CHAPTER IV
SYNTHESIS AND ENCAPSULATION OF MAGNETITE NANOPARTICLES IN PLGA

4.1 Abstract

Hydrophobic superparamagnetic iron oxide nanoparticle (SPION) encapsulate within poly(D,L-lactide-co-glycolide) (PLGA) particles were prepared by the w/o/w emulsion technique using PVA as a surfactant. The oleic acid surface of the SPIONs and their suspension were to ensure sufficient entrapment in the PLGA matrix at the first oil phase. The amounts of PLGA entrapment were varied (5 mg/ml, 15 mg/ml, 30 mg/ml, 45 mg/ml, and 60 mg/ml) to investigate the efficiency of composite SPION-PLGA nanoparticles for drug loading and drug release. The obtained composite particles were almost spherical, with the magnetite particles ranging in diameter from 6 nm to 17 nm, individually dispersed into the PLGA particles, as confirmed by transmission electron microscopy (TEM). Sizes of the composite particles varied from 300 nm to 400 nm, measured by dynamic light scattering (DLS), while the zeta potential remained about -25 mV, independently of SPION presence. Saturation magnetization was measured by a vibrating sample magnetometer (VSM), the magnetization properties were found to be proportional to the amount of magnetite in PLGA. The high magnetizations (36 emu/g to 50 emu/g) under external magnetic field demonstrated that the biodegradable composite nanoparticles were suitable as a potential platform for a model of magnetic drug carrier for targeted delivery

Keywords: Nanoparticles/ Magnetite/ Superparamagnetic iron oxide/ Double emulsion mathod

4.2 Introduction

Magnetic nanoparticles (MNPs) have been used in various biological and biomedical applications such as biomagnetic separation, hyperthermia treatment, and drug targeting [1]. They act as magnetic resonance imaging (MRI) contrast agent, which already received some clinical acceptance. Development of molecular and cellular imaging aims for visualizing the disease-specific biomarker at the molecular and cellular levels, which led to prevalent recognition the magnetic nanoparticle as MRI contrasts [2]. Superparamagnetic iron oxide nanoparticles (SPIONs) have ability as an important material to enhance magnetic resonance contrast. For example, maghemite iron (II) nanoparticles attached to bentonite clay surface will result in a highly efficient negative oral contrast agent for MRI diagnostics of the adjacent abdominal areas and small bowel including pancreas and choledoch [1]. Although not yet capable of reaching levels of safety and efficacy for regulatory approval, pre-clinical studies indicated that some of the short coming of magnetic drug targeting, such as their toxicity [3], poor penetration depth, and diffusion of the released drug from the disease site [4], can be overcomed by improvement in magnetic targeted carriers design. Furthermore, encapsulate MNPs as a drugs carrier that mean of real-time monitoring of drug delivery is an area of intense interest.

The use of external magnetic field with SPIONs as effectively direct to specifics tissue in the body, encapsulated magnetic nanoparticle with a layer of biodegradable polymer or evenly distributed in the matrix of polymer have also been reported and widely research to attain controlled release with respect to both time and location [5]. The coated SPIONs with biocompatible polymer aim to form stable non-toxic aqueous dispersion of magnetic nanoparticle. The covered polymers have resulted in reduce aggregation problem of the uncoated MNPs and lower its toxicity [6]. These biodegradable and biocompatible include hydrophobic polyester such as poly(D,L-lactide-co-glycolide) (PLGA), Poly(D,L-lactide) (PLA), and poly(glycolide) (PGA) [7].

Poly(D,L-lactide-co-glycolide) (PLGA) are significant for tissue engineering applications and delivery system because they are biodegradable, biocompatible, non-toxic [8], high regarding for safety biodegradability in the body

and approval by the U.S. Food and drug Administration (FDA) [9]. When microspheres formulated, drug depot was formed as advantage by direct injection in a tissue from which the slowly released of drug occurred. PLGA microparticles encapsulating a decoy oligodeoxynncleotide (ODN) against nuclear facto-κB (NF-κB) able to release decoy ODN at a constant rate for about 40 days [10]. PLGA encapsulation of SPIONs was used to study the distribution of nanoparticle in chinchilla cochleae, the nanoparticle were distributed in the chinchilla round window membrane [11]. However, microsphere size has a significant effect on the efficient of drug loading and drug release rate and can potentially be varied to design a drug delivery system controlled with desired release profile. Mathematical modeling provide the effect of mean diameter of PLGA microsphere on drug release, Piroxicam-loaded PLGA microsphere having mean size from 13.5-76 μm, result in drug initial release rate decreased with an increase in microsphere size increase [12].

