CHAPTER II
LITERATURE REVIEW

2.1 Theoretical Background

The goal of any drug delivery system is to deliver the amount of drug to the specific sites in the body and then sustain the drug concentration along the period of time. In order to induce the drug into specific site, we had to modify the drug, which have magnetic property. Iron oxide (Fe_3O_4) was dispersed in biocompatible and biodegradable polymer such as poly (D, L-lactic-co-glycolide) (PLGA) to create the magnetic property by using the water-in-oil-in-water (w/o/w) double emulsion method. Superparamagnetic iron oxide nanoparticle encapsulated within poly (D, L-lactic-co-glycolide) (PLGA) particles were created to use in drug delivery sys-tem for human body. However, there were some problems with these materials such as low surface area, less drug stability and accelerated polymer degradation. So, currently the researchers are focusing on improve and modify drug (carrier) to have the suitable and better properties such as high surface area, more drug stability and prolong release behavior.

2.2 Literature Review

2.2.1 Poly (D, L-lactic-co-glycolide) (PLGA)

PLGA, a copolymer of PLA and PGA, is the most widely used as biodegradable polymer for drug delivery. PLGA is synthesized by a random ring-opening copolymerization of the cyclic dimers of glycolic acid and lactic acid, whereby successive monomeric units of PGA or PLA are linked together by ester linkages. One of the outstanding advantages of PLGA over the other biodegradable synthetic polymers is that the ratio of PLA to PGA used for the polymerization can be adjusted to alter the biodegradation rate of the product. PLGA is degraded in vivo, through hydrolysis and enzymatic action, to lactic acid and glycolic acid, which are ultimately converted via the citric acid cycle to water and carbon dioxide. Since PLGA is biocompatibility, biodegradability and non-toxicity, it has been used in the

production of a variety of biomedical devices. PLGA can be used to construct biomaterials of various types and shapes such as resorbable suture materials; rods, screws, plates, and pins for orthopedic surgery; vascular grafts and stents; and surgical meshes and scaffolding for tissue regeneration. Moreover, PLGA has also been widely utilized in the development of various types of drug-release systems. In fact, drug release from PLGA polymeric particles occur in three steps.

I. Burst release: Drug release from the implant surface occurs, creating a short period of high drug release.

II. Diffusion and chain scission: Diffusional drug release, which is governed by the inherent solubility of the drug in the surrounding media, occurs. Random chain scission of polymers occurs by hydrolytic cleavage, which increases the porosity and surface area for drug diffusion.

III. Biodegradation and mass loss: Drug release is associated with biodegradation of the polymer matrix, mass loss initially occurring in the central core of the implant, and a final burst in some delivery systems.

2.2.2 Superparamagnetic Iron Oxide (Fe_3O_4) Nanoparticle

The functionalized magnetite nanospheres are usually formulated by encapsulation of magnetic nanoparticles (e.g. magnetite) into biodegradable polymeric matrix such as PLGA, which is an excellent record of biocompatibility, biodegradability and non-toxicity, and use gradually for biomedical applications including drug delivery, diagnostic magnetic resonance imaging (MRI), magnetic cell separation, tissue repair, hyperthermia and magnetofection (Liu *et al*, 2007). Several techniques for synthesis of superparamagnetic iron oxide (Fe_3O_4) have been reported. The technique, which is known as bottom-up technique, uses to form a magnetic core with a polymer shell nanoparticle. Another interesting technique is the top-down technique, which is based on hydrophobic–hydrophilic interactions, as well as ionic and Van der Waal's forces, for nanoparticles formation. The common top-down technique to create polymeric nanoparticles is emulsion techniques including emulsion evaporation, emulsion diffusion, salting out, and nanoprecipitation (Astete *et al*, 2006). The emulsion evaporation method was used to prepare PLGA nanoparticles with oleic acid stabilized superparamagnetic iron oxide nanoparticles,

using sodium dodecyl sulfate (SDS) as a surfactant. The nanoparticles size ranged from 78.8 to 115.1 nm. The magnetite with oleic acid into the PLGA nanoparticles were calculated by entrapment efficiency varied from 57.4 to 91.9 % (Astete *et al*, 2006). The nanoparticles of PLGA loaded with magnetite/maghemite nanoparticles were prepared by emulsion/evaporation technique. These sub-micron particles were nearly spherical in shape with a mean size near 280 nm. The highest incorporation rates of ferrite (up to 13.5 %w/w) were observed with initial ferrite/polymer ratio of 1:1 w/w (Fillafer *et al*, 2010). The highly magnetic biodegradable PLGA nanospheres were prepared by a modified single oil-in-water emulsion solvent evaporation method. The mean diameter was 360–370 nm with polydispersity indices of 0.12–0.20. High magnetite content (40–60 %) and high magnetization (26–40 emu/g) were obtained when magnetite loadings were 15 %, 39 %, and 61 % by mass for samples 50, 100, and 200, respectively (Liu *et al*, 2007). The oleic acid-coated superparamagnetic iron oxide (Fe_3O_4) nanoparticles encapsulated within PLGA particles were synthesized by the water-in-oil-in-water (w/o/w) double emulsion method as a dispersant was poly(vinyl alcohol). The weight percent of 7–16 nm diameter magnetite in the particles varied only from 56 to 62 % (23–28 vol.%). The superparamagnetic iron oxide nanoparticles were essentially spherical with magnetite spatially uniformly dispersed in individual PLGA particles (Bootdee *et al*, 2012).

