



CHAPTER III

MATERIALS AND METHODS

3.1 Materials

1. Triton X-100® (Fluka)
2. Methylene blue (Lab Scan)
3. Standard ICP Cadmium 1000 ppm in 2-3% HNO₃ (Merck)
4. Standard ICP Copper 1000 ppm in 2-3% HNO₃ (Merck)
5. Dodecylamine 98% (ACROSS ORGANICS)
6. Tetraethoxysilane 98% (ACROSS ORGANICS)
7. 3-aminopropyltriethoxysilane (Fluka)
8. 3-mercaptopropyltrimethoxysilane (Chisso)
9. Dimethyloctylchlorosilane (Fluka)
10. Powder activated carbon
11. Ethyl alcohol absolute (CARLO ERBA)
12. Ammonium nitrate
13. Potassium persulfate (UNI LAB)
14. Potassium nitrate (Merck)
15. Potassium bromide
16. Sodium hydroxide (Lab Scan)
17. Sodium chloride (Lab Scan)
18. Hydrochloric acid 37% (CARLO ERBA)
19. Nitric acid 65% (CARLO ERBA)
20. Potassium dihydrogenphosphate (Riedel-de-Haën)
21. Dipotassium hydrogenphosphate (Riedel-de-Haën)
22. Sodium hydrogencarbonate
23. Sodium carbonate

3.2 Instruments

1. Inductive Couple Plasma Optical Emission Spectroscopy, Varian
2. UV-Visible Spectrophotometer, Thermo Electron Corporation
3. Microwave Digestion and Extraction System, Milestone
4. Shaker, GFL
5. Oven, WTB Binder
6. Benchtop pH meter, HACH
7. Soxhlet Extraction, Isopad
8. Balance 2 digits, Sartorius
9. Balance 4 digits, Sartorius
10. Magnetic Strirrer 3 position, Clifton
11. Magnetic Strirrer 10 position, IKA
12. Hotplate Stirrer, Thermolyne
13. Furnace, CARBOLITE
14. Shaking Water Bath, GFL
15. Water Purification System (15 M Ω), ELGA
16. Water Purification System (18 M Ω), ELGA
17. Autoclave, HIRAYAMA
18. Hood
19. Vacuum Pump, KNF Neuberger
20. Ultrasonic cleaner, Bandelin
21. Laboratory Refrigerator, Puffer Hubbard
22. Desiccators
23. Glass wares

3.3 Adsorbates

3.3.1 Heavy metals

In this studies, used heavy metals in the phosphate buffer pH 3 and 5. They had the many studied and confirmed that in pH more than 5, both of Cd and Cu had opportunity to precipitate.

3.3.1.1 Copper (Cu(II))

Copper is a chemical element with the symbol Cu (Latin: cuprum) and atomic number 29, atomic weight 63.546, group IB. It is a ductile metal with very high thermal and electrical conductivity. Pure copper is rather soft and malleable and a freshly-exposed surface has a pinkish or peachy color. Gold, caesium and copper are the only metallic elements with a natural color other than gray or white. It is used as a thermal conductor, an electrical conductor, a building material, and a constituent of various metal alloys. Distinctive reddish color, specific gravity 8.96, melting point 1083°C, boiling point 2595°C, dissolves in nitric acid and hot concentrated sulfuric acid, dissolve slowly in hydrochloric and dilute sulfuric acid but only when exposed to the atmosphere. The toxicity of metallic copper is very low. However, inhalation of dusts, fumes, mists or salt can cause adverse health effects. Many copper (II) salts are toxic.

3.3.1.2 Cadmium (Cd(II))

Cadmium is a chemical element with the symbol Cd and atomic number 48, atomic weight 112.4, group IIB. The soft, bluish-white transition metal is chemically similar to the two other metals in group 12, zinc and mercury. Similar to zinc it prefers oxidation state +2 in most of its compounds and similar to mercury it shows a low melting point for a transition metal. Cadmium is a relatively abundant element. Becomes brittle at 80°C. Resistant to alkalis; high neutron absorber. Specific gravity 8.642, melting point 320.9°C, boiling point 767°C, soluble in acids, especially nitric and ammonium nitrate solutions. Cadmium is a high toxic metal, especially by inhalation of dust or fumes. It is a known carcinogen (OSHA).

