CHAPTER II
LITERATURE REVIEW

2.1 Magnetic Nanoparticles (MNPs) in Drug Delivery

Nanoscale structure and materials (e.g., nanoparticles, nanofibers, nanotube, and nano wiers) have been explored in many biological applications such as biological separation, biosensing, molecular imaging, and/ or anticancer therapy because their functions differ drastically from their counterparts and novel properties. Especially, their high surface volume, improved solubility, and multifunctionality to open a large of new possibilities for biomedicine (Kluchova *et al.*, 2009)

Nanovecters able to both carrier drug and cancer detected have also been interested, mainly using the outstanding superparamagnetic properties of iron oxide nanoparticle. Superparamagnetic Iron Oxide Nanoparticle (SPIONs) are usually used as magnetic nanoparticles since their superparamegnetic properties, depending on nanosize, make them magnetic in an external magnetic field as a presence, but no magnetic resonance remains after remover external field. Magnetic nanoparticles offer additional attractive possibilities in biomedicine since their magnetic properties allow them to be manipulated by an external magnetic field gradient, further chemical improving and biological drug delivery strategies, in addition to magnetic resonance imaging (MRI) contrast enchantment detection of localization of their sites (Challa S. *et al.*, 2006). Magnetic nanoparticles (MNPs) added components for specific feature, ligand such as targeting agent, therapeutic agent, and permeation enhancer can incorporated within these nanostructures (Figure 2) to used as *in vivo* cell (Sun *et al.*, 2008).

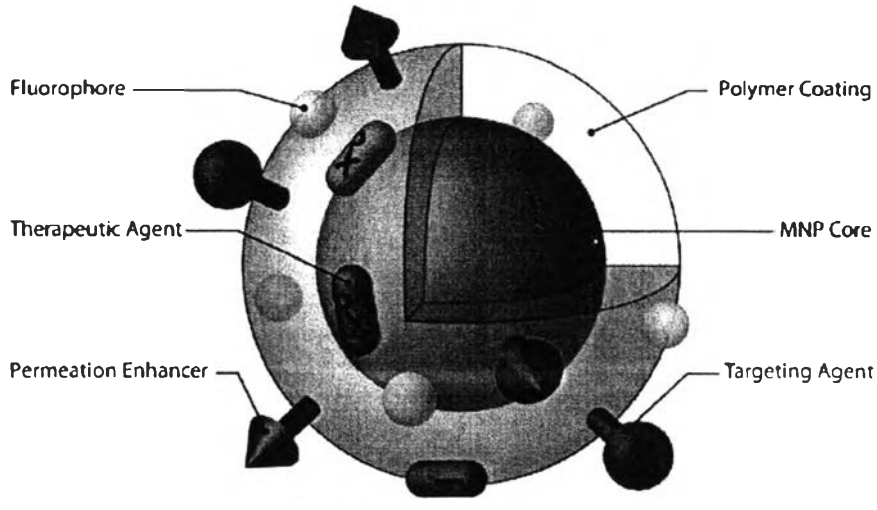


Figure 2.1 MNP with various ligands to enable multifunctionality from a single nanoparticle platform (Stark *et al.*, 1998).

SPIONs biocompatible have been developed *in vivo* biomedical application, mostly in magnetic resonance imaging, and have been only preclinically evaluated in the tissue-specific therapeutic agent release (Challa S. *et al.*, 2006). Previous experiments using this approach have been performed by superparamegnetic iron oxide (ferrite) evaluated as contrast agent for magnetic resonance (MR) imaging, doses ranging from 10 to 50 mumol/kg were administered intravenously to 15 partient, some positive effect were obtained, increase the number of hepatic lessons detected and reduce the threshold size for detection with the standard pulse sequence techniques. Degradation from the liver of ferrite was demonstrated as early as 12 hour after injection suggestion that lack of chronic toxicity observe in animal (Stark *et al.*, 1998).

Xu *et al.* (2007) developed a new type of engineered nanomaterials, FePt@CoS_2 yolk–shell nanocrystals as a potent agent to kill the cancer cells, synthesized by the mechanism of the Kirkendall effect when FePt nanoparticles serve as the seeds. The nanocomposites have directly to specific cell and resulting in the high cytotoxicity to cancer cell are shown in the Figure 2.2, when the cellular uptake, the multiple white small bud occurred on the surface of cell indicated that the cancer cells were almost dead.

