CHAPTER III

EXPERIMENTAL SECTION

3.1. Reagents

Tetraethoxysilane (TEOS) was used as a silica source, while hexadecyltrimethylammonium bromide (CTAB) served as a cationic surfactant. Aqueous sodium hydroxide (NaOH) was used as a catalyst. TEOS and CTAB were obtained from Fluka. Sodium hydroxide (NaOH), nitric acid (HNO$_3$), hydrochloric acid (HCl), hydrogen peroxide (H$_2$O$_2$) and organic solvents were purchased from Merck. Stock solutions of metal ions were prepared from analytical reagent grade Zn(NO$_3$)$_2$·6H$_2$O, Pb(NO$_3$)$_2$, Cd(NO$_3$)$_2$·4H$_2$O and Cu(NO$_3$)$_2$·3H$_2$O. All aqueous solutions were prepared with high purity water obtained with a Milli-Q system (>18 MΩ/cm^3 deionized water from Millipore system, ZMQS5V00Y). 1-Phenyl-3-methyl-4-stearoyl-5-pyrazolone (HPMSP) was synthesized according to Jensen [48].

3.2. Equipments

Powder X-ray diffraction patterns were measured on a Riguku DMAX 2200/Ultima X-ray diffractometer with nickel filtered CuK$_\alpha$ radiation. The BET surface area and pore size distribution were determined by N$_2$ adsorption-desorption isotherm at 77 K using Quantachrome Autosorb-1 nitrogen adsorptometer. A Perkin Elmer AAnalyst 100 atomic absorption spectrometer equipped with Perkin Elmer single element hollow cathode lamps and air-acetylene burner was used for determination of metals. The operational conditions were those recommended by the manufacturers. A HP8453 UV-visible spectrophotometer was used for spectra recording and absorbance measurements. The pH was determined with a Denver pH-meter calibrated againt two standard buffer solutions of pH 4.0 and 7.0.
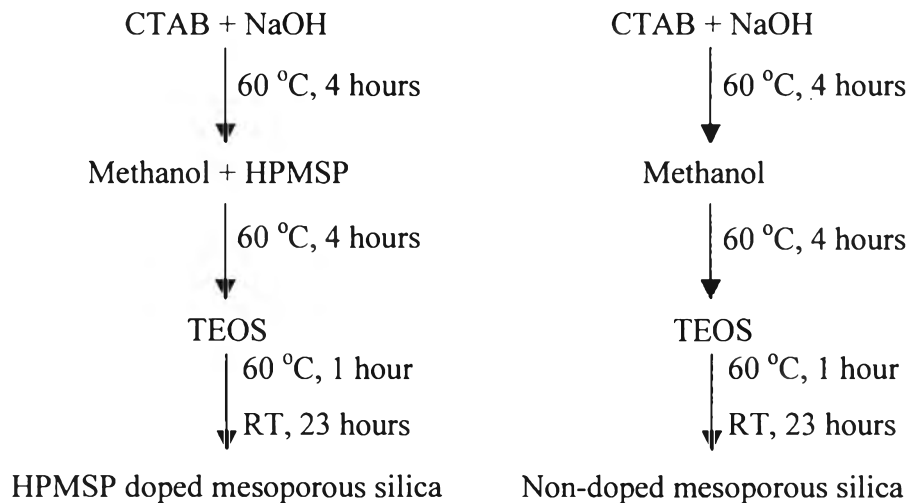
3.3. Experimental

The experiment was separated into three parts. Experiment began with synthesis and characterization of materials followed with the study of the extraction properties. Conditions for effective extraction were optimized with respect to different experimental parameters in both batch and column methods. The parameters including type of metal, pH of sample solution, selectivity of HPMSP doped mesoporous silica to metal extraction and effect of divers ions in metal solution were

investigated by batch method. Various conditions evaluated by column method were amounts of metal ions, concentration and volume of eluent. In the final part, the application of HPMSP doped mesoporous silica to metal extraction from food sample was studied.

3.3.1. Synthesis of materials

The synthesis of HPMSP doped mesoporous silica, based on procedure reported by Boos and co-worker [18], was carried out with the aid of cationic surfactant following this general procedure.

CTAB was dissolved in an aqueous solution of 0.1 M NaOH by stirring at 60 °C for 4 hours to give a clear solution. MeOH and extracting molecules (HPMSP) were then added to the template solution under stirring for 4 hours. After its dissolution, TEOS was added. After further stirring at 60 °C for 1 hour and at ambient temperature for 23 hours, the resulting synthesis gel of composition 1 TEOS : 140 H_2O : 13 MeOH : 0.18 CTAB : 0.06 HPMSP was filtered, washed with 0.001 M HNO_3 and water. The silica was then dried in an oven at 110 °C overnight. The synthesis of non-doped mesoporous silica was also performed using the same procedure. Schematic diagram for the synthesis of both materials were summarized in Figure 3.1.

<div align="center">

CTAB + NaOH   CTAB + NaOH

↓ 60 °C, 4 hours   ↓ 60 °C, 4 hours

Methanol + HPMSP   Methanol

↓ 60 °C, 4 hours   ↓ 60 °C, 4 hours

TEOS   TEOS

↓ 60 °C, 1 hour   ↓ 60 °C, 1 hour
↓ RT, 23 hours   ↓ RT, 23 hours

HPMSP doped mesoporous silica   Non-doped mesoporous silica

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Figure 3.1 Schematic diagram for the synthesis of HPMSP doped and non-doped mesoporous silica.