3.3.2 Methylene Blue (MB)

Methylene blue (MB) is a heterocyclic aromatic chemical compound with molecular formula: $C_{16}H_{18}ClN_3S$ in the group of thiazine dye of the quinonoimine, molecular weight 373.9 g/mol, pKa of methylene blue is less than 1 (Elogou *et al.*, 1997). It is a cationic dye. It has many uses in a range of different fields, such as biology and chemistry. At room temperature it appears as a solid, odorless, dark green powder, that yields a blue solution when dissolved in water. The hydrated form has 3 molecules of water per molecule of MB. Methylene blue should not be confused with methyl blue, another histology stain, new methylene blue, nor with the methyl violets often used as pH indicators.

In this studies, used methylene blue in the phosphate buffer between pH 5 and 9. From its properties due to pKa, it can be confirmed that in any pH of this studies methylene blue is always positive charge.

3.3.3 Alkylphenol polyethoxylates (APnEOs)

A commercially available APnEO, Triton X-100s (ICN Biomedicals Inc., TX-100), was used as a model substance of APnEOs. TX-100 is an iso-octylphenol ethoxylate surfactant with an average polyethylene-oxide chain length of 9.5 monomer units and was used as received.

TX-100 had maximum contactable surface area around 1.48 nm^2 , aggregation size 105.7 nm^2 , aggregation diameter 11.6 nm and aggregation density $8.15 \times 10^{15} \text{ aggregate/m}^2$ (Punyapalakul and Takizawa, 2006).

3.4 Preparation of Adsorbents

3.4.1 HMS Synthesis (Punyapalakul P. and Takizawa S., 2004)

Mix 29.6 mol of water with 0.27 mol of dodecylamine and 9.09 mol of ethanol to form as organic template of HMS. Add 1.0 mol of tetraethoxysilane (TEOS) in the mixture and were then mixed under vigorous stirring. The reaction mixture was aged at an ambient temperature for 18 h. The resulting was filtered and air-dried on a glass plate. The product was calcined in air under static condition at 650°C for 4 h to remove organic template.

3.4.2 Synthesis of modified Hexagonal Mesoporous Silicates (HMSs) (Punyapalakul P. and Takizawa S., 2004)

3-aminopropyltriethoxysilane

Mix 50 mol of water with 0.25 mol of dodecylamine and 10.25 mol of ethanol to form as organic template of HMS. Add 1.0 mol of tetraethoxysilane (TEOS) in the mixture and were then mixed under vigorous stirring for 30 min. Then 0.25 mol of 3-aminopropyltriethoxysilane was added in the mixture. The reaction mixture was vigorously stirred for 20 h at ambient temperature and the resulting were filtered and air-dried on a glass plate for 24 h. Residual organosilane and organic template were removed by solvent extraction for 72 h with ethanol.

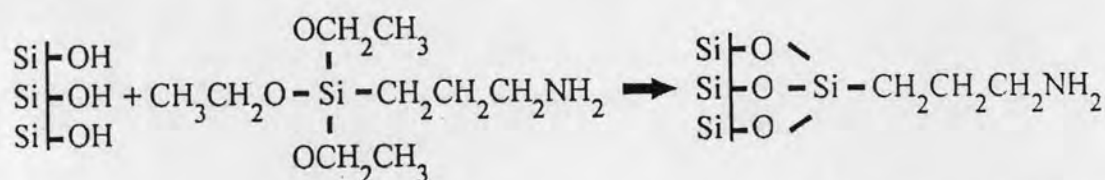


Figure 3.1: Silanization reaction of 3-aminopropyltriethoxysilane and HMS (A-HMS).

3-mercaptopropyltrimethoxysilane

Mix 50 mol of water with 0.25 mol of dodecylamine and 10.25 mol of ethanol to form as organic template of HMS. Add 1.0 mol of tetraethoxysilane (TEOS) in the mixture and were then mixed under vigorous stirring for 30 min. Then

0.25 mol of 3-mercaptopropyltrimethoxysilane was added in the mixture. The reaction mixture was vigorously stirred for 20 h at ambient temperature and the resulting were filtered and air-dried on a glass plate for 24 h. Residual organosilane and organic template were removed by solvent extraction for 72 h with ethanol.

