthracycline antibiotic class, closely related to the natural product daunomycin [35]. Doxorubicin is widely used for the treatment of several solid tumors while daunorubicin and idarubicin are used exclusively for the treatment of leukemia. Doxorubicin interferes with the growth of cancer cells and slows their growth and spread in the body. It acts mainly by sticking in between DNA base pairs and obstructing replication of the genetic material, thereby inhibiting polymerase activity (topoisomerase II). It is given through a vein by intravenous injection. Doxorubicin is classified as an antitumor antibiotic that has been isolated from natural sources produced by species of the soil fungus Streptomyces. However, it lack the specificity of the antimicrobial antibiotics and thus produce significant toxicity. [36], [37].

2.2.1 Mechanism of action:

Doxorubicin has antimitotic and cytotoxic activity through a number of proposed mechanisms of action: Doxorubicin forms complexes with DNA by intercalation between base pairs and blocks DNA synthesis and transcription. In addition, doxorubicin has also been shown to inhibit DNA topoisomerase II which is critical to DNA function, preventing the religation portion of the ligation-religation reaction that topoisomerase II catalyzes. Both of these mechanisms result in DNA disruption that ultimately can lead to the death of the cell.

2.2.2 Physicochemical properties

Doxorubicin is a red, crystalline solid that will appear as a red fluid when it is mixed and ready to be given. It is soluble in water and aqueous alcohols, moderately soluble in anhydrous methanol and insoluble in nonpolar organic solvents. Neutral aqueous solutions of doxorubicin are stable when stored at 5°C. When heated to decomposition, doxorubicin emits toxic fumes of nitrogen oxides and hydrogen chloride.

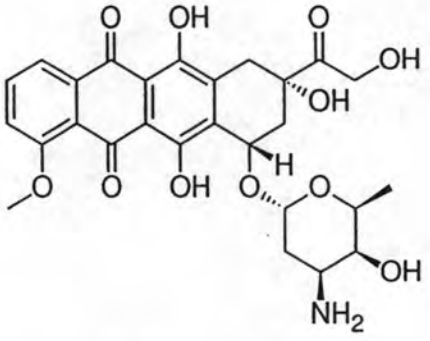

Figure 2.2 Chemical structure of doxorubicin

Chemical data [35]

Formula	:	$C_{27}H_{29}NO_{11}$
Molecular weight	:	543.52
IUPAC name	:	(8*S*,10*S*)-10-(4-amino-5-hydroxy-6-methyl-tetrahydro-2*H*-pyran-2-yloxy) -6,8,11-trihydroxy-8-(2-hydroxyacetyl) -1-methoxy-7,8,9,10-tetrahydrotetracene -5,12-dione
Synonyms	:	Adriamycin, Hydroxydaunorubicin, Rubex

Pharmacokinetic data [35]

Bioavailability	:	5% (oral)
Metabolism	:	Hepatic
Half life	:	12–18.5 hours when released from liposomes
Excretion	:	Biliary and fecal
Route	:	Intravenous

Adverse effects

The degree and severity of the side effects depend on the amount and schedule of the administration of doxorubicin. Some of the significant side effects are [38]:

- Pain
- Nausea or vomiting
- Soreness of the mouth, difficulty swallowing
- Diarrhea
- Low white blood counts

- Low platelet count
- Anemia
- Heart problems
- Damage to the veins (It can cause redness and irritation at the site of injection, despite proper injection.)
- Severe damage to the tissues if it leaks from the injection site (Extravasation)
- Red urine, which is due to excretion of the medicine by the kidneys

2.3 Topoisomerase

DNA Topisomerases are nuclear enzymes that catalyze changes in the topological state of DNA by breaking and rejoining of DNA strands. These enzymes have important roles in DNA metabolism such as replication, recombination, transcription, and chromosome condensation. In human, DNA topoisomerase are classified into two types: Topoisomerase I and Topoisomerase II. Both of them can remove DNA supercoiling by catalyzing DNA swiveling and relaxation. All physiological functions of DNA depend on its tertiary configuration. Topoisomerase I cuts one strand whereas topoisomerase II cuts both strands of the DNA to relax the coil and extend the DNA molecule. DNA is a double helix and the supercoiled which require relaxation before replication and translation. A variety of antitumor agents currently used in chemotheraphy are known to inhibit DNA toposiomerase.

DNA topoisomerase II forms a covalent linkage to both strands of the DNA helix at the same time, making a transient double-strand break in the helix. These enzymes are activated by sites on chromosomes where two double helices cross over each other. When the topoisomerase binds to such a crossing site, it (1) breaks one double helix reversibly to create a DNA "gate," (2) causes the second, around double helix to pass through this break, and (3) reseals the break and dissociates from the DNA. DNA Topoisomerase II can efficiently separate two interlocked DNA circles (Figure 2.3). The same reaction prevents the severe DNA tangling problems that would otherwise arise during DNA replication [39].

