# CHAPTER III

# EXPERIMENTAL

## 3.1 Materials

Chicken eggshell was obtained from Petchburi soi 5 Market, Bangkok. Polyvinyl alcohol (Mw~108,000g/mol and 99.7 mole% hydrolysis) was purchased from Polysciences. Nitric acid (65%w/w, AR grade) was purchased from RCI Labscan. Glutraldehyde (50%w/w aqueous solution) was purchased from Sigma-Aldrich. Orthophosphoric acid (85%w/w) and ammonium hydroxide (35%w/w) were purchased from Merck Chemicals. Distilled water was used throughout the experiment.

## 3.2 Equipments

3.2.1 Centrifugal ball mill S100/ Ball Mill Machine

The collected chicken eggshell was grinded by ball mill machine, using 300 rpm for 1 h.

3.2.2 Carbolite Furnace/ Furnace Equipment

A furnace was used to prepare calcium oxide from eggshell and hydroxyapatite from calcium oxide. The heating rate used to prepare calcium oxide from eggshell was set at 5 °C/min from 25° to 900 °C and held at 900 °C for 1 h. The heating rate for preparing hydroxyapatite was 10 °C/min from 25° to 700 °C and held at 700 °C for 30min.

3.2.3 LSI Lyophilization/ Freeze Dryer

A freeze dryer was used to fabricate calcium oxide or hydroxyapatite-polyvinyl alcohol hybrid aerogel.

3.2.4 Thermo Nicolet, Nexus 670/Fourier Transform Infrared (FTIR) Spectrophotometer

FTIR spectra were obtained to determine the functional groups of chemical composition of eggshell, calcium oxide, hydroxyapatite, and calcium oxide/ hydroxyapatite-polyvinyl alcohol hybrid aerogels, using an analysis range of 400–4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The sample preparation was carried out by mixing fine powder of a sample with KBr powder.

3.2.5 Perkin Elmer Thermogravimetric analyzer (TGA)

TGA thermograms were analyzed to investigate thermal stability, composition, and mechanism of degradation of eggshell, calcium oxide, hydroxyapatite, and calcium oxide/hydroxyapatite-polyvinyl alcohol hybrid aerogels. Approximately 5 mg of samples were analyzed, using a platinum pan and a temperature range from 30° to 900 °C in dynamic oxygen atmosphere with a flow rate of 80 ml/min and a heating rate of 10 $°C.min^{-1}$.

3.2.6 DMAX 2200 HV/X-Ray Diffractrometer (XRD)

XRD spectra were obtained to determine a phase composition, a fraction of crystallinity, and an average crystalline size. Samples were analyzed using a double-crystal wide-angle goniometry, scanning range of 20°–80° 2θ at a scan speed of 5° 2θ/min, and CuKα radiation (λ = 0.154 nm). Peak positions were compared with the International Center for Diffraction Data Standard (JCPDS) patterns to identify crystalline phases.

3.2.7 Hitachi FE-SEM S4800/Scanning Electron Microscope (SEM)

SEM was used to investigate morphology of eggshell, calcium oxide, hydroxyapatite, and calcium oxide/hydroxyapatite-polyvinyl alcohol hybride aerogels.

3.2.8 Quantachrome Autosorb-1/Surface Area Analyzer (SAA)

The specific surface area, pore volume, and pore diameter of calcium oxide/hydroxyapatite-polyvinyl alcohol hybride aerogels were determined with nitrogen adsorption-desorption by the Brunauer-Emmett-Teller (BET) method on Quantachrome Autosorb-1.

3.2.9 Quantachrome, Ultrapycnometer1000/ Pycnometer

The porosity of calcium oxide/hydroxyapatite-polyvinyl alcohol aerogels was characterized by a pycnometer.

3.2.11 Lloyd instrument/ Universal Testing Machine

Mechanical properties of calcium oxide/hydroxyapatite-polyvinyl alcohol hybride aerogels were determined using compression testing. The test was conducted using cylindrical specimens equipped with a universal testing machine fitted with a 1 kN load cell and a compression rate of 2 mm/min.

## 3.3 Methodology

3.3.1 Synthesis of calcium oxide (CaO) from eggshells via thermal Treatment

Raw eggshell was washed, separated membrane, and dried at room temperature. Eggshell was coarsely crushed by a motar and then finely ground by a ball mill. The obtained eggshell powder was separated by a sieving machine to collect particles less than 180 micron, followed by calcination in a furnace from room temperature to 900 °C with a heating rate of 5 °C/min and held at 900 °C for 1 h. White powder of CaO was kept in a desicator.

