SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

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DRUG RELEASE STUDY OF PLA MICROSPHERES LOADED WITH GENTAMICIN

By

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DECLARATION

I hereby declare that I have conducted and completed the research work and wrote the dissertation entitle "**Drug Release Study of PLA Microspheres Loaded with Gentamicin**". I also declare that it has not been previously submitted for award of any degree or diploma or other similar title of this for any other examining body or university.

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LIST OF ABBREVIATIONS

DCA	Dynamic Contact Angle
DCM	Dichloromethane
ESE	Emulsion and solvent Evaporation
HCl	Hydrochloric acid
NaOH	Sodium Hydroxide
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(L-lactic acid)
PSD	Particle size distribution
PVA	Poly(vinyl alcohol)
SEM	Scanning electron microscopy
UV-vis	Ultraviolet visible

KAJIAN PELEPASAN DADAH UNTUK MIKROSFERA PLA YANG DIMUATKAN DENGAN GENTAMISIN

ABSTRAK

Mikrosfera poli(laktik asid) (PLA) telah disediakan melalui kaedah emulsi dan pemeruapan pelarut (ESE). Rawatan hidrolisis permukaan telah dilakukan untuk mengubah suai permukaan dan memperkenalkan kumpulan hidroksil (-OH) pada PLA. PLA yang dimuatkan dengan gentamisin berkepekatan berbeza direndam ke dalam 0.5 M larutan NaOH selama 48 jam. Kepekatan gentamisin yang digunakan adalah 0.125 %, 0.25 % dan 0.375 %. PLA mikrosfera yang telah dirawat dengan larutan NaOH telah dikaji menggunakan beberapa kaedah pencirian termasuk Spektrometer Infra-Merah (FTIR), mengimbas mikroskop electron (SEM), analisis saiz zarah, sudut sentuh dan sinaran ultraungu (UV) spektroskopi boleh dilihat. Nilai puncak gentamisin telah ditentukan oleh FTIR. Mikrosfera PLA yang telah dirawat mempunyai permukaan yang kasar dan berliang selepas rawatan hidrolisis permukaan dilakukan. SEM telah membuktikan perubahan morfologi permukaan mikrosfera PLA. Nilai sudut sentuh kurang dari 90° selepas rawatan permukaan dan membuktikan terdapat penambahbaikan sifat hidrofilik bagi permukaan mikrosfera PLA yang terawat. Peratus kecekapan pengkapsulan (% EE) gentamicin ke dalam mikrosfera PLA telah diuji dengan menggunakan sinaran ultraungu (UV) spektroskopi boleh dilihat. Keputusan menunjukkan kepekatan gentamisin yang tinggi iaitu 0.375 % telah menghasilkan peratus kecekapan pengkapsulan (% EE) yang paling tinggi iaitu 15.22 %. Pembebasan ubat gentamisin dari mikrosfera PLA menunjukkan kesan pecah dalam masa 8 jam pertama. Selepas itu, kadar pelepasan dadah adalah secara perlahan dan berterusan bagi tempoh pemantauan hari berikutnya.

DRUG RELEASE STUDY OF PLA MICRSPHERES LOADED WITH GENTAMICIN

ABSTRACT

Poly(lactic acid) (PLA) microspheres were fabricated through emulsion and solvent evaporation (ESE) technique. PLA loaded with different concentration of gentamicin were immersed in 0.5M NaOH solutions for 48 hours. The concentration of gentamicin were used are 0.125 %, 0.25 % and 0.375 %. Treated PLA microspheres were investigated using several characterization methods including Fourier Transform InfraRed (FTIR), Scanning Electron Microscopy (SEM), Particle size analysis, Contact Angle and Ultraviolet (UV) visible spectroscopy. The peak of gentamicin was determined and confirmed by FTIR. The treated PLA microspheres has becomes rough and porous surface after surface hydrolysis treatment. SEM proved the surface morphology changes of surface modified PLA microspheres. The value of contact angle was less than 90° after surface hydrolysis treatment and confirmed the improved hydrophilicity properties of surface modified PLA microspheres. The percentage of encapsulation efficiency (% EE) of gentamicin within PLA microspheres was evaluated by using UV-vis spectroscopy. The results recorded that higher concentration of gentamicin, which is 0.375 % gentamicin has the higher encapsulation efficiency around 15.22 %. The drug release of gentamicin from PLA microspheres showed the burst-effect manner within first 8 hours. After that, the drug release rate was slow and sustained release for the remainder days monitoring period.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Drug delivery technology offers an intelligent approach in the medical field. It is based on encapsulating drug into a carrier particle such as micrsopheres, nanospheres or liposomes (Kassab *et. al.,*2013). Drug delivery systems are engineered technologies for the targeted delivery or controlled release of therapeutic agents. Microencapsulation is a very common method for preparing controlled release systems such as carriers for drugs and vaccines. Microspheres are one of the multiparticulate drug delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites.

Drug delivery systems possess many advantages including ease of application, site-specific action, prolonged delivery periods and decreased body drug dosage with concurrent reduction in possible undesirable side effects. In recent years, much research has been focused on the usage of biodegradable polymeric microspheres as novel drug delivery systems. Using biodegradable and biocompatible polymeric matrices in microspheres has many benefits for developing successful drug delivery systems. They provide sustained delivery of the drug, its localized delivery and its stabilization (Kassab *et. al.,*2013).

The great challenges for research in material sciences is the development of biomaterials for medical application. Biodegradable and bioabsorbable polymers have been identified as alternative materials for biomedical applications, since these polymers can be degraded by a simple hydrolysis to produce by products that can be metabolized by the human body. These biomaterials are substances that can be derived from natural or synthetic origins which can interact with biological systems on a temporary or

permanent manner. It is also offer a possible alternative to treat and to repair the loss of tissues and organs from diseases.