Several methods have been developed to prepare magnetic polymeric nanoparticles including water-in-oil (w/o) single emulsion, water-in-oil-in-water (w/o/w) double emulsion, coaservation [13], inverse microemulsion polymerization, and miniemulsion polymerization [14]. The main requirement is that the all the magnetite nanoparticles are transfered evenly into the resulting polymeric nanoparticles. The most interesting one is w/o/w double emulsion that best suited to encapsulated water-soluble drug like vaccines, proteins, and peptides [13].

In this work we encapsulated SPIONs with PLGA microparticles by using an emulsification diffusion technique. This technique forms a water-in-oil emulsion at the first which the SPIONs and the polymer are in the oil phase and dissolving the hydrophobic bioactive agent in the water phase [5]. Also in the study, characterization of the resulting effect of amount encapsulates nanosphere on encapsulation efficiency were carried out.

4.3 Experimental

4.3.1 Materials

Poly(D,L-lactide-co-glycolide) (50:50 lactide/glycolide, Mw 40–75 kDa) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyvinyl

alcohol (PVA) (Mw 30–70 kDa), ferrous chloride tetrahydrate (99%), feric chloride hexahydrate (99%), Oleic acid (90%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Chloroform was purchased from ACI Labscan. Ammonium hydroxide (25 wt% NH_3 in water) was purchased from Suksapanpanich Co.

4.3.2 Hydrophobic Magnetite

The hydrophobic magnetite is prepared by the following procedure: 24.3 g of $FeCl_3.6H_2O$ and 12 g of $FeCl_2.4H_2O$ are dissolved in 50 ml of deionized water under nitrogen gas. Then, 40 ml of NH_4OH (25%) are added at 70-80 °C. And, Oleic acid (OA) (40%, w/w of formed magnetite) is added dropwise during 10 min and heat for 30 min. The black lump-like gel is separated by magnetic decantation and cooled to room temperature and then washed several times with deionize water and acetone to remove the excess oleic acid

4.3.3 Preparation of PLGA Nanoparticles

Nanosphere containing SPIONs are prepared by double-emulsion technique. The desired concentration of SPIONs (5 mg/ml) is dispersed into 1 ml of chloroform. Then the amount of PLGA (5, 15, 30, 45, and 60 mg/ml) was dissolved in the solution, and emulsified 200 microliters of deionised water in the PLGA/SPIONs/chloroform solution by sonification on ice for 1 min to form a water-in-oil emulsion. This first emulsion is emulsified again by adding 6 ml of deionised water containing 2% PVA. The resulting w/o/w emulsion is sonificated on ice again for 5 min and stirred for ~24 h to allow solvent evaporation and nanoparticles formation. The PLGA nanoparticles are isolated by centrifugation at 15,000 rpm for 30 min at 4 °C, washed four times in demonised water to remove any access PVA and SPIONs, and dispersed in 1 ml of deionised water in 2 ml cryotubes. The PLGA nanoparticles then lyophilized for 2 days and stored until use

4.3.4 Hydrophobic Magnetite Nanoparticles Characterization

4.3.4.1 Fourier Transform Infrared Spectra (FTIR)

FTIR spectra of the magnetite nanoparticles were collected on a FT-IR spectrometer (Thermo Nicolet, Nexus 670). The sample powders were ground with KBr and compress into a pellet whose spectra were record. A drop of neat OA was on the ZnSe plate, and the spectra were recorded as a reference.

4.3.4.2 Transmission Electron Microscopy (TEM)

The particles morphology, size and structure of OA-coated Fe_3O_4 were determined by Hitachi transmission electron microscopy. The aqueous dispersed was drop-case onto formvar-copper grid. The sample was dried prior to placing it in the TEM instrument for analysis.