2.2.3 Water-Oil-Water (w/o/w) Double Emulsion Solvent Evaporation Technique

The water-in-oil-in-water (w/o/w) double emulsion method having the internal and external aqueous phases was separated by an oil layer. For their formation and stability, at least two surfactants, one having a low HLB to form the primary water in oil (w/o) emulsion and the other of higher HLB to achieve secondary emulsification are required to emulsify water in oil emulsion into water. These emulsion water-in-oil-in-water (w/o/w) systems being less viscous are excellent candidates for controlled release of hydrophilic drugs due to the existence of a middle oil layer that acts as a liquid membrane.

In the water-in-oil-in-water (w/o/w) double emulsion solvent

evaporation method, an aqueous solution or suspension of the drug (internal aqueous phase, w_1) is emulsified in a solution of polymer in organic solvent. The resulting primary emulsion (w_1/o) is then dispersed in a second aqueous phase (external aqueous phase, w_2) containing suitable emulsifier(s) to form double emulsion ($w_1/o/w_2$). The removal of the volatile organic solvent leads to the formation of solid micro or nanoparticles. The solid micro or nanoparticles are separated by filtration or centrifugation, washed several times in order to assuredly eliminate the residual emulsifier and dried under vacuum or freeze-dried. The volatile organic solvent used in the preparation of the micro or nanoparticles by the water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method should be of low boiling point to facilitate the removal of residual solvent. The various solvents that can be used for the preparation of micro or nanoparticles are acetonitrile, ethyl acetate, chloroform, benzene and methylene chloride (Giri *et al*, 2013). The crucial matter, effecting on the properties of micro or nanoparticles, is stabilizer. In this method can use the different types of stabilizer such as PEG, Tween-80, gelatin, dextran, pluronic L-63, PVA and DMAB.

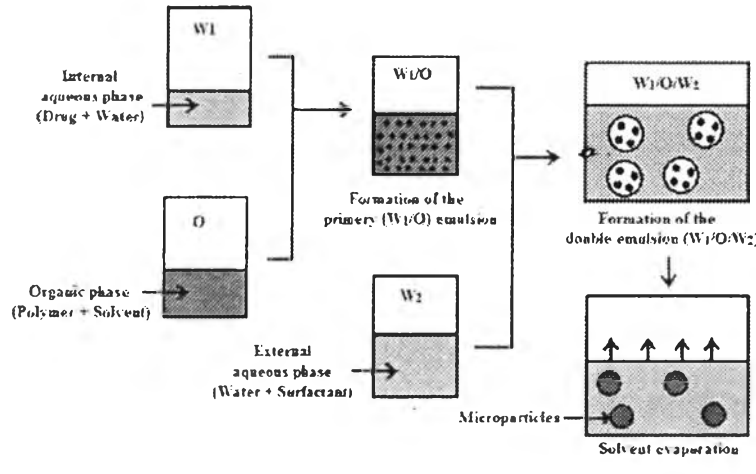

Figure 2.1 The double emulsion solvent evaporation method.

2.2.4 <u>The Factors affect Surface, Structure Morphology, Loading Efficiency and Encapsulation Efficiency</u>

2.2.4.1 Molecular Weight of Poly (D, L-lactic-co-glycolide)

The molecular weight of poly (D, L-lactic-co-glycolide) is a factor for a release behavior of the prepared polymeric microparticles of ovalbumin. The rate of release of ovalbumin from polymeric microparticles decreased with an increase in molecular weight of PLGA. The blending of low molecular weight (M_w) and high molecular weight (M_w) PLGA on microparticles prepared by double emulsion solvent evaporation technique effecting on loading efficiency. The loading efficiency increased significantly when high molecular weight (M_w) PLGA (RG 506) was mixed with low molecular weight (M_w) PLGA (RG 502) at a ratio of 1:7. However, this formulation also showed significantly high burst release, which implies that a major portion of the loading drug was deposited near the surface (Graves *et al*, 2004).