Among the different classes of biodegradable polymers, the thermoplastic aliphatic esters as poly(lactic acid) (PLA) and its glycolic copolymer poly(lactic-co-glycolic acid) (PLGA) are most commonly used as drug carriers due to their excellent biocompatibility, biodegradability and mechanical strength. They can be degraded by non-enzymatic hydrolysis of the ester backbone in body fluids yielding metabolic compounds. PLA was extensively studied in medical implants, sutures and drug delivery systems. They are mostly focusing on the encapsulation of large molecules, e.g., peptides, proteins and plasmid Deoxyribo Nucleic Acid (DNA) for potential used as vaccines or as last-acting release drug formulation. The major advantages of biodegradable systems are they was eventually absorbed or excreted by the body. The drug release of microspheres was controlled by the polymer degradation rate and particle size of microspheres (Ulery *et al.,* 2011). One of the major considerations in microspheres fabrication was the particle size distribution as it determined the drug administration route, drug release properties, patient compliance and safety during the application period (Berkland *et al.,* 2002).

Drug loaded microspheres are prepared through microencapsulation technique. Microencapsulation is the term used to describe the technique to surround or coat certain chemical entity (in the form of either solids, liquids or gaseous) within a material that able to release the content at certain conditions. Among the several technique has been used, emulsion solvent evaporation (ESE) technique was reported the most successful method to load either insoluble or poorly soluble drugs in biodegradable microspheres (O'Donnell and McGinity, 1997). Gentamicin belongs to a class of drugs known as aminoglycoside antibiotics. It works by stopping the growth of bacteria. It is an antibiotic used to treat several types of bacterial infection. Thus, microspheres loaded with Gentamicin will be formulated using ESE technique, with using biodegradable

polymer of poly(lactic acid) (PLA). Then, they will be characterized for drug encapsulation efficiencies, drug stability and drug release profile.

1.2 Problem Statement

Poly(lactic acid),(PLA) is a polymer which is well suited for use in drug delivery which is having desired properties including biocompatible, biodegradable and has good mechanical properties. Their degradation products, lactic acids, is biocompatible and is easily eliminated from the body. However, the major drawbacks of PLA limiting its applications in drug delivery system (DDS) is its poor hydrophilicity. This poor hydrophilicity may resulting in low cell affinity, and can elicit an inflammatory response from the living host upon direct contact with biological fluids. Therefore, there is a need to modify the PLA microspheres in order to enhance it biologically properties for drug delivery application. There are several steps to modify the surfaces of PLA which is classified as non-permanent or permanent. In this research, the alkaline hydrolysis surface treatment by using sodium hydroxide (NaOH) is used to modify surface of PLA microspheres by improving hydrophilicity properties of microspheres.

The microspheres fabrication technique used in this research is emulsion and solvent evaporation (ESE) technique, because this technique is conceptually simple in experimental setup. However, the quality of microspheres (e.g. particle size, drug loading, encapsulation efficiency and etc.) prepared from this technique are highly dependent on the system nature. The understanding of ESE technique are required to ensure the successful of microspheres fabrication.

Thus, the aims of this research is to study of the NaOH effect on the encapsulation and drug stability within PLA microspheres. Additionally, the drug release profile also will be investigated.

1.3 Objectives

The objectives of this research are:-

1. To study the effect of different gentamicin loading on the percentage of encapsulation efficiency (% EE) within PLA microspheres.
2. To study the effect of surface modification on the stability of gentamicin loaded PLA microspheres.
3. To study the drug release profile of PLA microspheres loaded with Gentamicin.

1.4 Scope of Study

Drug delivery systems are engineered technologies for the targeted delivery or controlled release of therapeutic agents. Drugs have long been used to improve health and extend lives. The practice of drug delivery has changed dramatically in the past few decades and even greater changes are anticipated in the near future. There are various types of drug delivery, such as oral, injection based, transdermal and carrier based.

In this research, PLA is used to produce microspheres for drug delivery. Biodegradable polymer such as poly(lactic acid) (PLA) finds widespread use in the drug delivery industry. This polymer is well suited for use in drug delivery because they are biocompatible with living tissue, and their degradation products, lactic acids, is biocompatible and is easily eliminated from the body. PLA has been used to make microspheres loaded with Gentamicin for drug delivery.

PLA microspheres was produced by using Emulsion Solvent Evaporation (ESE) technique. Surface modification was done to induce hydrophilic behaviour on the PLA microspheres surface. Several material characterization were done to investigate the properties of the PLA microspheres. FTIR was done to determine the functional groups present in the PLA microsphere which is hydroxyl and SEM was performed to observe the surface structure and size morphology of PLA microspheres. Contact angle was used to determine hydrophobicity or hydrophilicity of the PLA microspheres. While, UV-

vis spectrometer was used to determine encapsulation efficiency and drug release profile of PLA microspheres.

1.5 Thesis Outline

This research is about study of drug release profile of PLA microspheres loaded with gentamicin.

Chapter 1 Introduction. Briefly describe the background study of this research include the problem statement and the objectives of this research.

Chapter 2 Literature review. Establish a theoretical framework for the research. Give generally information about the research.

Chapter 3 Materials and methods. Give information about materials used in this research and method used to conduct the experiment.

Chapter 4 Results and discussions. Describe the results obtain throughout the research. Several characterization are described: FTIR, SEM, particle size analysis, Contact angle, UV-vis spectrometer

Chapter 5 Conclusion. Summary and conclusion on this present work as well as suggestions for the further research.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

When a drug is taken by a patient, the resulting biological effects, for example lowering of blood pressure. These biological effects are usually produced by an system is to enhance or facilitate the action of therapeutic compounds. Ideally, a drug delivery system could deliver the correct amount of drug to the site of action at the interaction of the drug with specific receptors at the drug's site of action. The purpose of any delivery correct rate and timing, in order to maximize the desired therapeutic response (Hillery *et al.,*2001).