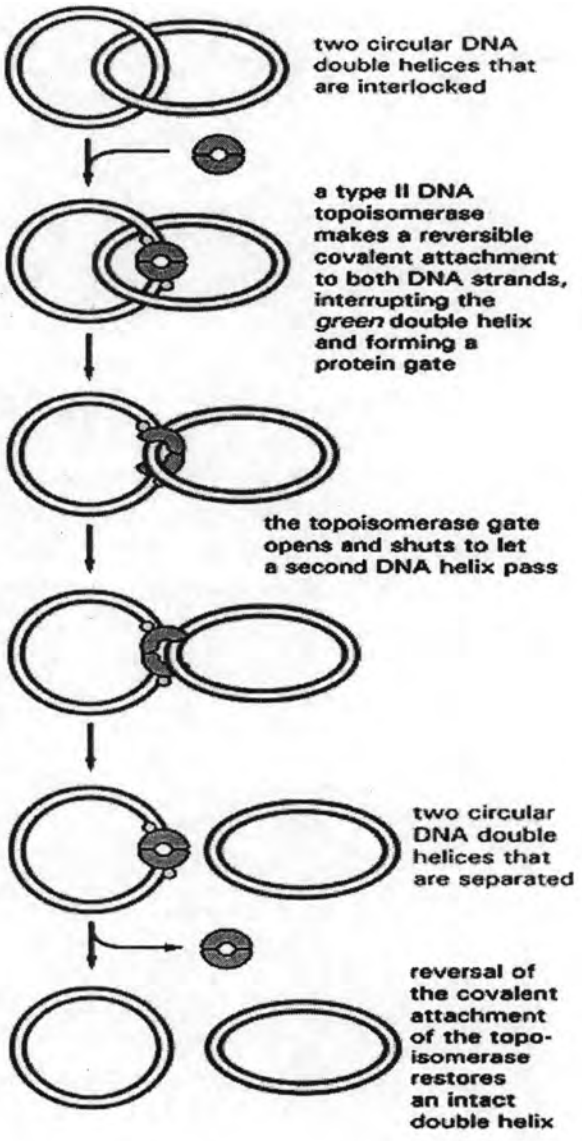

Figure 2.3 DNA topoisomerase II mechanism [39]

Anticancer drugs targeting topoisomerase II are some of the most widely used chemotherapeutic agents. These drugs include doxorubicin, etoposide, mitoxantrone, and others [40]. In a broad sense, the drugs targeting topoisomerase II can be classified as either topoisomerase "poisons" or topoisomerase cata-lytic inhibitors. As previously discussed, a trans-ient double strand DNA break is characteristic of the topoisomerase II enzymatic reaction. The topoisomerase II poisons increase the

steady state levels of this transient intermediate. They do this either by increasing the rate of DNA cleavage (ellipticine, genistein) or by inhibiting the rate of DNA religation (etoposide, amsascrine, teniposide). In either case, these drugs convert the enzyme into a DNA damaging agent. This is a stochiometric relationship because there is the potential for one DNA double strand break for every drug stabilized topoisomerase II enzyme. Thus sensitivity to the topoisomerase II poisons is dependent on high levels of enzyme. The more enzyme, the more DNA damage. On the other hand, the topoisomerase II catalytic inhibitors act like other types of anti-cancer drugs and inhibit the activity of the enzyme. Drugs interfering with the catalytic activity of the enzyme deprive the cell of the enzyme's ability to decatenate chromosomal DNA strands prior to mitosis. Cell growth is therefore altered. For these catalytic inhibitors, the reverse sensitivity pattern holds. Cells with low levels of topoisomerase II are the most sensitive to the topoisomerase II catalytic inhibitors and cells with high topoisomerase II levels are most resistant.

2.4 Polymer in pharmaceutical field

Polymers are becoming increasingly important in field of pharmaceutical industry as both drug encapsulants and vehicles of drug delivery in order to either protect an active agent during its passage through the body until its release, or control its release. Carrier technology obtained the drug delivery system by coupling the drug to the carrier polymers in various dosage forms such as beads, microspheres, nanoparticles, liposomes. Those formulations could delay the release of drug and also generate a response in a specific area or organ of the body requiring treatment. Moreover, a target drug, encapsulated in a polymer can be released sustainedly to improve drug therapeutic efficacy and decrease the dosing time and side effect [11].