4.3.5 Characteristic of PLGA/SPION Particles

4.3.5.1 Size and Zeta Potential

Dynamic light scattering was used for particles size and surface charge (zeta potential) measurements (Malvern Zetasizer Nano Series). Typically, a sample of 1.5 ml was place in a cuvette. The measurements were performed at 25 °C. The viscosity and refraction index of the continuous phase were set equal to those specific to water. Zeta potential measurements were made with the disposable capillary cell with a volume of 1 ml.

4.3.5.2 Morphology

Particle size and the qualitative state of aggregation of the SPIONs inside PLGA nanoparticles were determined by transmission electron micrographs (TEMs) (H-7650 Hitachi transmission electron microscope). The composite particles were droped on the formvar-coated copper grid and place to TEM measurement.

4.3.5.3 Magnetization Measurement

The magnetization of encapsulated SPIONs was measured by vibrating sample magnetometer (VSM, LakeShore 7404) at room temperature.

4.3.5.4 Thermogravimetric analysis (TGA)

The magnetite loading in the PLGA nanoparticles was determined by thermogravimetric analysis (TGA). The sample was placed in the furnace of thermogravimetric analyzer (DuPont, model TGA 2950) using an aluminum pan under air at 30 °C to 800 °C with increments of 20 °C/min.

4.4 Results and Discussion

4.4.1 Hydrophobic magnetite

The presence of OA on the surface of Fe_3O_4 was measured by FTIR. Figure 4.1 shows the typical FTIR spectrum of pure oleic acid (4.1a), and the iron oxide nanoparticles coated with OA (4.1b). In curve (4.1a), two bands at 2925 and 2855 cm^{-1} were attributed to the asymmetric CH_2 stretch and the symmetric CH_2 stretch, respectively. The peak at 1713 cm^{-1} was derived from the existence of the C=O stretch, and the band at 1285 cm^{-1} was presence the C–O stretch. The O–H in-plane and out-of-plane bands appeared at 1465 and 936 cm^{-1}, respectively. In the curve (4.1b), the asymmetric CH_2 stretch and the symmetric CH_2 shifted to 2924 and 2853 cm^{-1}, respectively. The shifted to lower frequency region indicated that the layer of surfactant molecules was absorbed to the solid surface, the hydrocarbon chains surrounding the nanoparticles were in a closed-packed, crystalline state. It was noted that the band of C=O stretch, which was present at 1713 cm^{-1} in the curve (4.1a) was almost absent in the curve (4.1b) and there are appear in two new bands at 1592 and 1531 cm^{-1}, which were characteristic of asymmetric COO^- and the symmetric COO^- stretch, instead. This result can be explained the bonding of the carboxylic acids on the surface of the nanoparticles (Figure 4.2). The result was consistence with oleic acid coated SPIONs. Further studied carried out is to characterize the size of the hydrophobic SPIONs using TEM.

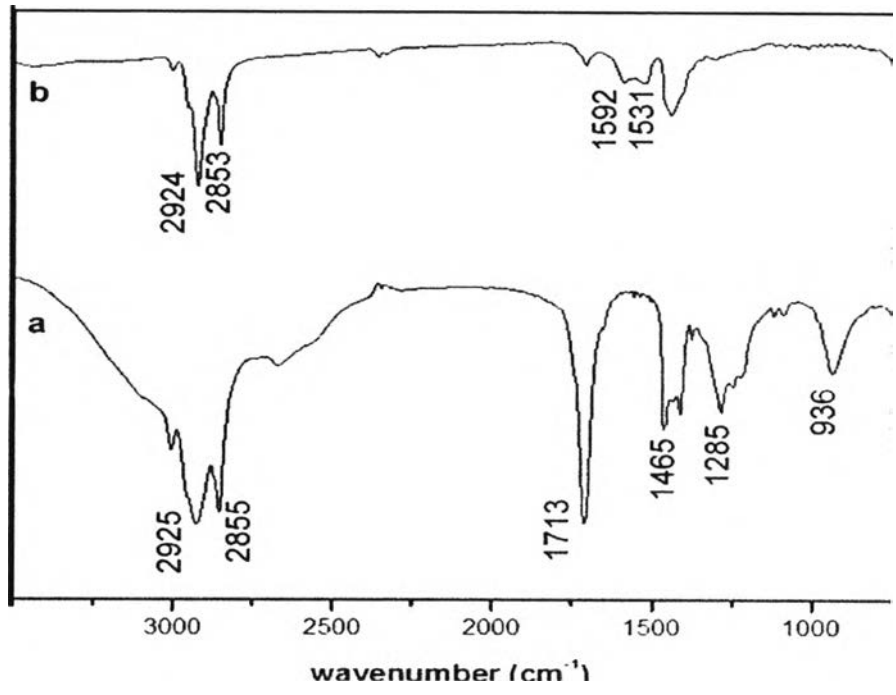

Figure 4.1 FTIR spectra of (a) pure oleic acid, (b) Fe_3O_4 coated with oleic acid.