2.2.4.2 Stabilizer

Stabilizer is a crucial key for the encapsulation efficiency of pBC 264 in microparticles. The encapsulation efficiency was very low (<20 %) when the inner aqueous phase did not contain any stabilizing agent. However, it was improved to about 41 % by the addition of ovalbumin used as stabilizer of the inner emulsion (Blanco-Prieto *et al*, 1997). The ovalbumin loaded PLGA microparticles were prepared using water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method by the different of external aqueous phase surfactant. PVP stabilized microparticles exhibited higher protein loading, about 8.2 %w/w, compared to PVA stabilized microparticles, about 4 %w/w. The use of PVP instead of PVA to prepare microparticles also resulted in reduction in the initial burst release of ovalbumin. Since the PVP was more soluble in organic polymer solution than PVA, The PVA stabilizer molecules could be expected to be confined to the surface of the microparticles formed in the water-in-oil-in-water (w/o/w) technique. Moreover, the diffusion of PVP molecules from the external aqueous phase increased the viscosity of the droplets of polymer solution and consequently improved the protein loading and delivery characteristics (Rafati *et al*, 1997). When increasing the concentration of emulsion stabilizer (PVA), a high stable double emulsion was obtained which increased the encapsulation efficiency. The use of PMMA polymers for controlled delivery of diclofenac sodium was investigated. PMMA coated

microparticles were prepared by a modified water-in-oil-in-water (w/o/w) emulsion solvent evaporation method using sodium alginate as a matrix material in the internal aqueous phase. The microparticles were spherical with diameters ranging from 213.45 to 308.60 µm and entrapment efficiency from 28.71 % to 72.16 % (Giri et al, 2013).

2.2.4.3 pH

The pH values of the internal aqueous phase can affect encapsulation efficiency. The encapsulation efficiency (90 %) was observed when pH of the internal aqueous phase was basic (pH 8 corresponding to the optimal solubility of the pBC 264) and when the pH of the external aqueous phase was acid (pH 2.5 corresponding to a lower solubility of pBC 264) (Coombes, Yeh et al. 1998). The highest encapsulation efficiency was achieved when the internal aqueous phase was maintained at pH 6. The ophylline loaded enteric-coated microparticles were prepared using the water-in-oil-in-water (w/o/w) solvent evaporation technique for targeted oral drug delivery. Initially, the cellulose acetate phthalate (CAP) was used as a pH sensitive polymer for the preparation of microparticles. The desired release pattern was achieved but not the encapsulation efficiency. Then, the CAP was replaced by a novel synthesized pH sensitive PMMA copolymer (Dalmoro *et al*, 2010).

2.2.4.4 Concentration of Polymer Matrix

The concentration of polymer matrix also has the effect on the encapsulation efficiency because the increasing matrix material improved the viscosity of internal aqueous phase minimizing the leaching of the drug into external aqueous phase thus the drug entrapment efficiency increased consequently. The PLGA microspheres containing fluorescin isothiocyanate labeled BSA and horseradish peroxidase were prepared by using the water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method. The prepared microcapsules were spherical and more than 90 % encapsulation efficiencies (Cohen *et al*, 1991).

2.2.4.5 Stirring Rate

The stirring rate is the point on encapsulation efficiency. The encapsulation efficiency was reversely related to the stirring rate. The particle size of microspheres was not affected by stirring time period and almost 6.0-7.0 µm. The

microspheres prepared with stirring time period between 30 and 90s has shown the drug-loading efficiencies to be almost 20 %. The longer stirring over 90 s caused the decrease of the loading efficiency (Ito *et al*, 2012).

2.2.5 Dexamethasone Phosphate (DEX)

Dexamethasone phosphate (as sodium) is a white or slightly yellow, very hygroscopic, crystalline powder. It is odorless or has a slight odor of alcohol. Dexamethasone phosphate (as sodium) is soluble 1 in 2 of water, slightly soluble in alcohol, practically insoluble in chloroform and ether, and very slightly soluble in dioxan.

Dexamethasone is indicated for therapy of the diseases such as Collagen diseases, Pulmonary disorders, Blood disorders, Rheumatic diseases, Skin diseases, Gastrointestinal disorders, Oedema, Eye disorders, Neoplastic states, and Endocrine disorders.

