CHAPTER 3 EXPERIMENT

3.1 Equipment and Materials

3.1.1 Experimental apparatus

1. UV-visible spectrophotometer (Hitachi U-1900)
2. Water-bath shaker
3. Orbital shaker
4. Hot–air oven
5. Analytical Balance
6. Hot plate
7. Magnetic stirrer
8. Stopwatch (Casio HS-5)
9. Thermometer
10. Magnetic bar
11. Volumetric Flask
12. Vials
13. Pipette and volumetric pipette
14. Dropper
15. rubber bulb and trip
16. Filter Funnel Glass

3.1.2 Chemicals and Materials

1. Green tea
2. Corn cob
3. Caffeine
4. Gallic acid
5. Tannic acid
6. Polyvinyl polypyrrolidone (PVPP)
7. Lead (II) acetate ($Pb(CH_3COO)_2$)
8. Sodium carbonate (Na_2CO_3)
9. Hydrochloric acid (HCl)
10. Sulfuric acid (H_2SO_4)
11. Sodium hydroxide (NaOH)
12. Calcium chloride ($CaCl_2$)
13. Folin-Ciocalteu reagent
14. Deionized-water
15. Filter paper No.1
16. Tea bag 5×8 cm^2.

3.2 Experimental Procedures

3.2.1 Adsorbent Preparations

3.2.1.1 Unmodified corncob

Corn cob was ground and sieved. The particles ranging from 16-18 mesh (particle size, 1-1.4 mm) were dried in a hot air oven at 50 °C, and kept in a desiccator until used.

3.2.1.2 Modified corncob

10 g of unmodified corn cob was immersed in 200 ml of 0.1 M NaOH solution at 50 °C for 30 minutes by using water bath shaker. Immersed in NaOH solution, hydroxyl groups of corn cob reacted with NaOH through basification as shown by [25] equation 1.

$$[C_6H_7O_2(OH)_3]_n + nNaOH \rightarrow [C_6H_7O_2(OH)_2ONa]_n + nH_2O \quad (1)$$

It was subsequently allowed to shaking to room temperature. After corn cob was reacted with NaOH for the desired time, it was removed from the NaOH solution and washed with DI water until the pH of water became neutral. It was then dried in a hot air oven at 50 °C and kept in a desiccator until used.

3.2.1.3 Corncob incorporated with ions

10 g of modified corn cob was incorporated with Ca^{2+} ions by immersing in 300 ml of 1 M $CaCl_2$ solution at room temperature for 12 hours. Two Na^+-form oxy groups were needed to exchange for one Ca^+ ion as shown by [26] equation 2.

$$2[C_6H_7O_2(OH)_2ONa]_n + nCaCl_2 \rightarrow [(C_6H_7O_2(OH)_2O)_2Ca]_n + 2NaCl \quad (2)$$

It was then washed with deionization water until the pH of water was neutral. The corn cob was dried at 50 °C.

3.2.2 Adsorbent Characterization

To determine the ion exchange capacity (IEC) of the modified corn cob, 0.5 g of modified corn cob was equilibrated with 25 cm^3 of 0.025 M HCl for 6 h. After the modified corn cob was removed from the solution, the remaining solution was titrated with 0.025 M NaOH. Ion exchange capacity (IEC) was calculated by using the equation

$$\frac{meq}{g} = \frac{(N_1V_1) - [(V_1/V_2)(N_2V_3)]}{M}$$

Where

N_1 = Concentration of HCl (N)

N_2 = Concentration of NaOH (N)

V_1 = Volume of HCl used in equilibration (cm^3)

V_2 = Volume of HCl taken for titration (cm^3)

V_3 = Volume of NaOH used in titration (cm^3)

M = Dried mass of corn cob (g)

3.2.3 Adsorption experiments

3.2.3.1 Adsorption without tea bag

In the adsorption experiments, 110 ml of deionized water in a flask was heated to 80 °C. 2 g of green tea leaf and adsorbent were placed in the hot water. The flask was then shaken (150 rpm) in a water bath shaker at 80 °C for various times. Green tea solution was sampled and diluted to ratio 1:50 with DI water. After that, it was analyzed to determine the amount of caffeine, tannin, and total polyphenol by UV-Visible spectrophotometer.

3.2.3.2 Adsorption in tea bag

Tea bag, 5 cm × 8 cm in size, was used in experiments. It was made of wood pulp. 2 g of green tea and 2 g of adsorbent were packed in the tea bag. The tea bag was immersed in 110 ml of 80 °C water, while shaking at 150 rpm all the time. 1 ml of the solution was periodically sampled for 20 minutes. The sample was analyzed for caffeine, tannin, and total phenolic compounds using a UV-Visible spectrophotometer.