3.3.2 Synthesis of hydroxyapatite from calcium oxide via sol gel process

In this study, calcium nitrate ($Ca(NO_3)_2$) solution was first prepared by adding the obtained calcium oxide from 3.3.1 to nitric acid until pH of the mixture became 7. To obtain ammonium phosphate ($(NH_4)_3PO_4$) solution, orthophosphoric acid was slowly added into ammonium hydroxide until the mixture pH became 10. Then, the prepared calcium nitrate solution was slowly added into the prepared ammonium phosphate solution by controlling the mixture pH ~10. The mole ratio of Ca/P was at 1.67. The solution was aged at room temperature in a close system for 24 h to obtain gel, followed by drying at 65 °C for 24 h in an oven. The dried gel was washed with distillated water to obtain white powder which was calcined from 30° to 700 °C for 30 min under air condition using a heating rate of 10 °C/min.

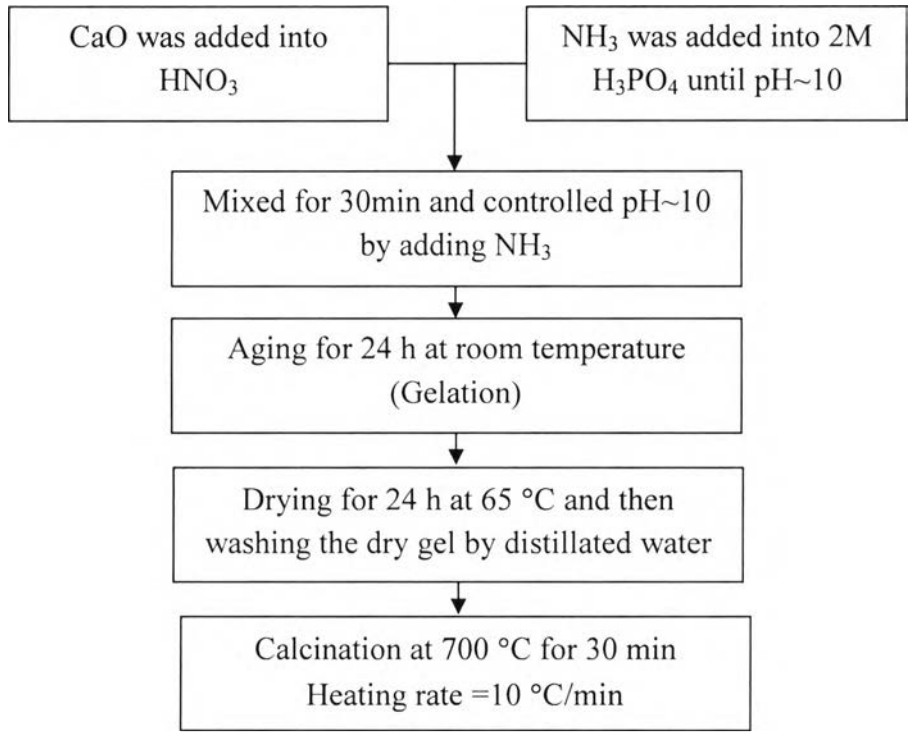


**Figure 3.1** Flow diagram showing synthesis of hydroxyapatite via sol-gel process.

3.3.3 Preparation of calcium oxide/hydroxyapatite - polyvinyl alcohol hybrid aerogel

Polyvinyl alcohol was refluxed in water. Glutaraldehyde acting as crosslinking agent was added into polyvinyl alcohol solution at 70 °C for 30 min, followed by calcium oxide or hydroxyapatite. The mixture was homogenized by a magnetic stirrer. Various the weight ratio of calcium oxide and poly vinyl alcohol (40:60, 30:70 and 20:80) and then finding the optimum properties of weight ratio and following various amount of polyvinyl alcohol in water (3, 3.5, 4, 4.5and 5% weight in water) by fixing the optimum of weight ratio of calcium oxide and hydroxyapatite.. After mixing, the samples became highly viscous solution and were added into a mold before placing into a refrigerator at 4 °C for 24 h, followed by placing into a freeze dryer to eliminate water.