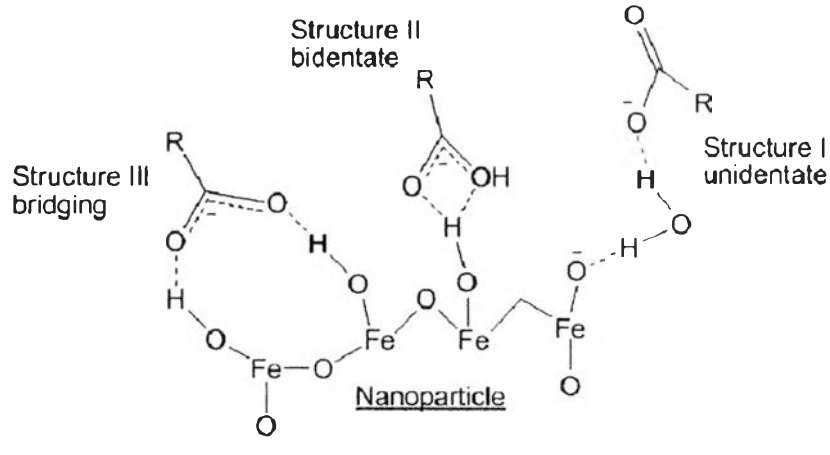

Figure 4.2 Three possible interactions between oleic acid and Fe_3O_4 (from Lazare and Hervé).

Figure 4.3 shows TEM image of magnetite nanoparticles coated with oleic acid. These sizes of faceted particles were raging from 7 to 16 nm that suitable for embed inside the polymer. The obtained oleic acid coated was later encapsulated

with PLGA to study physical properties of nanocomposite materials (PLGA nanoparticles)

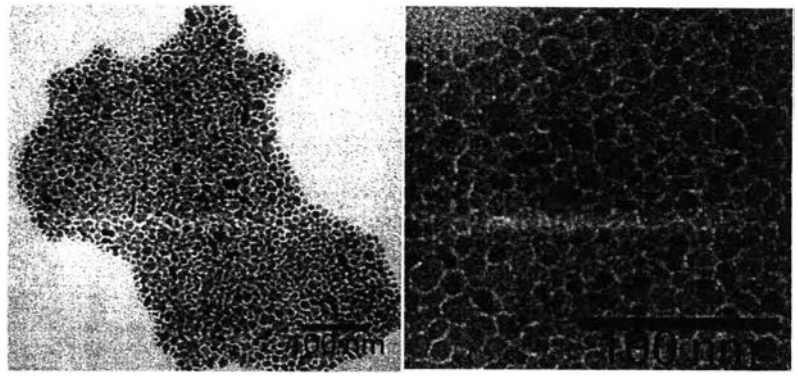

Figure 4.3 TEM image of OA-coated magnetite.

4.4.2 PLGA Nanoparticles

4.4.2.1 Morphology of PLGA Nanoparticles

The size and morphology of the PLGA nanoparticles were determined by TEM. Figure 4.4 show the spherical shape of the particles with an approximate diameter around 200 nm. The surface was primarily smooth on the PLGA nanoparticles without magnetite inside (Figure 4.4b), although some roughness could be identified in certain areas of some spheres. Figure 4.4c and 4.4d were the magnetite encapsulated with 30 mg/ml and 5 mg/ml, respectively. These images obviously demonstrate that the PLGA nanoparticles were well and high magnetite incorporated with magnetite equally distributed in the polymer rmatrix. Although some agglomerated were occurred but not surprised for high magnetite loading.