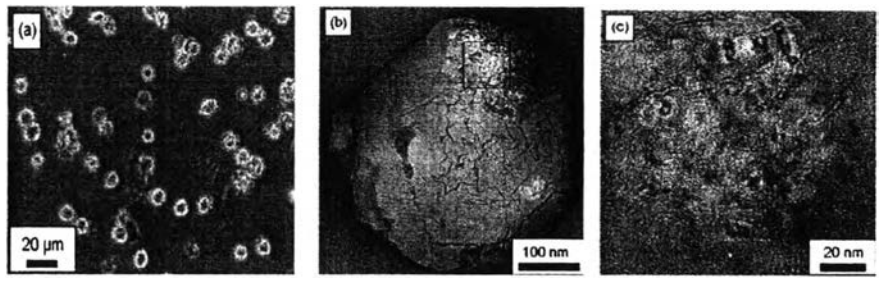
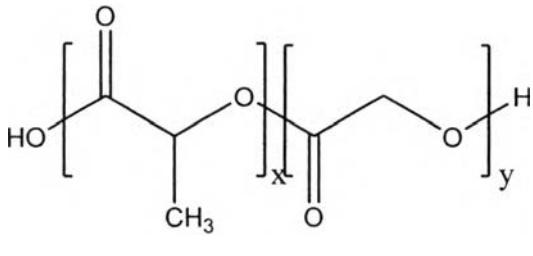


Figure 2.2 Transformation of FePt@CoS_2 yolk—shell nanoparticles after incubated with HeLa cells; (a) the optical image of HeLa cells incubated with 5 μg/mL of FePt@CoS_2 nanoparticles for 3 days, (b) a representatiive TEM image of mitrocondria from HeLa cells being incubated with FePt@CoS_2 yolk—shell nanoparticles for 3 days, (c) magnefied TEM image.

2.2 PLGA in Drug Release

PLGA or Poly(D,L-lactide-co-glycolide) is a copolymer of lactide and glycolide (Figure 1), also known as lactic acid-glycolic-acid copolymer that have success in the clinic as degradable sutures and drug delivery matrices (Ijeoma *et al.*, 2006). PLGA is synthesized by ring opening co-polymerization of two different monomers, the cyclic dimmers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. The catalysts used in the preparation of this include tin(II) 2-ethylhexanoate, tin(II) alkoxides, or aluminum isopropoxide. The polymerized successive in the linked together of unit monomer (glycolic or lactic acid) by ester linkages, thus yielding a linear, a product as aliphatic polyester (Astete *et al.*, 2006).



x = Number of units of Lactic acid
y = Number of units of Glycolic acid

Figure 2.3 Poly(D,L-lactide-co-glycolide)

Depending on the ratio of copolymer, each of homopolymer is crystalline and degrades relatively slowly, but the copolymers tend to have less crystallinity, and thus they degrade more rapidly. One composition used in drug delivery microparticle in the mixture ratio of 50:50 lactide and glycolide, but the other ratio have been used, especially in sutures, where the strength of the suture and its retension during early time period of wound healing is a more important factor than degradation rate (Ijeoma *et al.*, 2006)

The degradation of PLGA is a hydrolytic mechanism of its ester linkages in the present of water. It has been show that the monomer ratio of copolymer is related to the required time for degradation of PLGA: the higher content of glycolide units, the lower time required for degradation. An exception to this rule is the copolymer with 50:50 unit monomers which exhibits the faster degradation (about two months) (Astete *et al.*, 2006). However, adding a more hydrophobic comonomer, such as poly(carpolactone) PCL, which slows down the degradation rate by its lower water uptake (Ijeoma *et al.*, 2006)

PLGA has been successful as a biodegradable polymer because by products from PLGA degradation in the body are natural substrate for cell metabolic processes, and sidestep the need for removing spent particles from the body (Cu *et al.*, 2008). The major application include resorbable sutures, drug delivery systems and orthopaedic fixation devices such as screws, rods and pins (Pathiraja *et al.*, 2003), antiadhesive coating, and tissue-engineering scaffolds (Kwon *et al.*, 2005). PLGA-chitosans cationic have been used for DNA delivery (Ravi *et al.*, 2004).