Development of drug-releasing materials has been carried out for the last three decades. Their suitability for use in fields such as pharmaceutical therapy, tissue engineering especially for bone and cartilage tissue engineering and cancer therapy have been well assessed (Nikkola, 2009).

Drug delivery devices have several advantages compared to conventional drug administration methods. Drug delivery has been developed in order to maintain effective drug concentration in blood over longer periods, maximise efficiency and minimize side effects of drugs (Jain, 2008).

Poly(lactic acid) (PLA) is widely used in the biomedical field due to its biodegradability, biocompatibility, thermal plasticity and suitable mechanical properties. PLA is obtained from lactic acid and converted back to the latter one when hydrolytically degraded. Lactic acid is a naturally occurring organic acid that can be produced by fermentation of sugars obtained from renewable resources such as sugarcane (Lopes *et. al.,* 2012). PLA microspheres is produced by using emulsion solvent evaporation (ESE) technique.

2.2 Drug Delivery System (DDS)

A drug delivery system (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance into the body and improves its efficiency and safety by controlling the rate, time and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. The therapeutic agent can also be an agent such as gene therapy, which will induce *in vivo* production of the active therapeutic agent. Despite the fact that gene therapy has its own special regulatory control, gene vectors may need to be introduced into the human body by novel delivery methods (Jain,2008).

During the past two decades, it has been recognized that biodegradable polymers have become increasingly important in the development of drug delivery system (DDS). Massive researches have been done to designed appreciate devices like microspheres (MSc) and nanospheres, injectable form, wafer, tablet and so on using synthetic and natural biodegradable polymers. Beginning of 1980s, rapid and innovative progress in the area of biotechnology, especially cell and cloning technology, made possible the successive and massive production of therapeutic peptides and protein like cytokine, monoclonal antibody, hormone and growth factors. So, DDS using biodegradable polymers were extensively studied and commercialized for these peptides and proteins to achieve efficiency and to increase patient compliance. It can be avoided the difficulties associated with parental and oral delivery and patient compliance problems (Toi *et. al.,*, 2012).

Biodegradable polymers for local delivery system for DDS has been applied. Among of these biodegradable polymers, one of the most significant candidates for the development of the biodegradable polymeric controlled release system is the poly(a-hydroxy acid)s family such as poly(glycolide) (PGA), poly(L-lactide acid) (PLA) and its

copolymers as poly(L-lactide-co-glycolide) (PLGA) which is only approved by the Food and Drug Administration (FDA) due to its controllable biodegradability and relatively good biocompatibility. It provides many advantages such as regulating varying degradation period according to the molecular weight and mole fraction of lactide and glycolide in the copolymer (Toi *et. al.,* 2012).

2.2.1 Types of Drug Delivery System

The first generation drug delivery systems appeared toward the end of the nineteenth century and in the twentieth century, and they have consistency and uniformity. These drug delivery systems (conventional dosage forms) include tablets, capsules, elixirs, syrups, suspension, emulsion, and solution and topical administration of ointments, lotions and cream, suppositories or injection of suspensions and solutions. Though these conventional drug delivery systems are still with us, the need for more efficient drug delivery systems was realized with time (Ghosh *et. al.,*, 2004).

The treatment of acute disease or chronic illness has been achieved by delivery of drugs to the patients. For many years conventional drug delivery systems included tablets, injections, suspensions, creams and aerosols, still widely used for therapeutic approaches. The ultimate aim of pharmacy and medicine is the delivery of any drug at the right time, within the ideal therapeutic window and outside the systemic toxic range, in a safe and reproducible manner, to a specific target, at the required level (Korting, 2010).

Conventional types of dosage such as oral delivery or injection are the predominant routes of administration. However, this approach is only rarely useful to control the rate of drug delivery, is often associated with an immediate, rapid drug release, and does not regulate the target area of the applied drugs. Consequently the initial concentration of the delivered drug in the organism rises and may reach the level of toxicity, decreasing over time to a sub-therapeutic level. Toxicity is observed for peaks

of drug concentrations, rendering traditional methods of drug delivery ineffective. With a controlled drug release system, the rate of compound delivery should match the rate of drug elimination and, therefore, the drug concentration remains in the therapeutic window for the vast majority of the 24 h period (Korting, 2010).

According to Keservani *et. al*., (2016), conventional dosage forms have been found to have serious limitations in terms of higher dosage required, lower effectiveness, toxicity and adverse side effects. New drug delivery systems have been developed to overcome the limitation of the conventional drug delivery systems. These systems can be called as controlled drug release systems and targeted drug delivery systems. The therapeutic benefits of these new systems include:

- Increased efficacy of the drug site specific delivery
- Decreased toxicity/side effects
- Increased convenience
- Shorter hospitalization
- Better patient compliance

Controlled drug delivery technology represents one of the frontier areas of science in biopharmaceutical and biomaterials applications (Singh et. al., 2010). The idea of DDS is aimed to maximize efficiency of the current available drugs, and further delivery drugs to diseased site in the body it in a more effective and less invasive way. A controlled DDS possessed advantages in maintained the drug concentration within therapeutic window and sustained for longer period of time. The prolong drug release is able to reduce the drug administration frequency and also reduced the risk of toxic effect.

2.3 Concept of Drug Delivery

In 1930s, the concept of drug delivery can be traced when the first studied reported delivery of therapeutic agents from implanted compressed estrogen delivery pellets implanted subcutaneously in livestock. The hormonal implantation was already in common practice in the 1950s and since then research into implantable drug delivery devices has grown rapidly (Dash and Cudworth, 1998).