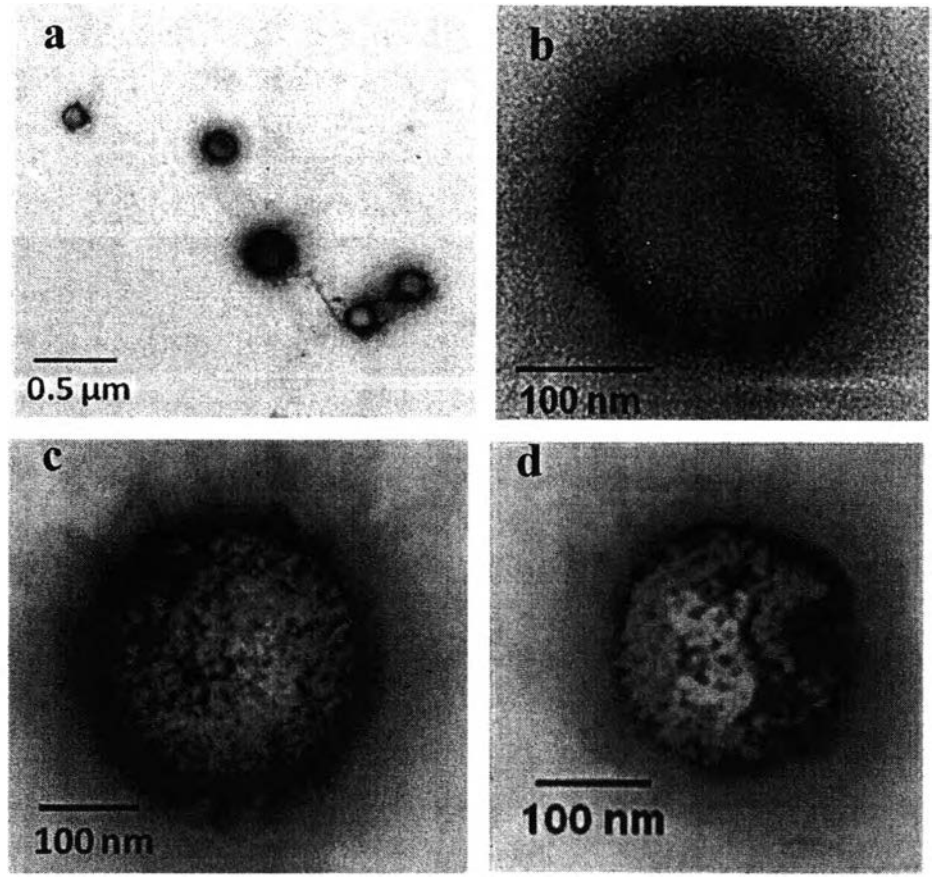

Figure 4.4 TEM images of synthesized PLGA nanoparticles; (a) PLGA nanoparticles, (b) pure PLGA, (c) 30 mg/ml PLGA with magnetite 5 mg, (d) 5 mg/ml PLGA with magnetite 5 mg.

4.4.2.2 Size and Zeta Potential

The size and zeta potential of the nanospheres from dynamic light scattering was shown in Table 4.1, these spheres had an average size varied from 300 nm to 400 nm and there were independence on amount of encapsulated polymer (PLGA) used. The hydrophobic magnetite appeared to range in size from 7 to 16 nm yet incorporation the polymer spheres did not significantly affect the size of PLGA particles. In comparison with the size obtain with TEM imaging, the hydrodynamic diameter is probably larger because the PVA is expected to have a much smaller configuration dried on TEM grid versus in water [5]. The zeta potential was about -25 mV, surface charge did not difference between the bare magnetite and

magnetite-loaded PLGA particles and not difference when increased amount of polymer encapsulation.

Table 4.1 Size and surface charge of PLGA nanoparticles prepared with different amount of PLGA

Weight of PLGA/ 5 mg OA-SPIONs	Mean diameter (nm)	Zeta potential (mV)
pure PLGA	358 ± 43	-26.8 ± 0.9
5 mg	379 ± 38	-25.5 ± 2.3
15 mg	380 ± 19	-24.6 ± 1.5
30 mg	400 ± 13	-25.9 ± 0.3
45 mg	382 ± 52	-25.5 ± 0.7
60 mg	394 ± 50	-24.1 ± 0.5
Avg.	382 ± 35	-25.5 ± 1.5