Naturally occurring polymers are attractive as drug delivery system since they possess the biocompatibility, biodegradability and non-toxicity required for used in human [41].

2.4.1 Mucoadhesion

Mucoadhesion is the ability of synthetic or nature macromolecules such as proteins and peptides to adhere at mucosal surfaces which are the moist surfaces lining the walls of various body cavities.

Mucoadhesive drug delivery systems, designed to adhere to mucosal surface, become interesting nowadays for transmucosal routes such as pulmonary, nasal, and oral routes due to their several advantages such as:

1) Prolong residence time of the dosage form on mucosal tissue for increasing drug absorption and drug's bioavailability.

2) High concentration gradient drug at the site of adhesion-absorption membrane which will create a driving force for the paracellular passive uptake.

3) Immediate absorption from the bioadhesive drug delivery system without previous dilution and possible degration in luminal fluids of body [42].

4) Enhancement of topical action of certain drugs such as antibiotic against certain bacteria that colonize the stomach such as *H.pylori* [43]. Better stability and longer residence time allow more of antibiotic to penetrate through the gastric mucus layer to act on *H.pylori*.

2.4.2 Mucoadhesive polymers

Mucoadhesive polymers have been also used for coating medical devices. As an example a new generation of intestine inspection device has been recently developed in which mucoadhesive polymer coating make intestinal locomotion possible.

Numerous polymers adhere to mucosal tissues. These include synthetic polymers, for instance, poly(acrylic acid) (PAA) [44], hydroxypropyl methylcellulose, poly(methylacrylate) derivatives and thiolated polymers [45], as well as naturally occurring polymers such as hyaluronic acid [46] and CS [47]. Among these various possible bioadhesive polymeric hydrogels, PAA has been considered as a good

mucoadhesive. However, due to a high transition temperature and higher interfacial free energy, PAA does not wet the mucosal surface to the optimal level, causing loose interdiffusion of the polymer. Therefore, PAA is copolymerized with polyethylene glycol (PEG) or poly(vinyl pyrrolidone) (PVP) to improve these properties. It is important to realise that balanced adhesive and cohesive properties for a polymer is essential for its application in a transmucosal drug delivery systems, especially for the removable devices.

Table 2.1 The relative bioadhesive property of various polymers

Polymer	**Bioadhesive property**
Carboxy methyl cellulose	+++
Carbopol 934	+++
Sodium alginate	+++
Chitosan	+++
Gelatin	++
Guar gum	++
Pectin	+
Polyvinyl Pyrrlidone	+

NOTE: +++ : Excellent

++ : Moderate

+ : Poor

2.4.3 Chitosan (CS)

Chitosan is a natural cationic biopolyaminosaccharide that was discovered by Rouget in 1859 and gave a name by Hoppe-Seyler in 1894 [48]. It is produced commercially by deacetylation of chitin in strong alkaline solution, which is noticeably present in outer skeletons of arthropods in particular, for example, in the epidermis of crustaceans such as crabs and shrimp shells, lobsters, prawns and cell walls of some fungi such as *aspergillus* and *mucor*. In plants, chitin is present in hyphae or spores of molds. The degree of deacetylation (%DA) can be determined by NMR spectroscopy, and the % DA in commercial chitosan is in the range 60-100 %. It was reported that chitosan is a potentially useful pharmaceutical material owing to its good biocompatibility and low toxicity.

Structure of chitosan

Chitosan $(C_6H_{11}O_4N)_n$, is a natural polysaccharide derived from chitin by alkaline deacetylation, consists mainly of the repeating unit of 2-amino- and 2-acetamido-2-deoxy-β-D-glucopyranose (Figure 2.4) [11]. A fraction of the repeating units in the chitosan backbone contains hydroxyl groups (–OH) and amine pendent groups ($–NH_2$) while the rest contains acetamide group (–NHCO) in its place. Both reactive primary amine and hydroxyl group can be used to chemically alter its properties under mild reaction conditions. The polymer differs from chitin in that a majority of the N-acetyl groups in chitosan are hydrolyzed. The degree of hydrolysis (deacetylation) can be controlled by time, temperature and concentration of alkaline treatment of chitin [49], so has significant effect on the solubility and rheological properties of polymer.