In 2007, Matthew M. Arnold *et al.* used the large porous PLGA microparticles encapsulated nanoCipro, immiscible oil is added to PLGA dissolved in dichloromethane containing a nanosuspension of ciprofloxacin. Following the double emulsion, organic solvent is extracted to produce oil/PLGA/drug particles. The oil is then extracted to produce large porous particles. In the result, ciprofloxacin was distributed evenly throughout the particle matrix (Figure 2.4).

The duration of unencapsulated nanoCipro dissolution was ~5days, which was extended to 2–4weeks depending on the microparticle size and structure (figure 2.5). The release kinetics generally followed trends suggesting that the release of

ciprofloxacin may be attributable to nanoCipro dissolution coupled with the effective diffusion of ciprofloxacin through the degrading polymer matrix.

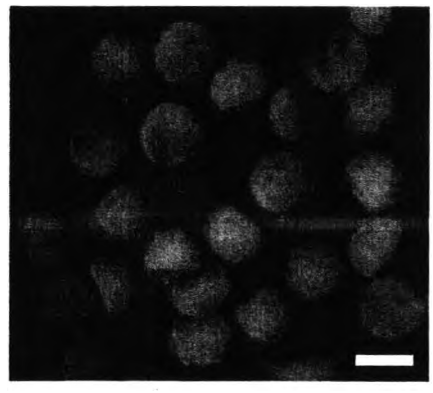


Figure 2.4 Laser scaninng confocal microscopy was used to detect the distribution of ciprofloxacin within large porous PLGA microparticle (Scale bar 10 μm).

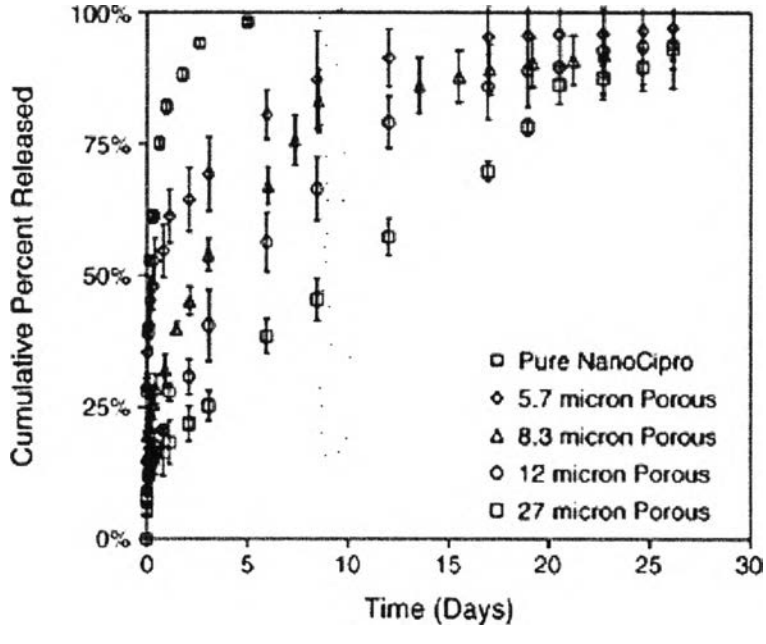


Figure 2.5 Release kinetics of encapsulated nanoCipro from porous porous microparticles.

2.3 Preparation of Nanoparticles for Delivery System

There are several method to prepare nanoparticles for delivery system. Three main methods are single emulsion process, double (multiple) emulsion process, and phase separation (coacervation).

2.3.1 Single emulsion process

The single emulsion process involves oil-in-water (O/W) single emulsion. At the first, the polymer dissolved in a water immiscible, low boiling point organic solvent (dicloromethane (DCM) most comonly used). Then the drug added to the polymer solution to produce a solution or dispersion of the drug particles. This polymer-solvent-drug dispersion/ dispersion is then emulsified in a larger volumn of water containing an emulsifier (such as poly(vinyl alcohol) (PVA)) to form an oil-in-water emulsion. The emulsion is then removed solvent by either extraction or evaporation process to harden the oil droplets. The obtained solid nanospheres are then washed and collected by sieving, filtration, or centrifugation. And these are dried under the condition or are lyophilized to give final free flowing injectable nanosphere partlcles (Jain, 2000).