2.3.1 Local Drug Delivery

Local drug delivery method is very beneficial as the drug can be effectively delivered to the target site. It can be performed in various ways, such as injection of drug to the site. However, injection of drug in a liquid dissolves easily and the drug can escape from target site relatively fast, thus enabling only short-term therapy. The administration of drug is prolonged and targeted to the specific site with the local drug delivery devices (Koort *et al.,* 2006).

2.3.2 Categorization of Drug Delivery Mechanism

There are several typical controlled release system to describe the release mechanism of an active agent from a drug delivery system. To be able to describe or predict the release profile, knowledge of the type of system and rate controlling mechanism is crucial. According to Heller (1996), drug delivery devices can be categorized as diffusion method, water penetration controlled, chemically controlled and regulated (magnetic or chemical) devices.

Diffusion controlled devices can be either monolithic, where the drug is dispersed in the carrying matrix and released by diffusion, or systems comprising an outer diffusion-controlling membrane and an inner drug-loaded core, also known as reservoir device. Figure 2.1 shows the mechanism of drug delivery in a reservoir and matrix system. In a monolithic device (matrix system), the drug is dispersed in a matrix and the release is controlled by diffusion from the system. While, reservoir devices are

where the drug is encapsulated or present as a core within a polymer film or coat. The diffusion occurs through a membrane that controls the movement of the drug or solvent between two sides. Modelling the release characteristics of reservoir devices as well as monolithic devices, in which the transport of the drug is by a solution-diffusion mechanism. When the device contains dissolved drug, the rate of release decreases exponentially with time as the concentration of the drug within the devices decreases. If however, the active agent is in a saturated suspension, then the driving force for release is kept constant (zero order) until the device is no longer saturated (Andersson et. al., 2008)

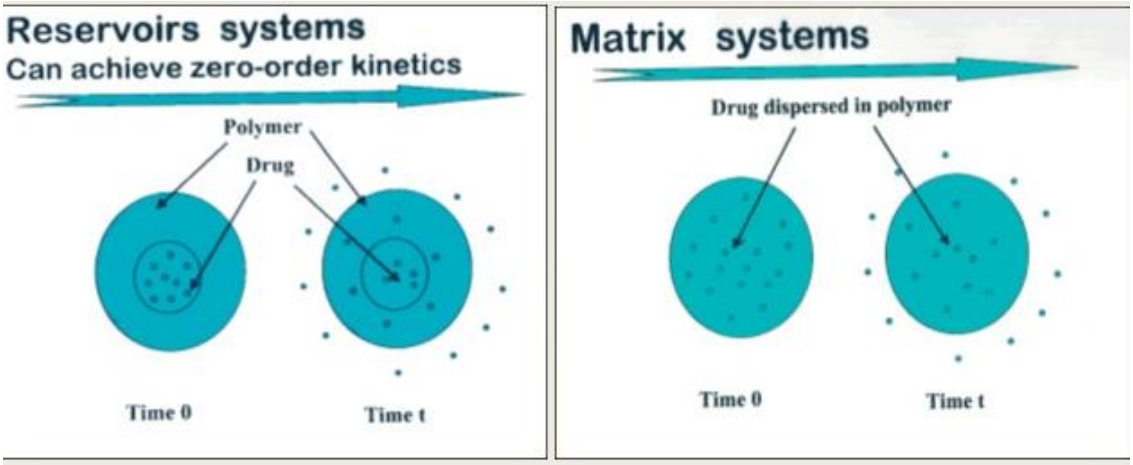


Figure 2.1: Mechanism of drug delivery by diffusion controlled devices

Water-penetration controlled systems are also of two types, being either swelling or osmotically controlled. In swelling controlled, the agent is dispersed in a hydrophilic polymer which is glassy in the dehydrated state. The polymer will release the agent during an aqueous environment. Osmotically controlled contain an osmotically active agent within a rigid housing separated from the therapeutic agent by a movable wall. Water is osmotically driven across the semipermeable wall of the housing will increase the pressure in the compartment of the osmotic agent (Harrison, 2007).

In chemically controlled systems, the therapeutic agent can be attached to a polymer backbone and the disintegration of the backbone by hydrolysis breaks the bond and release the agent. The drug also can be dispersed in a biodegradable polymer. It will not undergo transformation during the release period but later will be slowly degraded (Heller, 1996).