4.4.2.3 Magnetization of PLGA Nanoparticles and Magnetic Content

The weight percent of the magnetite nanoparticles incorporated inside PLGA nanoparticles was determined by TGA. The magnetite nanoparticles and PLGA nanoparticles were heated from 300 °C to 800 °C to make sure that all the PLGA and oleic acid were removed. The remaining weight of the samples will be equal to the weight of magnetite nanoparticles in the PLGA nanoparticles. From the results, the weight of magnetite nanoparticles encapsulated in PLGA is about 56.11-62% (Figure 4.5). With the hydrophobic magnetite, magnetite content/particle is higher when compared with the particles prepared from hydrophilic magnetite (56.11–62% w/w vs. 1%) [15] and also in our study magnetite content/particle is higher than previous studies using hydrophobic magnetite (13.5% w/w [15], and 40% w/w [16]. Moreover, the weight ratio of OA-SPION/PLGA is an important factor in the incorporated magnetite inside the polymer. It could be

suggested that the magnetite content decrease when increased the amount of encapsulated polymer. The highest incorporated OA-SPION inside the PLGA particles was obtained when the weight ratio of OA-SPION/PLGA was 1:1 (5 mg/ml PLGA with 5 mg SPIONs) and was decreased when increased the amount of polymer. However, the magnetite content not much change at large amount of polymer (30 mg/ml PLGA, 45 mg/ml PLGA, and 60 mg/ml PLGA). The solubility of a polymer in the solvent had effect on the encapsulated magnetite in the polymer and also related to the content of magnetite was incorporated. The large amount of PLGA was not completely dissolved in the CDM solvent. Some chains of PLGA that were dissolved could be further encapsulating magnetite, but non-dissolved chains could not encapsulate magnetite and phased out when collected the particles.

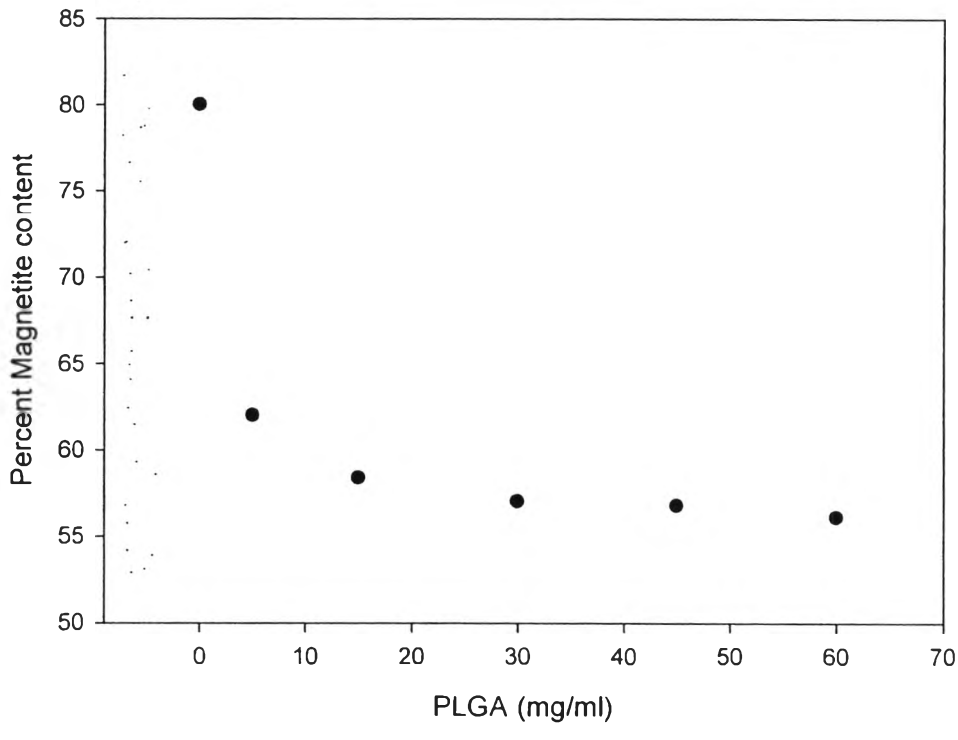

Figure 4.5 Percent of magnetite incorporated inside PLGA plotted vs. amount of polymer that used with 5 mg of magnetite.