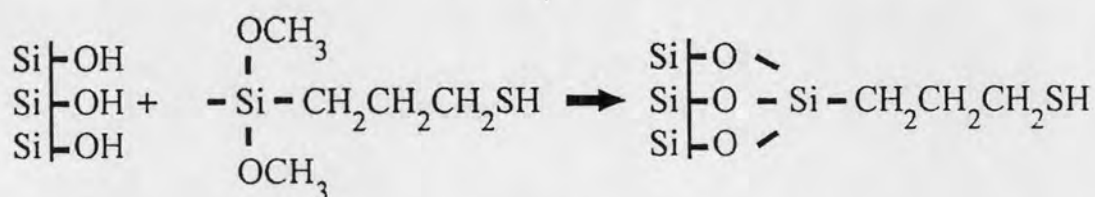


Figure 3.2: Silanization reaction of 3-mercaptopropyltrimethoxysilane and HMS (M-HMS).

Dimethyloctylchlorosilane

Silanization with dimethyloctylchlorosilane, denoted as OD-HMS, was prepared by drying 5 g of HMS at 120°C for 12 hours. Then added dried HMS in the mixture between 0.5 g of dimethyloctylchlorosilane and 30 ml of Toluene. The reaction mixture was vigorously stirred for 24 hrs at room temperature and the resulting were filtered, washed with toluene thoroughly, and dried at 90°C.

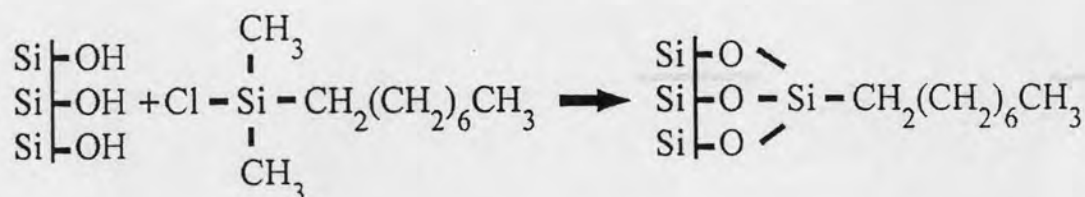


Figure 3.3: Silanization reaction of dimethyloctylchlorosilane and HMS (OD-HMS).

3.5 Physico-Chemical Characterization for Adsorbents

3.5.1 Pore Structure

Pore structures of adsorbents were determined by Powder X-Ray Diffraction (XRD) patterns of synthesized adsorbent. XRD patterns are recorded on Powder Diffractometer; Bruker AXS Model D8 Discover equipped with Cu K α radiation source in the 2 θ range 0.35-6°.

3.5.2 Surface Area and Pore Size

Adsorption-desorption isotherms (BET) using N₂ gas were measured at 77 K on a Surface area and Porosity Analyzer Micromeritic model: ASAP 2020 version 1.04H. The BET specific surface areas were calculated by using adsorption data in the relative pressure from 0.06 to 0.14. Pore size distributions will be calculated from adsorption branches of isotherms using the Barrett-Joyner-Halenda (BJH) formula.

The BET equation can be represented in Equation 9 as below.

$$\frac{p}{v(p_0 - p)} = \frac{1}{v_m c} + \frac{c-1}{v_m c} \frac{p}{p_0} \quad (9)$$

Where v = volume of N₂ adsorbed by the sample under pressure p , p_0 = Saturated vapor pressure at the same temperature, v_m = Volume of N₂ adsorbed when the surface is covered with a unimolecular layer, and c = Constant for a given adsorbate

The specific surface areas of samples are calculated from adsorption data by Equation 10.

$$S = \frac{N_0 v_m A}{22414m} \quad (10)$$

Where S = Specific surface area, N_0 = Avogadro number, m = Amount of solid adsorbent, A = Cross-section of the gas molecules (16.2 Å² for N₂)

The Barrett-Joyner-Halenda(BJH) formula with the corrected Kelvin equation. The Kelvin equation describe as follows:

$$\ln \frac{p_0}{p} = \frac{2\gamma V_L}{rRT} \quad (11)$$

Where p is the actual vapour pressure, p_0 is the saturated vapour pressure, γ is the surface tension, V_m is the molar volume, R is the universal gas constant, r is the radius of the droplet, and T is temperature.