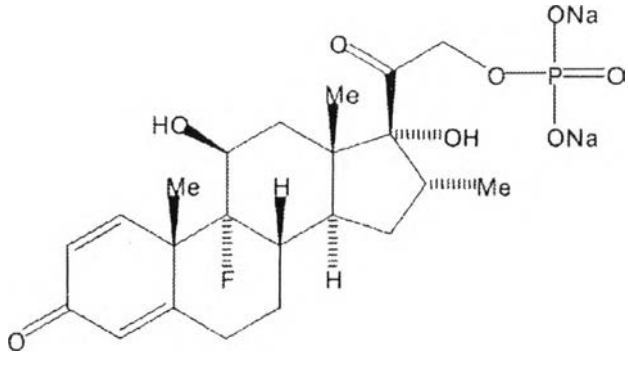

Figure 2.2 The chemical structure of dexamethasone phosphate (as sodium).

2.2.6 Application for Drug Carrier

2.2.6.1 Pharmaceutical

Anticancer drugs, Matsumoto and his coworkers prepared cisplastin loaded multireservoir type microparticles by s/o/w double emulsion method using PLA and PLGA as polymers. The resultant microparticles showed almost 100 % encapsulation efficiency. In vitro release study indicated that the

release of cisplastin continued for 45 days without initial burst (Matsumoto *et al*, 1997).

Anti-inflammatory drugs, Nagda and her coworkers prepared Aceclofenac loaded microparticles by using O/W/O double emulsion method. The entrapment efficiency was in the range of 32-47 %. Aceclofenac released from these microparticles was slow and extended over longer periods of time with fickian diffusion control (Nagda, *et al*, 2009).

Antibiotics drugs, Gentamicin is the most important aminoglycoside antibiotics and is used widely for the treatment of serious infections. Biodegradable PLA and/or PLA/PEG copolymer disk implants containing gentamicin sulfate were obtained by compression of microparticles prepared by a w/o/w double emulsion process for local treatment of bone infection. It has been observed that the biodegradable PLA/PEG gentamicin delivery system had a potential for prophylaxis of post-operative injection.

Steroidal drugs, Budesonide is a glucocorticoid and widely used for the treatment of asthma. The budesonide loaded porous PLGA microparticles were prepared by a water-in-oil-water (w/o/w) double emulsion method with ammonium bicarbonate as the porogen (Oh et al, 2011). The drug loading efficiency in the microparticles was about 60 %, and drug was released from the microparticles in a sustained rate for 24 h in vitro. It has been observed that the budesonide, which loaded microparticles, significantly, reduced bronchial hyper-responsiveness of asthmatic mice.

Anti HIV agents, Zidovudine is an antiretroviral drug used for the treatment of HIV/AIDS. The zidovudine encapsulated ethyl cellulose microparticles were prepared by w/o/o double emulsion solvent diffusion method (Das *et al*, 2006). The resultant microparticles showed that 32–55 % entrapment efficiency with free flowing properties.

2.2.6.2 Biopharmaceuticals

Bovine serum albumin (BSA) is a serum albumin protein derived from cows. The BSA loaded PLGA microparticles were prepared using a modified w/o/o double emulsion phase separation method (Zhang *et al*, 2005). Microparticles with highly yield (>80 %) and entrapment efficiency (>90 %) were

produced using petroleum ether containing 5 % (w/v) span 80 as coacervating agent.

Lysozyme is glycoside hydrolase that damages the bacterial cell walls. Lysozyme loaded PLA microparticles were prepared by combining Shirasu porous glass (SPG) membrane emulsification technique and water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method (Liu *et al*, 2005). The lysozyme loaded PLGA microparticles using a water-in-oil-in-water (w/o/w) double emulsion technique. It has been observed that the protein aggregation and inactivation were minimized by carefully selecting excipients efficient in stabilizing lysozyme against the major stress factors of w/o/w encapsulation (Perez-Rodriguez *et al*, 2003).

Insulin is poorly absorbed via the intestinal membrane because of extensive proteolytic degradation by intestinal enzymes and suffers from insufficient membrane permeability due to high molecular weight and low lipophilicity. Insulin loaded PLA-PEG microparticles were prepared using a water-in-oil-in-water (w/o/w) double emulsion solvent evaporation technique for controlled parenteral drug delivery (Sheshala *et al*, 2009). The insulin loaded PLGA microparticles by water-in-oil-in-water (w/o/w) and s/o/w double emulsion method for providing basal insulin level for an extended period of time. It has been observed that the insulin loaded PLGA microparticles controlled in vitro absorption of insulin to maintain the basal insulin level for a long period and the delivery system was biocompatible (Khan *et al*, 2011).

2.3 Objectives

2.3.1 Synthesize superparamagnetic iron oxide (Fe_3O_4) PLGA nanoparticles.

2.3.2 Study the effect of the initial concentration of dexamethasone sodium phosphate (DEX) on the loading efficiency and release behavior.

2.3.3 Compare loading efficiency and release behavior between low and high molecular weight of superparamagnetic iron oxide (Fe_3O_4) PLGA nanoparticles.