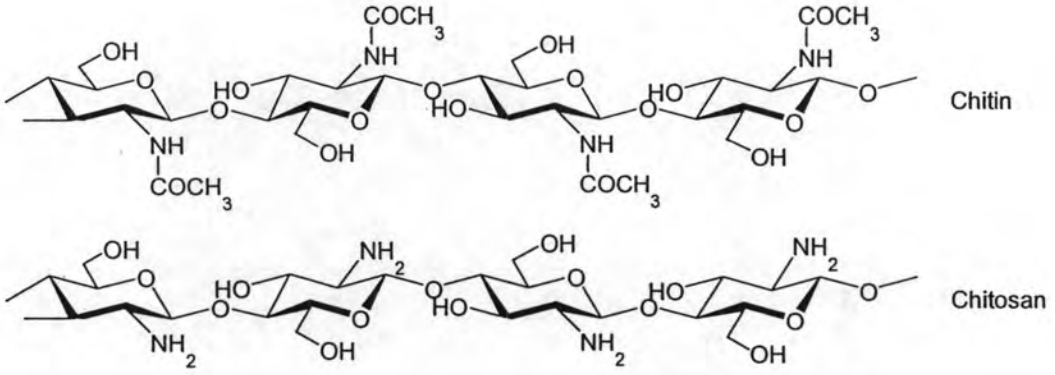

Figure 2.4 Structure of chitin and chitosan

Physicochemical properties of chitosan

Chitosan is the large polysaccharide which is various different the degree of deacetylation and high molecular weight. It is very important effect on the biological properties.

Chitosan is a cationic polysaccharide which is a weak base with pK_a value of the D-glucosamine residue of about 6.2-7.0. Therefore, is insoluble at neutral and alkaline pH values. However, make salts with inorganic and organic acids such as acetic acid, hydrochloric, glutamic acid, and lactic acid making it soluble in water. Because of in acidic medium, the amine groups of chitosan can undergo protonation, positively charged polysaccharide. Furthermore, the solubility of chitosan is depending on the degree of deacetylation and pH of solution.

The viscosities of chitosan are depending on concentration and temperature increasing the degree of deacetylatin increases the viscosity. As the chitosan concentration increases and temperature decreases, the viscosity increases. Because the hydrogen bonding in chitosan chains due to the presence of amine and hydroxyl groups causes the high viscosity of chitosan solutions.

Applications of Chitosan [50], [51, [52]

1. Cosmetics

Chitosan is a particularly effective hydrating agent which is able to supply water and avoid dehydration. Chitosan has fungicidal and fungistatic properties. Chitosan is the only natural cationic gum that becomes viscous on being neutralized with acid. Usually organic acids are used as good solvent for cosmetic application. Chitosan form a protective tensor film on the skin's surface that can fix and allow the active principles to be placed in close contact with the skin. This is a new double advantage that makes chitosan of great interest in cosmetic. Therefore, chitosan are now widely used in skin creams, shampoos, etc.

2. Agriculture

chitosan has plant protecting and antifungal properties. In case of very low concentration of chitosan, it can stilmukate defensive mechanisms in plants against infections and parasite attacks. In addition, they can be used as coatings of seeds which obtained to increase crop yield more than 20% in comparison with uncoated seeds.

3. Water treatment

Chitosan has been gaining interest for industry and nature conservation. Therefore, chitosan is cationic polymer that can be used as flocculating agent. It can also act as chelating agent and heavy meatls trapper.The Environmental Protective Agency (EPA) has already approved the use of chitosan in water at concentrations of up to 10 mg per litre. For sewage treatment, chitosan can be used at up to 5 ppm. It reduces the oxygen demand by 80 to 85% and reduces the phosphates level to less than 5 ppm.

4. Pharmaceutical and medical uses

Chitosan is still utilized in the pharmaceutical field. Due to its low-toxicity, biocompatibility with human body tissue, chitosan have displayed their effectiveness

for all forms of dressings-artificial skin, corneal bandages and suture thread in surgery-as well as for implants or gum cicatrization in bone repair or dental surgery.

Moreover, chitosan is an excellent medium for carrying and slow release of medicinal active principles in plants, animals and man. If degree of deacetylation and molecular weight can be controlled, it would be good advantage for developing size of chitosan for drug delivery system.

2.4.4 4-Carboxybenzenesulfonamide – Chitosan (4-CBS-CS)

The 4-CBS-CS gave higher mucoadhesive property, has a good swelling property, more resistant in an acidic condition of the stomach. Furthermore, it is non-toxic to Vero cell and inactive against to anticancer cell lines of KB cell line (epidermoid carcinoma of oral cavity), MCF-7 cell line (breast adenocarcinoma) and NCL-H187 (small cell lung carcinoma), which can be implied that the 4-CBS-CS is biocompatible to the human body. It also stronger inhibit *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) than that of chitosan [9]. Therefore, the mucoadhesive polymer of 1:0.05 4-CBS-CS is a suitable polymer for applying as a drug delivery system.