Magnetic properties of PLGA nanoparticles were determined by VSM which used to determine super paramagnetic behavior of the nanoparticles. The magnetization curves of the PLGA nanoparticles were shown in Figure 4.6. These

nanoparticles exhibit superparamagnetic behavior which no hysteresis loop, indicating that these particles had the single domain magnetic nanoparticles remained [15]. The highest saturation magnetization of nanocomposite was 44.950 at 5 mg/ml PLGA and was decreased when increased the PLGA concentration (Figure 4.7A). The saturation magnetization of magnetic PLGA nanoparticles was smaller than the bulk of OA-SPION. This significant loss in magnetitzation of the magnetic material is come from the presence of polymer matrix [15]. However, the saturation magnetitezation is higher than previously studied [3] and if plot between the maximum magnetitzation of each concentration and the weight percent of magnetite in PLGA particles (Figure 4.7B), the saturation magnetization was increase linearly with the content of magnetite inside the polymer. The saturation magnetitzation did not go down indicated that the SPIONs did not oxidize with encapsulation; the oxidation was occurred in the miniemulsion method described by Lanfester and Ramirez [5]. It demonstrated that the PLGA nanoparticles have sensitive to move toward an external magnetic field that seemed to have sufficient magnetization for targeting drug carrier.

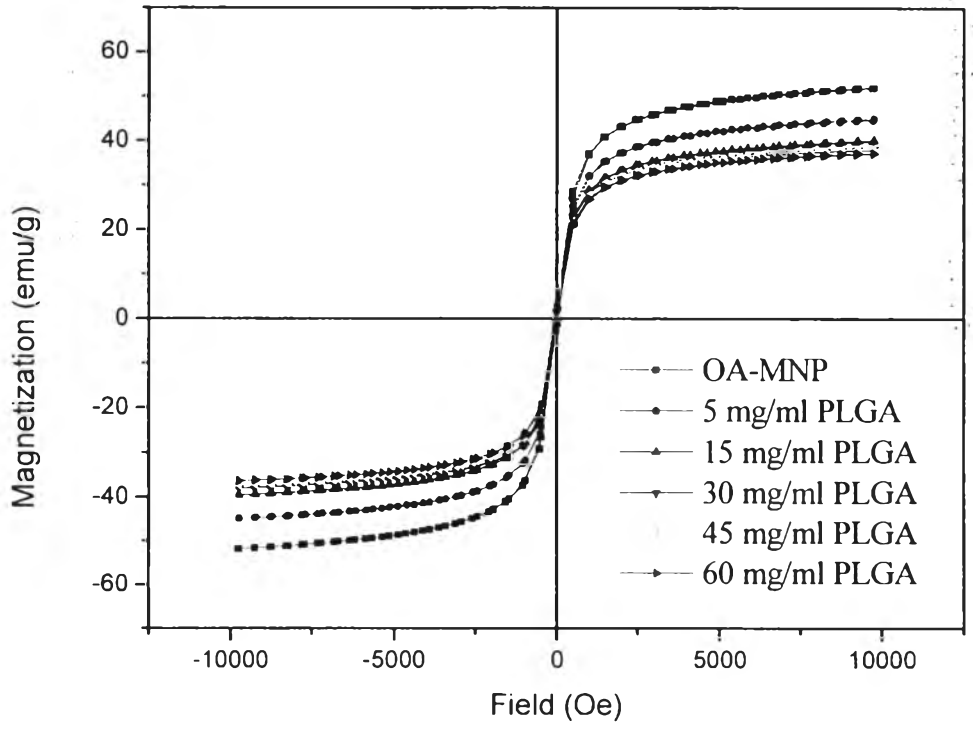

Figure 4.6 Magnetization of PLGA nanoparticles versus magnetic field with magnetite incorporated inside for different amount of PLGA.