3.2.4 Analysis

3.2.4.1 Determination of caffeine [27]

3.2.4.1.1 Reagents

1. Lead acetate solution

$CH_3COO)_2Pb$ (100 g) was dissolved and diluted to 200 ml with DI water.

2. Hydrochloric acid solution

Hydrochloric acid (36% HCl, specific gravity 1.18, 0.9 ml) was diluted to 1000 ml with distilled water.

3. Sulfuric acid solution

Sulfuric acid (98% H_2SO_4, specific gravity 1.84, 167 ml) was diluted to 1000 ml with distilled water.

3.2.3.1.2 Measurement

10 ml Tea solution, 5 ml hydrochloric acid solution and 1 ml lead acetate solution were mixed in a 100 ml volumetric flask and diluted to 100 ml with distilled water. The

solution was then filtered through Whatman No. 1 qualitative filter paper. After that, 25 ml filtered and 0.3 ml sulfuric acid solution were placed in a volumetric flask and diluted to 50 ml with distilled water. The solution was filtered using the same type of filter paper. The absorbance of the filtrate was measured using a Hitachi U 1900 UV-Visible spectrophotometer at 274 nm with a 10 mm quartz cell. The measurement was performed in triplicate.

3.2.4.2 Determination of total phenolic content [28]

3.2.4.2.1 Reagents

1. Folin Ciocalteu reagent (2 N)
2. Sodium carbonate (7%)

7 g sodium carbonate was dissolved in about 70 ml of DI water and made up to 100 ml with DI water.

3.2.3.2.2 Measurement

Samples were analyzed spectrophotometrically for contents of total phenolics by a modified Folin–Ciocalteu colorimetric method. 0.5 ml of deionized water and 0.125 ml of a known dilution of the extract were added to a test tube, followed by addition of 0.125 ml of Folin–Ciocalteu reagent. They were mixed well and then allowed to stand 6 min before 1.25 ml of a 7% sodium carbonate solution was added. The mixture was diluted to 3 ml with deionized water. The color was developed after 90 min at room temperature and the absorbance was measured at 760 nm using a spectrophotometer (Hitachi U 1900). The measurement was compared to a standard curve of prepared gallic acid solutions.

3.2.4.2 Determination of tannin [29]

3.2.4.2.1 Reagents

1. Folin Ciocalteu reagent (1 N)

Commercially available Folin-Ciocalteu reagent (2 N) was diluted with an equal volume of distilled water. It was stored in a brown bottle and kept in a refrigerator (4°C). It should be golden in color. Do not use it if it turns olive green.

2. Sodium carbonate (20%)

40 g of sodium carbonate (x10 H_2O) was dissolved in about 150 ml distilled water and made up to 200 ml with distilled water.

3. Insoluble polyvinyl pyrrolidone (polyvinyl polypyrrolidone, PVPP)
4. Standard tannic acid solution (0.1 mg/ml)

25 mg of tannic acid (TA) was dissolved in 25 ml distilled water and then diluted 1:10 in distilled water (always use a freshly prepared solution).

3.2.3.2.2 Measurement

1. Preparation of calibration curve

Calibration curve was prepared using standard tannic acid solution (0.1 mg/ml). Tannic acid, DI water, Folin-Ciocalteu and sodium carbonate solution were mixed in vial by vortex it. After 40 min, the mixture was recorded absorbance at 725 nm (Hitachi U 1900).

Table 3.1 Preparation of calibration curve

Tube	Tannic acid solution (0.1 mg/ml) (ml)	DI water (ml)	Folin reagent (ml)	Sodium carbonate solution (ml)
Blank	0.00	0.50	0.25	1.25
T1	0.02	0.48	0.25	1.25
T2	0.04	0.46	0.25	1.25
T3	0.06	0.44	0.25	1.25
T4	0.08	0.42	0.25	1.25
T5	0.10	0.40	0.25	1.25

2. Analysis of total phenols

0.1 ml of the tannin-containing extract in vial, make up the volume to 0.4 ml with DI water, and add 0.25 ml of the Folin-Ciocalteu reagent (1N) and then 1.25 ml of the sodium carbonate (20%) solution. Vortex the vial was measured at 725 nm using a spectrophotometer (Hitachi U 1900) after 40 min. Calculate the amount of total phenols as tannic acid equivalent from the above calibration curve. The concentrations obtained were tannin1.

3. Removal of tannin from the tannin-containing extract

0.05 g of PVPP, 0.5 ml distilled water and 0.5 ml of the tannin-containing extract add to in a centrifuge tube sized 1.5 ml. Vortex it. Keep the centrifuge tube at 4°C for 15 min, vortex it again, then centrifuge (12000 rpm) for 10 min and collect the supernatant. This supernatant has only simple phenolics. Measure the phenolic content of the supernatant as mentioned above. The concentrations obtained were tannin2. As a result, the tannin concentrations were tannin1- tannin2.