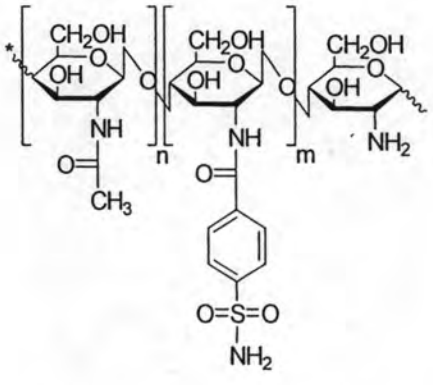

Figure 2.5 Structure of 4-carboxybenzenesulfonamide-chitosan

The formation of amide group of 4-CBS-CS conjugates by using EDAC

The formation of an amide of 4-CBS-CS conjugates using EDAC is straightforward, but with several side effect complicating the subject. The carboxylic acid will react with the carbodiimide to produce the key intermediate: the O-acylisourea, which can viewed as a carboxylic ester with an activated leaving group. The O-acylisourea will react with amines group of CS to give the desired amide group of 4-CBS-CS conjugates and urea.

Furthermore, the side reaction of the O-acylisourea can produce both desired and undesired products. The O-acylisourea can react with an addition carboxylic acid to give acid anhydride. The main undesired reaction pathway involves the rearrangement of the O-acylisourea to the stable N-acylurea . The use of solvents with low-dielectric constants such as dichloromethane or chloroform can minimize this side reaction.

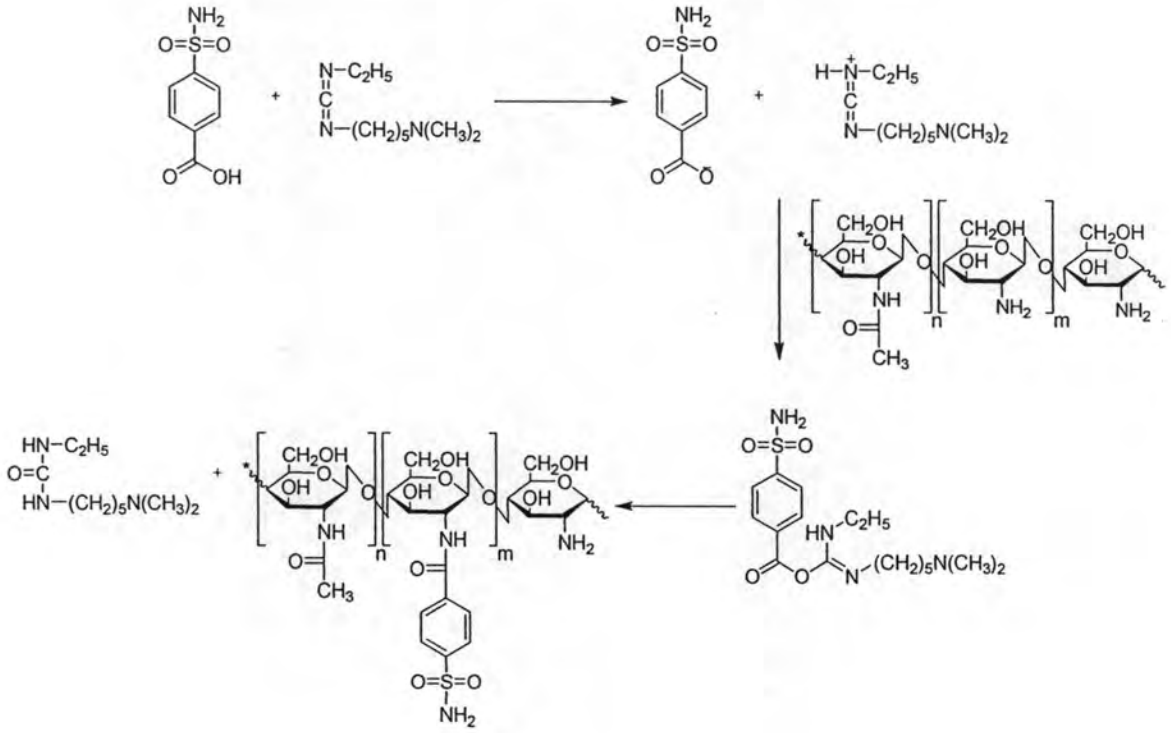

Figure 2.6 EDAC as coupling reagent for the formation of 4-CBS-CS conjugate

2.4.5 Poly (lactic acid)

Poly (lactic acid) (PLA) is a linear aliphatic polyester derived from lactic acid monomers (Figure. 2.7). PLA is widely used in micro/nanoparticulate drug delivery systems because of their biocompatibility and biodegradation properties which was approved by the Food and Drug Administration (FDA) for specific human clinical applications, such as surgical sutures and someimplantable devices. In addition to drug delivery devices, these polymers are used in several medical devices such as sutures, tissue screws and tacks, tissue regeneration membranes, bone fixation devices and systems for meniscus and cartilage repair [53], [54], [55].