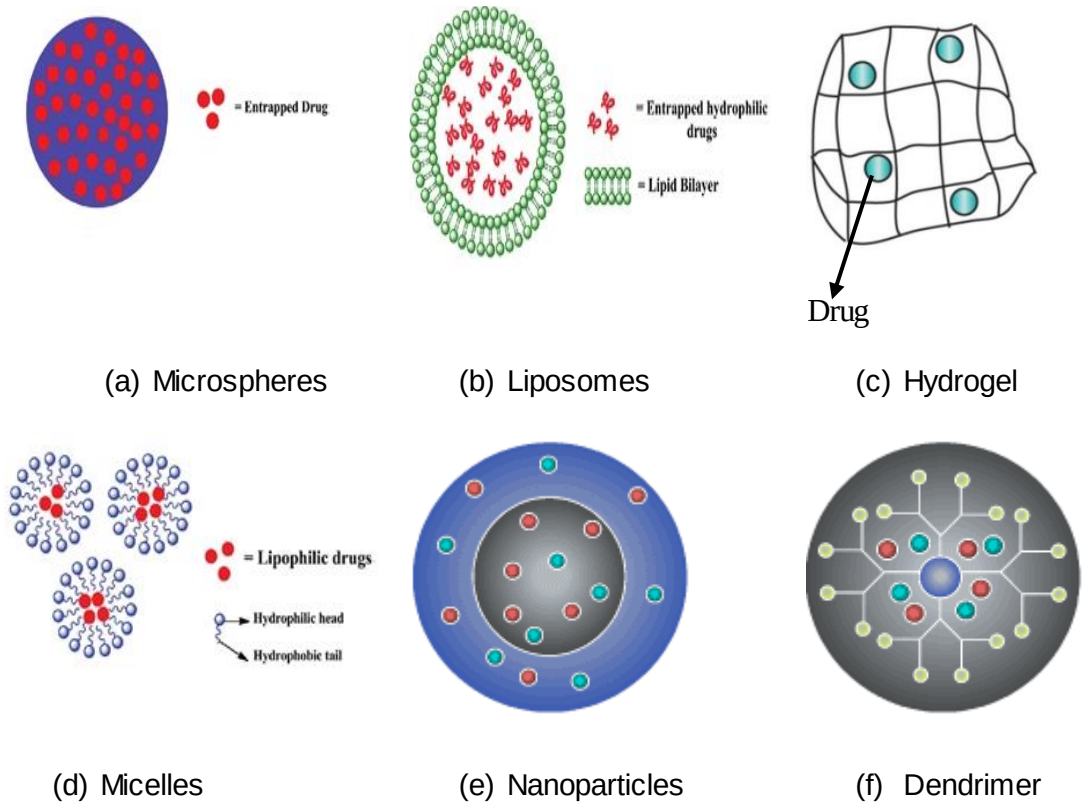
2.4 Drug Delivery Carrier

One of the main challenges for the biomedical scientific community is the design of novel drug-delivery systems (DDS) able to transport an effective amount of cargo specifically to the target cell or tissue. Currently, most clinically used drugs in oral or systemic administration are low-molecular weight that exhibit short half-lives in the bloodstream and a high overall clearance rate. Therefore, high initial drug doses are needed to maintain therapeutic concentrations over a prolonged time period.

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to target tissues. The new strategies were generated, which often called drug delivery system (DDS). Drug carriers are one of the important approaches among new drug delivery system. Drug carriers introduced into the field to achieve different goals like enhancement of bioavailability, stability, preventing the drug interaction (Srikanth *et. al.,*2012).

2.4.1 Types of Drug Carrier

There are several types of drug carrier, which are microspheres, liposomes, hydrogels, polymeric micelles, nanoparticles, dendrimers and etc. Figure 2.2 shows the illustration for all types of drug carrier.



(a) Microspheres (b) Liposomes (c) Hydrogel

(d) Micelles (e) Nanoparticles (f) Dendrimer

Figure 2.2: Schematic diagram of several types of drug carrier; (a)Microspheres, (b) Liposomes, (c) Hydrogel, (d) Micelles, (e) Nanoparticles, (d) Dendrimer

Microspheres – Microspheres are characteristically free flowing powders consisting of drug or synthetic polymers which are biodegradable in nature and ideally having a particle size less than $200\mu m$. These are developed using different methods like emulsion techniques, polymerization techniques, spray drying, spray congealing, solvent evaporation, etc. These are developed for variety of applications like controlled drug delivery, vaccine delivery, as drug carrier, etc. It is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects (Srikanth *et. al.,*2012).

Liposomes – Tiny pouches made of lipids, or fat molecules surrounding a water core widely used for clinical cancer treatment. Several different kinds of liposomes are widely employed against infectious diseases and can deliver certain vaccines. According to Manivannam *et. al.,* (2010), liposomes are the microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 20-100μm. Liposomes are manufactured by means of different methods like film hydration technique, ether injection method, reverse phase evaporation method, membrane extrusion technique, etc. All these methods involve two steps to manufacture, which are drying of lipids from lipid solution followed by dispersion of lipid film in aqueous medium. These have wide applications in drug delivery because they can administer from almost all routes, and can be used as carriers for all drugs (both lipophilic and hydrophilic drugs).

Hydrogels – Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical crosslinks (tie-point, junctions), or physical crosslinks, such as entanglements or crystallites. Hydrogels exhibit a thermodynamics compatibility with water, which allow them to swell in aqueous media. They are used to regulate drug release in reservoir-based, controlled release systems or as carriers in swellable and swelling-controlled release devices. In controlled drug delivery, hydrogels as enviro-intelligent and stimuli-sensitive gel systems modulate release in response to pH, temperature, ionic strength, electric field, or specific areas of the body or also via specific sites. Hydrogels as drug delivery systems can be very promising materials if combined with the technique of molecular imprinting (Manivannam *et. al.,* 2010).

Polymeric Micelles – These systems include amphiphilic block copolymers such as Pluronics (polyoxyethylene block copolymers that self-associate in aqueous solution to form micelles). Polymeric micelles offer a number of advantages in terms of

thermodynamic stability in physiological solution leading to their slow dissolution in vivo. Because of their core-shell structure, these serve as suitable carrier for water insoluble drugs, such drugs partition in the hydrophobic core of micelles and outer hydrophilic layer aids in dispersion in aqueous media making it an appropriate candidate for intravenous administration. Nanometric size range helps micelles to evade the RES, and aids passage through endothelial cells (Srikanth *et. al.,*2012).

Nanoparticles – Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. These are used as potential drug delivery devices because of their ability to circulate for a prolonged period time in systemic circulation and are useful to target a particular organ. Apart from these, they can also acts as carriers for DNA in gene therapy (Srikanth *et. al.,*2012).

Dendrimers – The term dendrimers derived from the words Dendron (tree.branches) and meros (part). These posses three distinguishing architectural components.

A) an interior core
B) Interior layers (generations) composed for repeating units of radially attached to the interior core
C) Exterior (terminal functionality) attached to the outermost interior generation.

Variety of dendrimers is available, which are simple dendrimers, liquid crystalline dendrimers, chiral dendrimers, micellar dendrimers, hybrid dendrimers, metallodendrimers, etc. Two fundamentally different methods have been developed for stepwise synthesis of dendritic polymers. They are divergent growth method and convergent growth method. These have wide applications in the pharmaceutical field like solubilisation, controllable gene therapy, drug carrier, magnetic resonance imaging contrast agents, as vaccines, artificial proteins, enzymes, etc.(Srikanth *et. al.,*2012).