2.3.2 Double (multiple) emulsion process

This is a water-in-oil-in-water (W/O/W) double emulsion and is best suited to encapsulated water-soluble drugs like protiens, vaccines, and peptides, unlike the o/w emulsion which is suitable for water-insoluble drug like steroids (Rajeev A., 2000). The typical w/o/w double emulsion process consists of four steps; (1) primary emulsification: an solution of bioactive agent (inner water phase, W_1) is emulsified into organic solution containing the biopolymer (oil phase, O), (2) re-emulsification: the primary emulsion is further emulsified into a larger volumn of aqueous phase containing stabilizer (outer water phase, W_2) to form $W_1/O/W_2$ double emulsion, (3) solidication: the organic sovent is removed by extraction or evaporation and then solid nanoparticles are formed, and (4) separation and purification: nanoparticles are collected by centrifugation and then lyophilized (Fan T. M. *et al.*, 2003).

2.3.3 Phase separation (coacervation)

This process consists of additioning a third component to polymer solution in an organic solution for decreasing the solubility of the encapsulating

polymer. At this point, the process yields two liquid phase (phase separation), the polymer containing coacervate phase and the supernatant depleted in the polymer. The polymer is first dissolved in an organic solution. The water-soluble drug are dissolved in water and dispersed in the polymer solution (W/O emulsion). The water-unsoluble drug are either solubilized or dispersed in the polymer solution. Then an organic nonsolvent is added to the polymer-drug-solvent system with stirring which gradually extracts the polymer solvent resulting in phase separation of the polymer and form very soft coacervate droplets which entrap the drug. This system is transferred to a large of another organic solvent to harden the nanodroplets and form the final nanospheres which are collected by sieving, filtration, washing, or centrifugation, and are finally dried (Jain, 2000).

2.4 Biodegradable Encapsulate of Magnetic Nanoparticle

The magnetic nanoparticles have attracted a great deal of attention due to their potential use as magnetic materials as well as magnetic resonance imaging (MRI) comtrast agent. However, poor penetration depth, poor diffusion of the released drug from release site (Sun *et al.* 2008), and relatively high toxicity of magnetic nanoparticles restricts the use of these materials in human beings (Seung-Lee *et al.*, 2004). To reduce the shortcomings, encapsulatation of the magnetic nanoparticle with polyester such as PLGA has been used because of the compatible and biodegradable properties as well as low toxicity and improves the ability of penetration and diffusion of drug released. The various methods can be used to incorporate nanoparticle in the biodegradable polymer with respect to their in vivo compatibility.

In 1996, Müller *et al.* subsequently prepared magnetite-loaded PLA/PLGA particle by mechanically mixing bare magnetic aggregate with the polymer matrix. Disperse the magnetite aggregates in ethanol and then incorporated in polyester polymers with the transition temperature (approx. 55 °C) under stirring, homogenous dispersion of the magnetite in the polymer matrix was perform. However, the investigator encountered difficulties in controlling both particle shape and size as well as magnetite exposure at the particle surface.

In 2005, Lee *et al.* reported magnetite/PLGA nanoparticle with good superparamagnetism made by an emulsification-diffusion method using aqueous ferrofluide. The encapsulation process involves the formation of a conventional oil-in-water emulsion which consists of an oil phase containing a stabilizer. The subsequent addition of oil phase into aqueous media forms an oil-in-water-type emulsion and then solvent diffuses out and dissolves at the external phase. Nevertheless, the hydrophobic nature of the biopolymer matrix (i.e. PLA, PLGA) did not permit encapsulation of large amounts of bare magnetite suspension due to its hydrophilic nature, ultimately resulting in aggregation of nanosized magnetite and poor magnetizations (0.075 emu/g with 0.18% volume fraction) of the composite particle.

In 2005, Marchais *et al.* incorporate SPIONs into PLGA using an emulsification-diffusion technique where SPIONs were in the oil phase via the use of fatty acid-coated SPIONs; however, their dispersion were poor and their PLGA particles showed an inconsistent distribution of SPIONs (Ronald A. *et al*).

In 2005, Okassa *et al.* investigated the magnetite entrapment efficiency by comparing aqueous magnetite suspensions and organic magnetite suspension showed more efficient entrapment as compare to the aqueous magnetite suspension. The organic magnetite suspension was obtained by redispersing the magnetite precipitate in oleic/ethanol solution in which oleic acid acted as a surfactant. In this context, the development of sub-micron PLGA biodegradable/biocompatible magnetic carriers with high magnetic loading, but remains an ongoing bioengineering challenge, without compromising in vivo applicability.