3.5.3 Surface Functional group

Surface functional groups of prepared HMSs will be investigated by Fourier Transform Infrared (FT-IR) Spectroscopy Nicolet Impact 410. KBr use as the background and sample mixer (KBr:Sample ratio = 10:1). Before measuring, sample is heated at 110°C for reducing the effect of H₂O at 3750 and 3457 cm⁻¹.

3.5.4 Elemental Analysis

3.5.4.1 Analysis of Nitrogen content

The amounts of nitrogen in synthesized HMSs will be measured by UV adsorption technique. Adsorbents will be digested by potassium persulfate (K₂S₂O₈) with base condition in autoclave. Chemical preparations were described as follows:

Mixture of sodium hydroxide (NaOH) and potassium persulfate was prepared by adding 3 g of potassium persulfate in solution of 4 g NaOH in 100 ml. This solution was newly prepared every time. Stock solution (0.1 mg N/ml) for total nitrogen standard curve was prepared by adding 0.722 g of dried potassium nitrate (KNO₃) in milli-Q water, and made volume to 1000 ml. HCl (1+500) was prepared by mixing of 1 HCl conc. in 500 ml of milli-Q water, and HCl (1+16) also was prepared by mixing 1 HCl conc. in 16 ml of milli-Q water.

Small amount of sample (50 mg) was added to autoclave glass bottle, and then 50 ml of milli-Q water was added. 10 ml of mixture of NaOH and K₂S₂O₈ were added into the mixture and autoclaved at 120°C for 30 min. Then sample was filtered and 25 ml of filtrate was taken. After that, 5 ml of HCl (1+16) were added to adjust pH to 2-3 and filled into 50 ml of colorimetric tube. Sample was diluted to 50 ml by milli-Q water. All samples were measured by UV spectrophotometer at 220 nm. Blank was prepared by adding 5 ml of HCl (1+500) into milli-Q water and diluted to 50 ml. Calibration curve was made by following procedure. 10 ml of prepared TN stock solution was taken and diluted ten time to 100 ml. Then 1-15 ml of obtained solution (0.01 mg N/ml) were added to each colorimetric tube and 5 ml of HCl (1+500) was added to each tube. All of tubes were made to 50 ml and measured by UV spectrophotometer with same condition. Amount

of TN were plotted against UV-absorbance. TN (mg/g) was calculated by the following equation:

$$\text{TN (mg/g)} = \{ [a \times (60/25)] / b \}$$

Where a is amount of TN obtained from calibration curve (mg) and b is amount of synthesized adsorbent (g).

3.5.4.2 Analysis of Sulfur content

The synthesized HMSs will be digested with 7 ml of HNO₃ 65% and 2 ml of HF 40% in Microwave equipment to analyze the amounts of sulfur. Microwave is temperature-programmed at 240°C in 10 minutes and held for 20 minutes. After microwave completion, samples were cooled by air until the solution reached room temperature. HF in solution was removed by adding 10 ml of H₃BO₃ 5% before analysis. The amounts of sulfur will be analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

3.5.5 Surface Charge

Acid/base titration of synthesized HMSs and PAC is carried out using 20 ml of adsorbent mixture (2 g/l). Varying amounts of 0.01 M HCl or 0.01 M NaOH solutions are added to a maximum of 9 samples to vary pH of each sample. There is also one sample with no acid or base addition. Each sample is then diluted to a final volume of 25 ml with Milli-Q water. The ionic strength is adjusted with NaOH solution at 0.01 M when diluted in 25 ml. Then the samples will be shaken in shaking water bath at 25°C for 12 hours. After equilibrating, the suspension pH of each sample is measured and the pH data of entire batch are plotted against surface charges that are calculated from principle of electroneutrality as follows:

$$\text{Surface charge (C/g)} = [(\text{HCl})_{\text{add}} - (\text{NaOH})_{\text{add}} - (\text{H}^+) + (\text{OH}^-)] \times 96500/\text{weight}$$

3.6 Adsorption Experiments

3.6.1 Adsorption Kinetic

3.6.1.1 Heavy metals (Cd(II) and Cu(II))

The equilibrium contact time for heavy metal adsorption will be performed by varying contact time from 0 to 72 h under batch condition at each heavy metals concentration of 20 mg/l, and amount of adsorbent of 0.33 g/l. The pH of solution will be fixed at pH 5 and ionic strength 0.1 M by phosphate buffer. Samples will be shaken in shaking water bath at 25°C, 150 rpm. After that they will be filtered by glass filter (GF/C) and the rest of heavy metal concentration will be digested by microwave digestion and analyzed by using ICP-OES.