3.3.2. Characterization of materials

The characterization of materials comprised the determination of organic matter contents in mesoporous silica and the investigation of physical properties of materials such as crystallinity, mesoporosity, surface area and pore size. All characterization procedures were described below.

3.3.2.1. Determination of organic matter contents

The organic matter contents in mesoporous silica was determined by calcination according to the following procedure.

The mesoporous silica after dried at 110 °C was placed in a muffle furnace. The sample was heated from room temperature to 100 °C at a rate of 1 °C/min and held for 1 hour at that temperature, then heated to 540 °C at a rate of 1 °C/min and kept at 540 °C for 10 hours. After calcination process, the sample weight loss was calculated. The thermal profile for calcination is demonstrated in Figure 3.2.
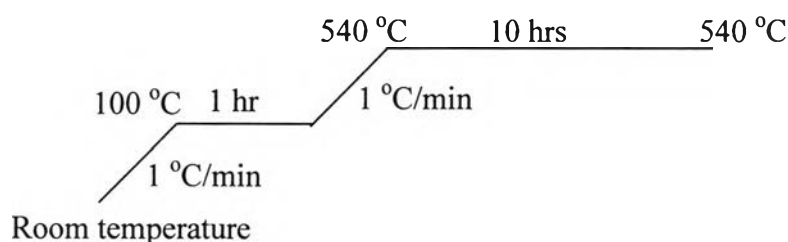

Figure 3.2. The thermal profile for calcination of HPMSP doped and non-doped mesoporous silica.

3.3.2.2. Crystallinity, mesoporosity, surface area and pore size

The crystallinity of materials was determined by X-ray diffraction. The samples were evenly dispersed onto glass slides. Patterns were collected using a nickel filtered CuK_α radiation (λ = 1.5406 Å) at 40 kV, 30 mA. Scattering patterns were collected from 1.0 ° to 10 ° with scan time of 1 min per 2 ° step.

The mesoporosity of materials was determined by N_2 adsorption-desorption isotherms at 77 K. Surface area calculation was made using the Brunaur-Emmet-Teller (BET) equation. Pore-size distribution was calculated using Barrett-Joyner-Halenda (BJH) method.

3.3.3. Extraction properties of materials

The sorption properties of non-doped and HPMSP doped mesoporous silica were studied under both batch and column methods. Various parameters such as types of metal, pH of sample solution and the presence of divers ions in metal solution were studied by batch method. For the column method, the operational variables such as amounts of metal ions, concentration and volume of eluent were optimized. The salts of all metal used for the extraction experiments was metal nitrate unless stated otherwise.

3.3.3.1. Batch method

All batch experiments were performed in triplicate and carried out according to the general procedure unless specified otherwise.

0.2 g of sorbents were added to 25 mL of 200 ppm metal solution. The mixture was stirred for 24 hours at 25 $^\circ$C in thermoregulated bath. The solid was then separated by centrifugation. The amounts of metal in the supernatant solution were determined by flame atomic absorption spectrometry. The metal extraction (mol/kg) was calculated by $N_f = (n_i-n_e)/m$ where N_f is the number of moles of metal extracted on the silica surface; n_i is the initial mole of metal ions in solution; n_e is the number of moles of metal ions in solution at equilibrium; and m is the mass of the silica.

3.3.3.2. Column method

0.7 g of silica was packed in a column (30 mm x 9.5 mm i.d.). The packed column was then washed with water and conditioned with 0.01 M HNO_3. An aliquot of the metal solution was passed through the packed column at a flow rate of 1 mL min^{-1}. The packed column was then washed with deionized water to remove the unextracted metal ions. For the desorption process, the adsorbed metal on the HPMSP doped silica was eluted with acid solution. The concentrations of the metal ions in the solution after passing the packed column and in the eluent were determined by flame atomic absorption spectrometry.

3.3.4. Application of HPMSP doped mesoporous silica to the metal extraction from food sample

As the major aim of this work is to create the new functionalized silica for being as an efficient sorbent in the metal extraction field. Thus, the feasibility of using HPMSP doped mesoporous silica for trace metal analysis in food was demonstrated. Two types of food such as fish and mussel were applied as matrix samples. One of the digestion methods described below was performed prior to the metal extraction process.

<u>Microwave digestion method</u>

A portion of 1.0 g samples was placed into the digestion vessel, then 5 mL of concentrated HNO_3 was added and the vessel were sealed and placed in a six-position pressurized bomb. The sample was mineralized in an Perkin Elmer microwave oven as follows: 5 min at 100 W, 5 min at 600 W and 5 min at 1000 W. After cooling, the digested sample was filtered through a Whatman No.1 filter paper, transferred to a 50 mL of volumetric flask and diluted to the mark with deionized water. The digested solution was then ready for the metal extraction experiment described previously in 3.3.3.1. In addition, a blank solution was treated in the same manner to assess the degree of contamination during the digestion process.

<u>Wet digestion method</u>

6 g of each sample were placed into a Philips beaker and dried in an oven at 60 °C for 30 min and at 100 °C for 3 hours. Then 10 mL of concentrated HNO_3 was added into the sample. The beaker was covered with a watch glass and placed on a hot plate. During the digestion, the concentrated HNO_3 and hydrogen peroxide may be added to the mixture in order to prevent the sample from drying out and to destroy the existing organic matrix. When the sample was completely digested, it was evaporated to near dryness and the solution was filtered through a Whatman No.1 filter paper, transferred to a 50 mL of volumetric flask and diluted to the mark with deionized water. The digested solution was then ready for the metal extraction experiment. In addition, a blank was prepared using similar procedure.