In 2007, Wessel *et al.* showed the SPIONs with a hydrophobic coating incorporate to PLGA particles by using emulsification-diffusion technique (oil-in-water-in-oil emulsion). The use of oleic acid-coated SPIONs and their suspension in the first oil phase to form a water-in-oil emulsion. Then this phase is added to an aqueous phase containing a stabilizer and emulsified to form a water-in-oil-in-water emulsion. Overtime the solvent diffuses out creating nanoparticles with the SPIONs and bioactive agent incorporated inside the polymer. In the result, encapsulation of the nanoparticles does not seem to affect the super paramagnetic nature of the SPIONs, as evidenced by the slope in the magnetization data in Figure 2.6.

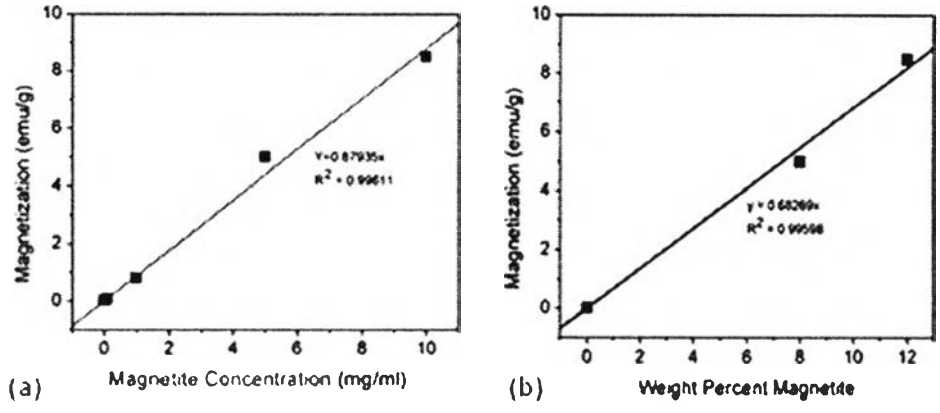


Figure 2.6 (a) magnetization of PLGA particles plotted vs. magnetite concentration in the organic phase prior to emulsification, (b) magnetization of PLGA particles plotted vs. weight percent of magnetite in PLGA particle.

TEM image showed the well dispersion, SPIONs were evenly distributed throughout the PLGA particle, both with respect to individual particles as well as respect to many particles (Figure 2.7)

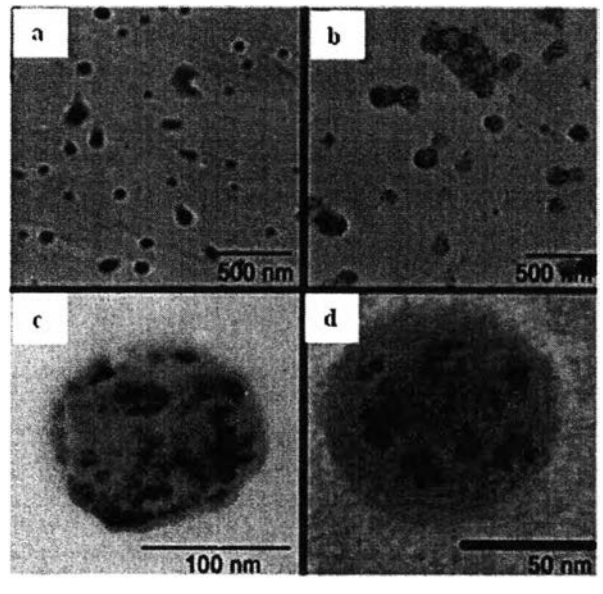


Figure 2.7 TEM images. (a) PLGA particles, (b) magnetite encapsulated PLGA, (c) PLGA particles with magnetite made from a 5 mg/m solution, (d) PLGA particles with magnetite made from a 1 mg/m solution.

Although many work successfully used the emulsification-diffusion method to produce sub-micron PLGA particle with encapsulated iron oxide nanoparticles that used oleic acid coated with magnetite nanoparticles disperse in the oil phase, there is no systematic studies carried out to determine the suitable ratio of encapsulates PLGA with magnetite nanoparticles. The obtained particles were further characterised by transmission electron micrographs (TEMs), Dynamic light scattering (DLS), vibrating sample magnetometer (VSM), and thermogravimetric analysis (TGA).