3.6.1.2 Ionic dye (Methylene Blue)

The equilibrium contact time for ionic dye adsorption will be performed by varying contact time from 0 to 72 h under batch condition at ionic dye concentration of 30 mg/l except PAC use concentration of 225 mg/l and amount of adsorbent of 0.33 g/l. The pH of solution will be fixed at pH 5 and ionic strength 0.1 M by phosphate buffer. Samples will be shaken in shaking water bath at 25°C, 150 rpm. After that they will be filtered by glass filter (GF/C) and the rest of ionic dye concentration then will be analyzed by using UV-Visible spectroscopy at 660 nm.

3.6.1.3 Non-ionic surfactant (TX-100)

The equilibrium contact time for non-ionic surfactant adsorption will be performed by varying contact time from 0 to 72 h under batch condition at non-ionic surfactant concentration of 400 mg/l and amount of adsorbent of 0.33 g/l. The pH of solution will be fixed at pH 5 and ionic strength 0.1 M by phosphate buffer. Samples will be shaken in shaking water bath at 25°C, 150 rpm. After that they will be filtered by glass filter (GF/C) and the rest of non-ionic surfactant concentration then will be analyzed by using UV-Visible spectroscopy at 270 nm.

3.6.2 Single solute adsorption isotherms

3.6.2.1 Heavy metal (Cd(II) and Cu(II))

The single solute adsorption isotherm for each heavy metal adsorption will be performed by varying heavy metal concentration of 0-20 mg/l under batch condition and amount of adsorbent of 0.33 g/l. The pH of solution will be

fixed at pH 5 and ionic strength 0.1 M by phosphate buffer. Samples will be shaken in shaking water bath at 25°C, 150 rpm until equilibrium. The mixtures will be filtered by glass filter (GF/C) and the rest of heavy metal concentration will be digested by microwave digestion and analyzed by using ICP-OES.

3.6.2.2 Ionic dye (Methylene Blue)

The single solute adsorption isotherm for ionic dye adsorption will be performed by varying methylene blue concentration of 0-30 mg/l except PAC which uses concentration of 60-150 mg/l under batch condition and amount of adsorbent of 0.33 g/l. The pH of solution will be fixed at pH 5 and ionic strength 0.1 M by phosphate buffer. Samples will be shaken in shaking water bath at 25°C, 150 rpm until the equilibrium. The mixtures will be filtered by glass filter (GF/C) and the rest of methylene blue concentration then will be analyzed by using UV-Visible spectroscopy at 660 nm.

3.6.2.3 Non-ionic surfactant (TX-100®)

The single solute adsorption isotherm for non-ionic surfactant (TX-100) will be performed by varying non-ionic surfactant concentration of 50-600 mg/l under batch condition and amount of adsorbent of 0.33 g/l. The pH of solution will be fixed at pH 5 and ionic strength 0.1 M by phosphate buffer. Samples will be shaken in shaking water bath at 25°C, 150 rpm. The mixtures will be filtered by glass filter (GF/C) and the rest of TX-100 concentration then will be analyzed by using UV-Visible spectroscopy at 270 nm.

3.6.3 Effect of pH

The effect of pH for all of single-solute heavy metals adsorption will be investigated by changing from pH 3 and 5, adjusted by phosphate buffer with ionic strength at 0.1M. And methylene blue and TX-100 adsorption isotherms were operated under varying pH from 5 to 9 by phosphate buffer. The amount of adsorbent of 0.33 g/l. Samples will be shaken in shaking water bath at 25°C, 150 rpm until equilibrium. After that they will be filtered by glass filter (GF/C) and the rest of heavy metal, non-ionic surfactant, and ionic dye concentration then will be analyzed by using ICP-OES and UV-Visible spectroscopy, respectively.