2.5 Drug Release Profile

According to Jones (2004), the drug can be dissolved, dispersed or partially dissolved in the polymer matrix. The release of a drug from stabile polymers is based on diffusion, which can occur as either zero or first order kinetics.

Figure 2.3 illustrate the release profile of zero and first order kinetic. According to Siegel and Rathbone (2012), Zero order, or constant rate of drug release is desirable in order to minimize swings in drug concentration in blood. Zero order release, in which a drug is released at a constant rate, is the ultimate goal of all controlled-release drug delivery mechanism. It leads, in principle, to the best control of plasma concentration and offers several advantages, including improved patient compliance and reduction in the frequency of drug administration. Although there are several techniques that formulation scientists can use to achieve zero-order release, most of them are complex, expensive, time consuming, and difficult to manufacture. In addition, most formulation techniques result in, at best, first-order release. In typical first-order release kinetics, the drug release rate depends on its concentration.

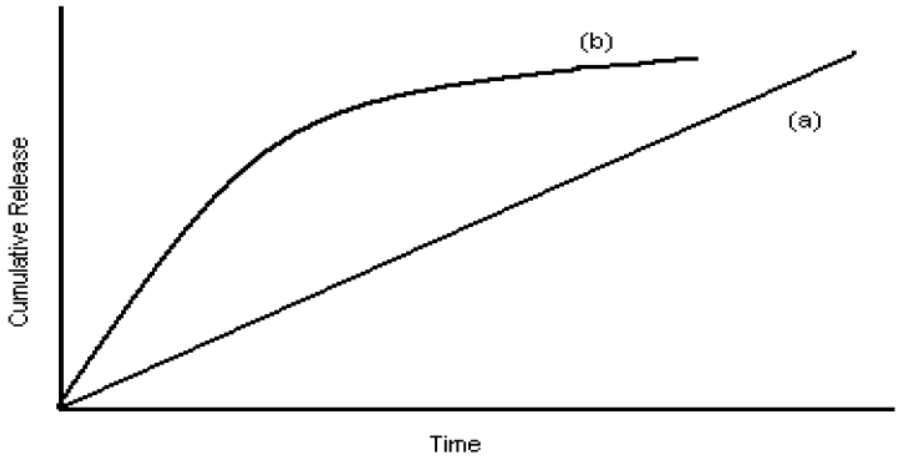


Figure 2.3: Schematic illustration of release profiles of (a) zero order kinetics and (b) first order kinetic release (Jones, 2004)

According to Jean (1993), drug is the chemical compound administered to the patient's organism and it develops a reciprocal interaction for therapeutic purposes. The drug is not used in the pure state for many reasons. The supply form of presentation of the drug, or dosage form is the completion medication.

All conventional dosage forms made of a drug dispersed in excipients and release the drug to the following pattern. The drug is very rapidly dissolved from the dosage form and quickly builds up to a maximum high concentration, which then falls exponentially with time because of the first order absorption. The limitations of conventional dosage forms made of drug and excipients appear then since they cause problems in maintaining therapeutic drug levels over only brief durations of time.

Simple oral dosage form capable controlling the release of the drug where the drug is dispersed in a biocompatible polymer. This polymer plays the role of a polymer matrix, which can be either biodegradable or non-degradable. It controls the release of the drug. Generally, the process is as follows; the liquid enters the polymer, dissolves the drug and enables the drug to leave out the dosage form through the liquid located in the dosage form. The matter transfer for the liquid and for the drug are controlled by transient diffusion, with concentration-dependent diffusivities, the diffusivity of the drug depending on the concentration of the liquid in the dosage form. The release of the drug being controlled by transient diffusion, exhibits a rather high rate at the beginning of the process which decreases with time in an exponential way. The process of released is controlled by diffusion and are very simple to prepare (Jane 1993).

Figure 2.4 shows a comparison between conventional drug release profile and controlled drug release profile. Conventional drug delivery systems have slight control over their drug release and almost no control over the effective concentration at the target site. This kind of dosing pattern may result in constantly changing, unpredictable

plasma concentrations. Drug can be delivered in a controlled pattern over a long period of time by the Controlled or Modified release drug delivery systems.

A controlled release system is a device for controlled the release time of a chemical, and/or the release rate. In the field of drug delivery, the amount delivered over a certain time frame is critical depending on the therapeutic purpose and the patient. If drug concentration are below this specific range, then there is no therapeutic benefit for the patient. However, if the drug concentration are too much, then the drug can have toxic effects and can possibly pose substantial danger or death to the patient.

Conventional drug delivery systems include tablets of injections that commonly results in a burst release of drug followed by a general decrease in drug concentrations. In a drug delivery profile in Figure 2.4, it can be seen as a sharp peak much larger than the therapeutic concentration range within the initial stages of delivery that immediately decreases below the therapeutic concentration range. An ideal form of drug delivery would be a sustained or "controlled" form of drug release that can be kept constant within the therapeutic range (Freiberg *et. al.,* 2004).

