

CHAPTER III EXPERIMENTAL

3.1 Materials

3.1.1 Polymer Chemicals

- Poly(D,L-lactide-co-glycolide) ((50:50): lactide/glycolide, 1.13 dL/g viscosity)
- Poly vinyl alcohol (PVA) (MW 30-70 kDa)
- Ferrous chloride tetrahydrate (99%)
- Feric chloride hexahydrate (99%)
- Oleic acid (90%)

3.1.2 Solvents

- Ammonium hydroxide (25 wt% NH₃ in water)
- Chloroform
- Deionised water

3.2 Equipments

1. Fourier Transform Infrared Spectroscopy (FTIR)
2. Dynamic light scattering (DLS)
3. Transmission electron micrograph (TEMs)
4. Vibrating sample magnetometer (VSM)
5. Thermogravimetric analysis (TGA)

3.3 Methodology

3.3.1 Hydrophobic Magnetite

The hydrophobic magnetite was prepared by the following procedure: 24.3 g of FeCl₃.6H₂O and 12 g of FeCl₂.4H₂O were dissolved in 50 ml of deionized water under nitrogen gas. Then, 40 ml of NH₄OH (25%) were added at 70-80 °C.

Oleic acid (OA) (40%, w/w of formed magnetite) was added dropwise for 10 min and heated for 30 min. The black lump-like gel was separated by magnetic decantation and cooled to temperature and then washed several times with deionized water and acetone to remove the excess oleic acid.

3.3.2 Preparation of PLGA Nanoparticles

Nanosphere containing SPIONs were prepared by double-emulsion technique. Initially, the desired concentration of SPIONs (5 mg/ml) was dispersed into 1 ml of chloroform. Then the amount of PLGA (5, 15, 30, 45, and 60 mg) 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 initial emulsion was emulsified again by adding 6 ml of deionised water containing 2% PVA. The resulting w/o/w emulsion was sonicated on ice again for 5 min and stirred for ~24 h to allow solvent evaporation and microparticles formation. The microparticles were isolated by centrifugation at 15,000 rpm for 30 min at 4 °C, washed four times in deionised water to remove any excess PVA and SPIONs, and dispersed in 1 ml of deionised water in 2 ml cryotubes. The PLGA particle then lyophilized for 2 days and stored until use.

3.3.3 Hydrophobic Magnetite Nanoparticles Characterization

3.3.3.1 *Fourier Transform Infrared Spectra (FTIR)*

FTIR spectra of the magnetite nanoparticles were collected on a Bruker spectrometer. The sample powders were ground with KBr and compressed into a pellet whose spectra were recorded. A drop of neat OA was placed on the ZnSe plate, and the spectra were recorded as a reference.

3.3.3.2 *Transmission Electron Microscopy (TEM)*

The particles morphology, size and structure of OA-coated Fe₃O₄ were determined by Hitachi transmission electron microscopy. The aqueous dispersed was drop-cast onto formvar-copper grid. The sample was dried prior to placing it in the TEM instrument for analysis.

3.3.4 Characteristic of PLGA/SPION particles

3.3.4.1 *Size and Zeta Potential*

Dynamic light scattering was used for size and surface charge 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.

3.3.4.2 Morphology

Particle size and the qualitative state of characteristics of the SPIONs were studied from transmission electron micrographs (TEM, H-7650 Hitachi transmission electron microscope). The composite particles were drop on the formvar-coated copper grid and place to TEM measurement.

3.3.4.3 Magnetization Measurement

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

3.3.4.4 Magnetite Loading

The amount of SPIONs in the PLGA particles were measured 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.