3.6.4 Bi solute adsorption isotherms

Two types of the pollutants will be mixed together by fixing concentration of one pollutant and varying concentrations of the other. Then adsorption experiment will be conducted under the same condition as single-solute adsorption experiment.

3.6.4.1 Effects of heavy metal (Cd(II) and Cu(II)) on TX-100 adsorption.

The experimental procedure was similar to that of single-solute adsorption isotherms of heavy metals, except that the heavy metal (Cd(II) and Cu(II)) was mixed with TX-100 that fixed initial concentration. The initial concentrations of TX-100 were 600 mg/l.

3.6.4.2 Effects of TX-100 on heavy metal (Cd(II) and Cu(II)) on adsorption.

The experimental procedure was similar to that of single-solute adsorption isotherms of TX-100, except that the TX-100 was mixed with heavy metal (Cd(II) and Cu(II)) that fixed initial concentration. The initial concentrations of heavy metal were 20 mg/l.

3.6.4.3 Effects of heavy metal (Cd(II) and Cu(II)) on methylene blue adsorption.

The experimental procedure was similar to that of single-solute adsorption isotherms of heavy metals, except that the heavy metal (Cd(II) and Cu(II)) was mixed with methylene blue that fixed initial concentration. The initial concentrations of methylene blue were 30 mg/l and 100 mg/l for HMSs and PAC, respectively.

3.6.4.4 Effects of methylene blue on heavy metal (Cd(II) and Cu(II)) on adsorption.

The experimental procedure was similar to that of single-solute adsorption isotherms of methylene blue, except that the methylene blue was mixed with heavy metal (Cd(II) and Cu(II)) that fixed initial concentration. The initial concentrations of heavy metal were 20 mg/l.

3.6.4.5 Effects of methylene blue on TX-100 adsorption.

The experimental procedure was similar to that of single-solute adsorption isotherms of methylene blue, except that the methylene blue was mixed with TX-100 that fixed initial concentration. The initial concentrations of TX-100 were 600 mg/l.

3.6.4.6 Effects of TX-100 on methylene blue adsorption.

The experimental procedure was similar to that of single-solute adsorption isotherms of TX-100, except that the TX-100 was mixed with methylene blue that fixed initial concentration. The initial concentrations of methylene blue were 30 mg/l and 100 mg/l for HMSs and PAC, respectively.

3.7 Analytical Method

A concentration of heavy metals, methylene blue and TX-100 were determined by using ICP-OES and UV-Visible spectroscopy as followed:

i.) Single solute

- Heavy metals (Cd(II) and Cu(II)): ICP-OES
- Methylene blue: UV-Visible spectroscopy (660 nm)
- TX-100: UV-Visible spectroscopy (270 nm)

ii.) Bi solute

- Concentration of heavy metals and TX-100 were measured by using ICP-OES and UV-Visible Spectroscopy, respectively.
- Concentration of heavy metals and methylene blue were measured by using ICP-OES and UV-Visible Spectroscopy, respectively.
- Concentration of methylene blue and TX-100 were measured by using UV-Visible Spectroscopy.

Methylene blue in mixed solute with TX-100 can be measured by UV-VIS spectrophotometer at wave length at 660 nm without interfere from the presence of TX-100. However, measurement of TX-100 in mixed solute by UV-VIS spectrophotometer at wave length at 270 nm can be affected by the benzene ring of methylene blue structure. Hence, methylene blue base line has to be added in

calibration curve for TX-100 measurement. The dye and surfactant mixtures were prepared in different proportions (Table 3.1).

Table 3.1 Concentration proportion of TX-100 and methylene blue in mixed solute calibration curve

TX-100 conc. MB conc.	0 ppm	50 ppm	100 ppm	200 ppm	400 ppm	600 ppm
0 ppm	0+0	0+50	0+100	0+200	0+400	0+600
2 ppm	2+0	2+50	2+100	2+200	2+400	2+600
4 ppm	4+0	4+50	4+100	4+200	4+400	4+600
8 ppm	8+0	8+50	8+100	8+200	8+400	8+600
10 ppm	10+0	10+50	10+100	10+200	10+400	10+600