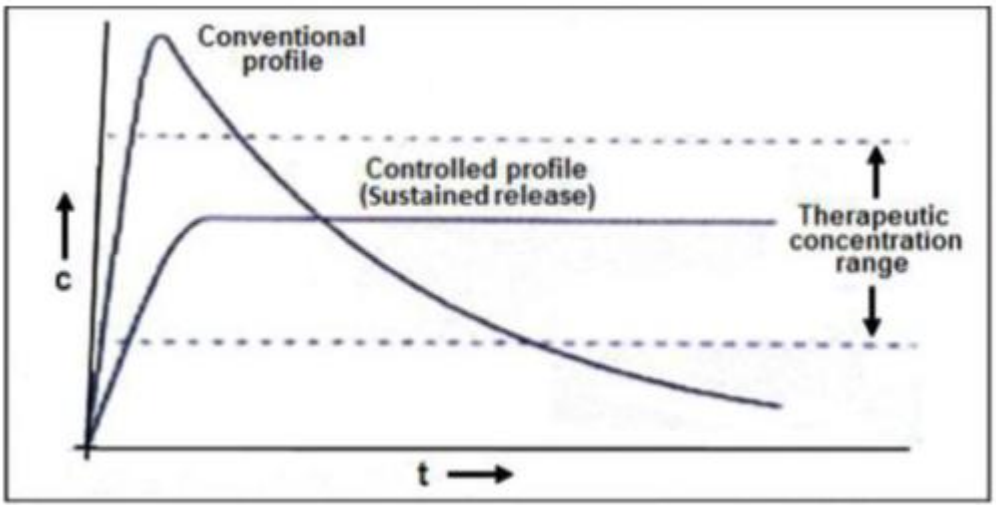


Figure 2.4: Theoretical plasma concentration in conventional and controlled release drug delivery, in which c is drug concentration and *t* is time

2.6 Drug Release and Polymer Degradation

The release from biodegradable microspheres is dependent on drug diffusion through microspheres matrix and polymer degradation. Generally, the drug release from biodegradable polymer matrix is unpredictable and erratic as the microspheres structure tends to breakdown after certain duration (Sinha and Trehan, 2003). The possible drug release mechanisms from biodegradable microspheres are listed, where all these mechanism together play a part in the release process.

- The initial release from microsphere's surfaces
- Release through the pores within the microsphere's bulk morphology
- Drug diffusion through a water swollen matrix which dependent of the polymer hydrophilicity and polymer molecular weight
- Release through the pores formation in the matrix that induced by the hydrolysis of the polymer chain.

2.6.1 PLA Hydrolysis Degradation

The major degradation mechanism of PLA is hydrolysis in either with or without catalyst. The term "degradation" is used to describe the chain scission of polymer chains from macromolecules to oligomers and finally to monomers. The polymers can be divided into surface erodible and bulk erodible polymers on the basis of their degradation behaviour.

Polyorthoesters (POE) and polyanhydrides (PAH) represent groups of surface erodible polymers, of which POEs have been developed specifically for use as drug release matrix materials. The release of active agent can be adjusted to follow zero order kinetics due to the erosion characteristics. One well known and widely applied group of biodegradable polymers is the synthetic polyesters, especially aliphatic α-hydroxy acids such as poly(lactic acid) (PLA) and polyglycolides (PGAs) and their

copolymers. These polymers degrade by bulk erosion, degrading by hydrolytic chain scission to produce acidic, though non-toxic degradation products, which are eliminated from the human body through natural body functions in the citric acid cycle.

Figure 2.5 shows the schematic of PLA degradation in bulk and surface erosion mode. “Bulk” degradation is the condition where the polymer chain scission occurred throughout the polymer structure, and ultimately the structural disintegration of polymer occurred. On the other hand, “erosion” is designated to describe the degradation occurred predominantly at polymer surface followed by the loss of oligomers or monomer from polymer surface. Water molecules are the main catalysis involved in PLA hydrolysis degradation process. Surface erosion degradation mode occurred when the surface degradation rate is higher than water absorption rate into PLA microspheres. While bulk erosion occurred as the water absorption rate is higher than surface degradation rate.

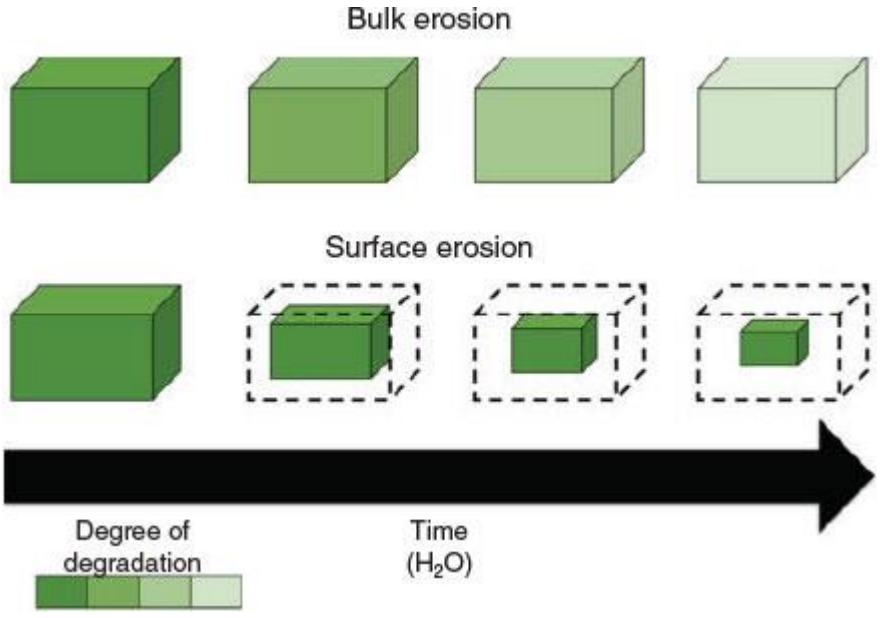


Figure 2.5: Schematic of PLA degradation in bulk and surface erosion mode

Recently, polymers that respond to changes in the environment have been developed. The changes, such as in pH or temperature, can induce conformational changes to the polymer chain, making them applicable for a variety of medical applications like tissue engineering scaffolds and drug delivery devices (Chan and Mooney, 2008).