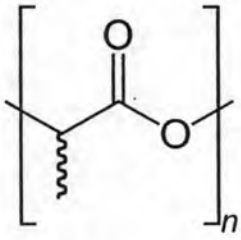

Figure 2.7 Structure of poly (lactic acid)

Physicochemical properties of poly (lactic acid)

Poly (lactic acid) exists in two optical forms. They are L(+) lactic acid and D(-) lactic acid. Both of them are partially crystalline, on the contrary; the racemic poly(D,L-lactic acid) is amorphous. PLLA has a crystallinity of around 37%, a glass transition temperature between 50-80 °C and a melting temperature between 173-178 °C.Those two enantiomers, the polymeric L-lactic acid is normally employed. Due to the of one degrading into the endogenous lactic acid is in the body. Low molecular weight (MW) PLAs (< 3 000 g/mol) are produced by direct condensation of lactic acid, whereas higher MW PLA polymers are obtained usually from ring opening polymerization of lactide. PLAs are soluble in organic solvents such as

chloroform and dichloromethane but insoluble in common alcohols like ethanol and also insoluble in water [56].

Applications of poly (lactic acid)

Poly (lactic acid) is a wide range of applications and has become a significant commercial bioplastic. Its clarity makes it useful for recyclable and biodegradable packaging, such as food packaging, bottles, cups, and wrappers. It has also been used for food service ware, lawn and food waste bags, coatings for paper and cardboard, and fibers-for clothing, carpets, sheets and towels, wall coverings and fiber. In biomedical applications, it is used for sutures, prosthetic materials, and materials for drug delivery.

2.5 Controlled release system

In its broadest sense, the concept of controlled or sustained release of biologically active agents has existed for over three decades. Early commercial applications of the technology occurred in both the pharmaceutical and agricultural industries.

Controlled-release technology emerged during the 1980s as a commercial sound methodology. The achievement of predictable and reproducible release of an agent into a specific environment over an extended period of time has much significant merit. It creates a desired environment with optimal response, minimum side-effects and prolonged efficacy.

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.

2.5.1 Advantages of controlled release

Controlled release system provides numerous benefits over conventional dosage form. Controlled release dosage forms are able to control the rate of drug

delivery, the target area of drug administration and maintain therapeutic levels of drug with narrow fluctuations (Figure 2.8). That can reduce toxic and/or undesirable side effects of the drug. The serum concentration of drug released from controlled release dosage form fluctuates within the therapeutic range over a long period of time. That makes it possible to reduce the frequency of drug administration to encourage patients to comply with dosing instructions and improvement in treatment efficiency.

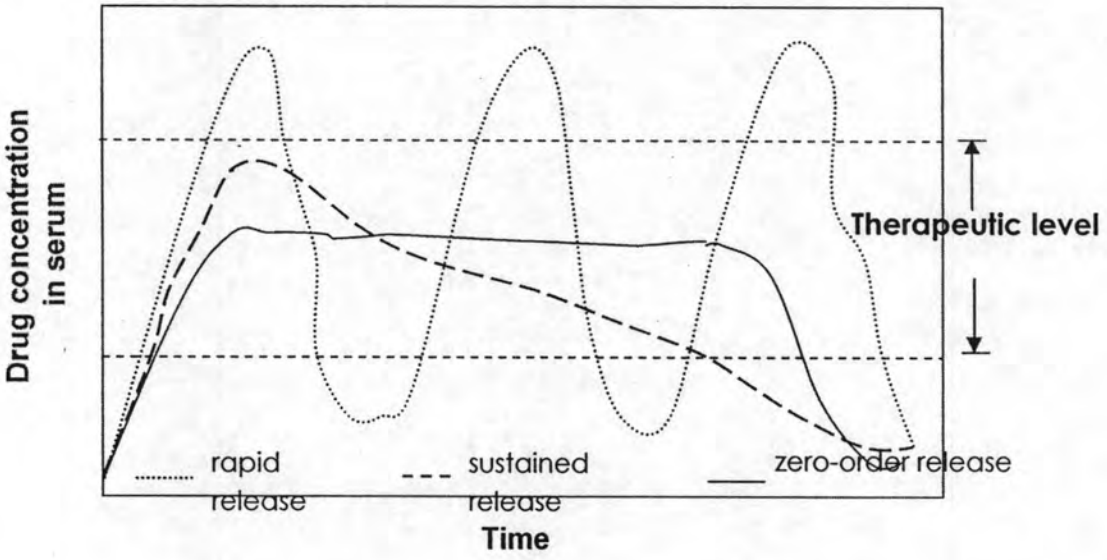

Figure 2.8 Hypothetical serum drug concentrations of various oral dosage forms [57]

2.5.2 Methods of achieving controlled release [57]

The drug can be released from the system by 3 mechanisms.

