CHAPTER III
METHODOLOGY

3.1 Material

PLGA (50:50 lactide/glycolide, M_w 5 kDa) was purchased from DURECT Co. (Cupertino, CA, USA) and. PLGA (50:50 lactide/glycolide, M_w 40- 75 kDa) was purchased from Sigma Chemical Co. Dexamethasone phosphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyvinyl alcohol (PVA) (M_w 30-70 kDa), ferrous chloride tetrahydrate (99 %), ferric chloride hexahydrate (99 %), and oleic acid (90 %) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Chloroform was purchased from ACI Labscan (Thailand). Ammonium hydroxide (25 wt% NH_3 in water) was purchased from Suksapanpanich Co (Thailand). All reagents were used as received.

3.2 Synthesis of Hydrophobic Magnetite

24.3 g of $FeCl_3 \bullet 6H_2O$ and 12 g of $FeCl_2 \bullet 4H_2O$ were dissolved in 50 ml of deionized water under nitrogen gas. Forty millilitre of NH_4OH (25 %) was then added at 70–80 °C. Oleic acid (OA) (40 %, w/w of calculated magnetite amount) was added dropwise during 10 min and heated for 30 min. The black lump-like gel was separated by magnetic decantation and cooled to room temperature and then washed several times with deionized water and acetone to remove excess OA.

3.3 Synthesis of Dexamethasone Phosphate (DEX) Loaded on Superparamagnetic Iron Oxide (Fe_3O_4) PLGA Nanoparticles

Dexamethasone sodium phosphate (DEX) loaded on non-porous superparamagnetic iron oxide (Fe_3O_4) PLGA nanoparticles were prepared by double-emulsion technique. 1 milligram per millilitre of magnetic nanoparticles was dispersed into 1 ml of chloroform. PLGA (27 milligram per millilitre) was then dissolved in the solution, and 200 µl of deionized water containing DEX (1, 2, 3 and

4 milligram per millilitre) were emulsified in the PLGA/DEX/Fe_3O_4/chloroform solution by sonification on ice for 1 min to form a water-in-oil emulsion. This first emulsion was emulsified again by adding 6 ml of deionized water containing 2 % PVA. The resulting w/o/w emulsion is sonicated on ice again for 5 min (56 % amplitude for sonicated at the first oil phase and 96 % amplitude at the second emulsion with probe size of 1½ inch and 3 inch vessel size in diameter) and stirred for ~24 hours to allow solvent evaporation and nanoparticles formation. PLGA nanoparticles were isolated by centrifugation at 15,000 rpm for 30 min at 4 °C, washed four times with deionized water to remove any access PVA and Fe_3O_4, and were dispersed in 1 ml of deionised water in 2 ml cryotubes. The PLGA nanoparticles were then lyophilized for 2 days and stored in a desiccator prior to use.

3.4 Characterization of OA-coated Magnetic PLGA Nanoparticles

3.4.1 Characterization of OA-coated Magnetite

FTIR spectra of the magnetite nanoparticles were collected on a FTIR spectrometer (Thermo Nicolet, Nexus 670). The powder samples were ground with KBr and compressed into a pellet whose spectra were record. A drop of OA was placed on a ZnSe plate, and the spectrum was recorded and used as a reference.

3.5 Characterization of Fe_3O_4 Loaded in PLGA Nanoparticles

3.5.1 Particle Sizing and Zeta Potential

The hydrodynamic particle size and zeta potential were determined by a Malvern Zetasizer Nano series. The measurements were performed at 25 °C. Viscosity and refraction index of the continuous phase were set to values appropriate for water.

3.5.2 Transmission Electron Microscopy (TEM)

Particle size and the qualitative state of aggregation of the Fe_3O_4 inside PLGA nanoparticles were determined by TEM (H-7650 Hitachi transmission electron microscope). Composite particles were dropped onto formvar-coated copper

grid and placed in the TEM for analysis.

3.5.3 Magnetization Measurement

Magnetization of encapsulated Fe_3O_4 was measured by vibrating sample magnetometer (VSM, LakeShore 7404) at room temperature.

3.5.4 Thermogravimetric Analysis (TGA)

Magnetite loading in the PLGA nanoparticles was determined by a thermogravimetric analyzer (TGA, DuPont, model TGA 2950). Samples were placed in an aluminum pan and subsequently heated from 30 to 900 °C at a rate of 20 °C/min in air.

3.5.5 High Performance Liquid Chromatography (HPLC)

The concentration of dexamethasone was determined by using HPLC. The analytical column was an Octdecyl C18 column, 5-micron bead size with column dimentions 4.6 x 150 mm. The mobile phase consisted of water: methanol (30: 70 (v/v)). The flow rate was set at 1 mL/min. The total run of HPLC analysis was 15 min.

3.5.6 Drug Loading

20 mg of particle dropped into 2 ml of dichloromethane and then 5 ml of PBS was added into the mixing solution. The mixing solution was votexed at 2500 for 1 min; afterward, it was spitted again at 1000 rpm for 2 hours at room temperature. The drug loading method is adapted from (Xu *et al*, 2008). Drug content was determined by comparing with the standard curve of dexamethasone, which was acquired from dexamethasone solutions in PBS. The drug loading and drug encapsulation efficiency was calculated as follows:

Drug Content in PLGA (%)

$$\text{DL (\%)} = \frac{\text{weight of drug}}{\text{weight of drug+PLGA+SPION}} \times 100$$

Encapsulation Efficiency (%)

$$\text{EE (\%)} = \frac{\text{acture drug encapsulate}}{\text{theoretical drug encapsulated}} \times 100$$

3.5.7 In Vitro Release Study

20 mg of sub-micro particles was suspended into test tubes containing 25 ml of phosphate-buffered saline (PBS, pH 7.4) at 37 °C. The test tubes were shaken by skanking water bath for 30 days. At appropriate day, 1 ml of supernatant was collected and 1 ml of fresh PBS was added back to the test tubes. The concentration of dexamethasone was determined by using HPLC.