Polymer degradation is counteract process involved molecular chains degradation, swelling, dissolution and diffusion of degraded oligomers and monomers and morphological changes. Generally, the drug release is known to be initiated by diffusion followed by degradation/erosion. As the hydrolysis degradation of PLA required water to be diffused into the matrices. Water adsorption occurred once the polymer in contact with water, however the absorption rate is very dependent on the nature of materials (e.g. hydrophilicity or crystallinity). The adsorbed water molecules are firstly occupied certain volume in the polymer matrices, further facilitate the mobility of molecular chains in amorphous region and thus allowed the polymer hydrolysis and drug diffusion to occurred.

In polymer degradation, the hydrolytically degradation of PLA occurred on the ester bond breakage in the PLA backbone chain. There are two possible cleavage route of ester linkage, which either at “acryl” carbon or “alkyl” carbon. March (1997) have reported that the cleavage of acryl bond are easier than alkyl bond in hydrolysis reaction. The cleavage of ester bond results in a shorter polymer chains with the formation of both acid and alcohol end-groups. Along the degradation process, the increase in the number of carboxylic acid end-groups have always been reported to increase the acidity at the site of degradation.

2.6.2 Factors Affecting Release Rate

According to Kim and Pack (2006), method of microsphere fabrication is a governing factor in the encapsulation and release of therapeutics. Another factors including the type of polymer, polymer molecular weight, polymer composition, nature of any excipients added to the microsphere formulation and the microspheres size can have a strong impact on the drug release rate.

The types of polymer used in microspheres fabrication and the way in which the polymer degrades affect drug release rates. Polymers can be categorized into two types, which are surface-eroding and bulk-eroding based on the rate of hydrolysis of their functional groups. Bulk-eroding polymers allow permeation of water into the polymer matrix and degrade throughout the microsphere matrix. While, surface-eroding, such as polyanhydrides, are composed of relatively hydrophobic monomers linked by labile bonds. They are able to resist the penetration of water into the polymer bulk, while degrading quickly into oligomers and monomers at the polymer/water interface via hydrolysis (Kim and Pack, 2006).

Besides that, polymer molecular weight also can affect polymer degradation and drug release rates. An increase in molecular weight will decreases diffusivity and therefore drug release rate. A major mechanism for release of many drugs is diffusion through water-filled pores, formed as polymer degradation generates soluble monomers and oligomers that can diffuse out of the particle.

The co-monomer ratios in many copolymers also can affect release rates. The release rate will increase with increasing the content of more rapidly degrading monomer. Similarly, when the drug release is controlled by polymer erosion, release rate typically increases with higher concentration of the smaller and/or more soluble monomer. Other than that, to stabilize the drug during fabrication and/or release, a

variety of excipients may be added to microsphere formulations and may impact drug release through several different mechanisms (Kim and Pack, 2006).

Lastly, the rate of drug release also strongly affected by the microsphere size. As size decreases, the surface area-to-volume ratio of the particle increases. Thus, for a given rate of drug diffusion through the microsphere, the rate of flux drug out of the microsphere, per mass of formulation, will increase with decreasing particle size. Water penetration into smaller particles also maybe quicker due to shorter distance from the surface to the center of the particle (Kim and Pack, 2006).

2.7 Microspheres

Drug delivery using biodegradable polymeric microspheres has gained increased in the last two decades. According to Sahil *et al.* (2011), microspheres are characterized as the powders prepared from either nature or synthetic polymer having a particle size range from 1-1000 μm. Microspheres are different from microcapsules. In microspheres, the drug is dispersed throughout the polymeric matrix, whereas in microcapsules, the drug is the core surrounded by a polymeric membrane. In the biomedical and bioprocess applications, microsphere have been routinely used. For example, in high performance liquid chromatography the polystyrene microspheres have been used (Kirkland *et al.,* 2000). Polyethylene microspheres are used as permanent or temporary fillers (Khan *et al.,* 2009). While, biodegradable microspheres have also been used a bulking agents in soft tissue to augment the efficiency of opening-close system, which included in treatment of stress urinary vessel in assist surgical operation (Saralidze *et al.,* 2010).

Microsphere are widely used as drug carriers for controlled release and the incorporation of drug molecules into biodegradable polymeric microspheres has many advantages. The polymeric matrix can protect drugs from physiological degradation.

Microspheres can control the delivery of drugs from days to months therefore reducing frequent administration and improving patient compliance and comfort (Alok *et al.,* 2009). Microspheres performed drug release along with degradation of polymers and no further surgical removal is required after treatment completed (Shaik *et al.,* 2012). Example of polymers that exhibit both biodegradable and biocompatible properties are included poly(lactic acid), poly(glycolic acid), polyanhydrides, polyester, poly(caprolactom) and etc. Among the biodegradable polymers, PLA are still the most potential used for delivery (Ulery *et al.,* 2011).

2.7.1 Biodegradable Polymer for Microspheres Preparation

Polymers used as matrices for drug delivery can be classified under three basic types, which are water soluble polymers, biodegradable polymers and non-biodegradable polymers. Table 2.1 show the example of biodegradable polymers used in drug delivery system. Both natural and synthetic polymers are used as matrix materials in the preparation of biodegradable microspheres.

Table 2.1: Example of Biodegradable Polymers Used in Drug Delivery System

Natural Biodegradable Polymers	Synthetic Biodegradable Polymers
• Polypeptides and proteins: Albumin, fibrinogen, gelatin, Collagen, etc • Polysaccharides: Hyaluronic acid, starch, chitosan • Virus and living cells: Erythrocytes, fibroblast, Myoblasts, etc	• Aliphatic polyesters of hydroxyl acids: PLA, PGA, PLGA, poly(hydroxybutyric acid), poly(e-caprolactone) • Polyorthoester • Polyaminoacids • Polyanhydrides • Polyacrylamides • Poly(alkyl-a-cyanoacrylate)s • Etc.