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.

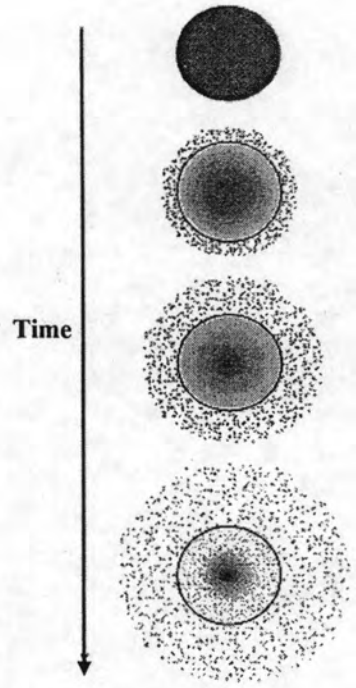

Figure 2.9 Presentation of diffusion controlled release

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.

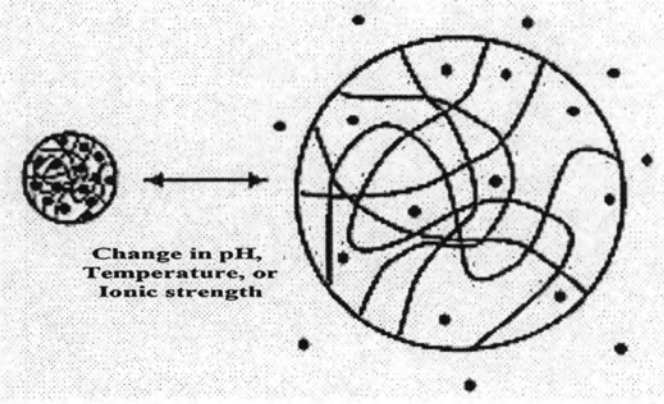

Figure 2.10 Presentation of swelling controlled release

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.

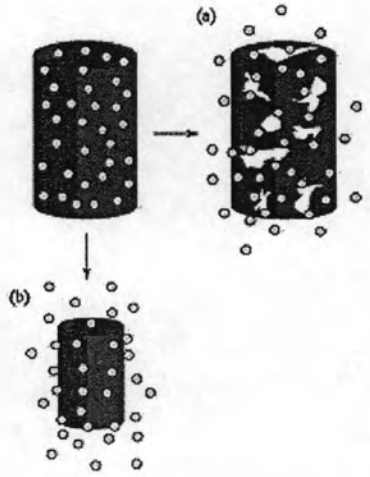

Figure 2.11 Presentation of erosion controlled release-(a) bulk erosion and (b) surface erosion

2.6 Preparation of polymeric nanoparticles

The various methods were used to prepare polymeric particles. It is important for the control of particle size which is affected to drug loading and efficiency of encapsulation.

The major types of method of preparation of micro/nanoparticle of polymer, as follow:

(1) Emulsion cross-linking
(2) Coacervaion/preparation
(3) Spray-drying

(4) Emulsion-droplet coalescence method

(5) Ionic gelation

(6) Reverse micellar method

(7) Sieving method

(8) Electrospray ionization method

This work focus on a new technique for preparation of drug loaded polymeric nanoparticles by using electrospray ionization.

2.7 Electrospray

Electrospray ionization has been used to generate nanoparticles and quantum dots [27] through electrical force which is a slightly modified form of the electrospinning process that is widely used for making micro/nanofibers [28]. In this process, liquid flowing out from a capillary nozzle maintained at high potential, is subjected to an electric field, which causes elongation of the meniscus to a form of jet or spindle. The jet deforms and disrupts into droplets due mainly to electrical force. In the electrospraying, no additional mechanical energy, other than that from the electric field alone, is needed for liquid atomization [31]. Figure 2.12 showed that a liquid is passing through a nozzle. The plume of droplets is generated by electrically charging the liquid to a very high voltage. The charged liquid in the nozzle becomes unstable as it is forced to hold more and more charge. Soon the liquid reaches a critical point, at which it can hold no more electrical charge and at the tip of the nozzle it blows apart into a cloud of tiny, highly charged droplets.