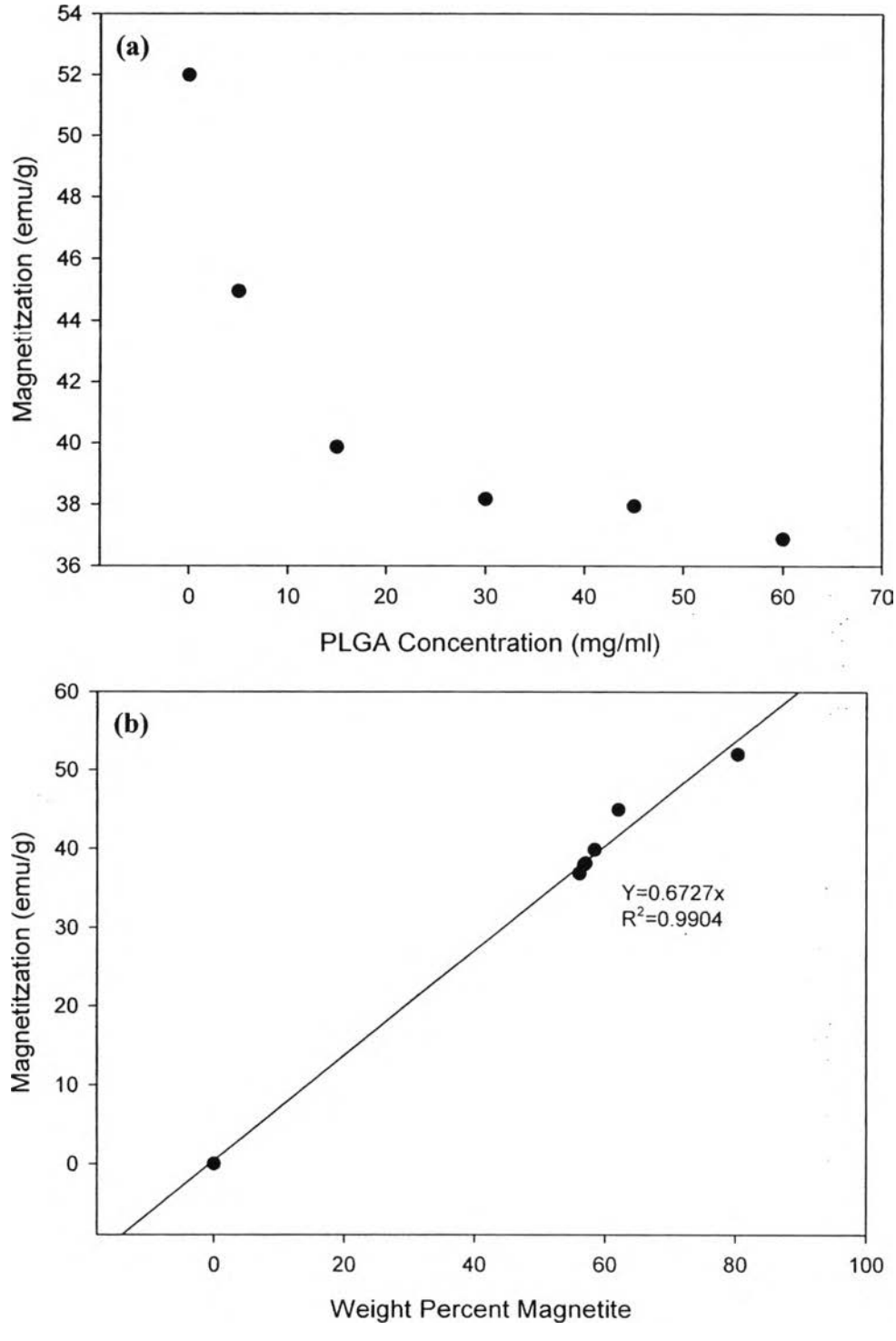

Figure 4.7 (a) magnetization of PLGA nanoparticles plotted versus OA-SPION/PLGA weight ratio, (b) magnetization of PLGA nanoparticles plotted versus weight percent of magnetite in PLGA particles.

4.5 Conclusions

Biodegradable poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles containing superparamagnetic iron oxide nanoparticles were successfully synthesized by a double-emulsion method that used the oleic acid-coated magnetite nanoparticles dispersed in the first oil phase led to SPIONs were equally distributed throughout the PLGA particles. Increasing the amount of encapsulated polymer (5mg, 15mg, 30mg, 45mg, and 60mg of PLGA/5mg of magnetite) did not significantly affect the particles size (300 nm to 400 nm) and surface charge (about -25 mV). The weight of magnetite incorporated in the PLGA was readily controlled by adjusting the amount of encapsulated PLGA, and amount incorporated is conversing with amount of encapsulated polymer. Saturation magnetizations were proportional to the magnetite loaded in polymer matrix with high magnetite loading up to 52 – 62 wt. %.

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