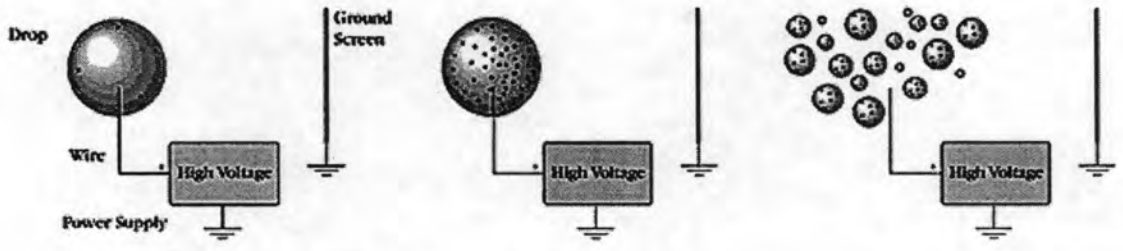

Figure 2.12 Generation of the droplets by electrical force [31]

Electrospray allows generation of fine droplets of charge magnitude close to one half of the Rayleigh limit. The Rayleigh limit is the magnitude of charge on a drop that overcomes the surface tension force, that leads to fission of the droplet.

Jaworek reported the charge and size of the droplet can be easily controlled by adjusting the flow rate and voltage applied to the nozzle [30].

Salata have been briefly reviewed about nanoelectrospray technologies that electrospraying can be widely applied to both industrial processes and scientific instrumentations. The interest in industrial or laboratory applications has recently prompted the search for new, more effective techniques which allow control of the processes in which the droplets are involved. Electrospray is used for micro- and nano-thin-film deposition, micro- or nano-particle production, and micro- or nano-capsule formation. Thin films and fine powders are used in modern material technologies, microelectronics, and medical technology. Research in electro-microencapsulation and electro-emulsification is aimed at developing new drug delivery systems, medicine production, and ingredients dosage in the cosmetics and food industries [27].

The electrospraying has some advantages over conventional mechanical spraying systems with droplet charged by induction such as

1. Droplet size is smaller than that available from conventional mechanical atomisers, and can be smaller than 1 mm.
2. The size distribution of the droplets is usually narrow, with small standard deviation that allows production of particles of nearly uniform size.
3. Charged droplets are self-dispersing in space (due to their mutual repulsion), resulting also in the absence of droplet coagulation.
4. The motion of charged droplets can be easily controlled (including deflection or focusing) by electric fields.
5. The deposition efficiency of a charged spray on an object is order of magnitudes higher than for un-charged droplets.

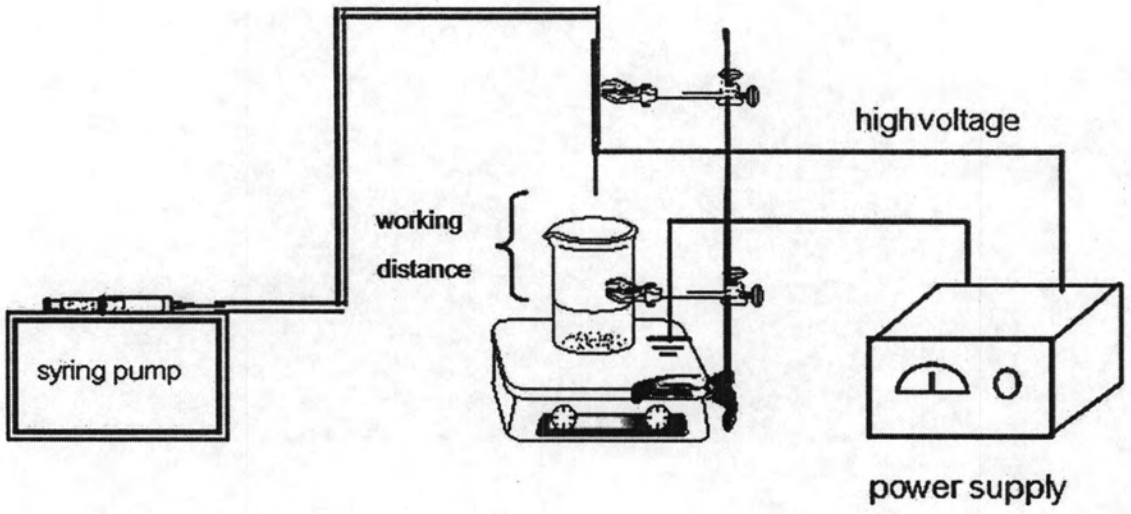

Figure 2.13 Electrospray ionization apparatus