CHAPTER III
EXPERIMENTAL

3.1 Material

Synthetic zeolite-A (Valfor 100 Zeolite) was supplied by PQ Chemicals (Thailand) Ltd. Low density polyethylene (LDPE, EL-LENE LD1905FA, low MI, MI= 5) was supplied from Siam Cement Group Chemicals Company Ltd. AgNO3, 100g, AR grade was purchased from Fisher Scientific Inc. $Cu(NO_3)_2 \cdot 3H_2O$, 500 g, AR grade was purchased from Lobachemie PVT LTD, India. Both Bacto™ Agar and Bacto™ Tryptic Soy Broth, soybean-casein digest medium were purchased from Becto, Dickinson and Company, USA.

3.2 Equipment

The absorption isotherm of Ag/zeolite-A and Cu/zeolite-A was obtained by collection the data of atomic adsorption from atomic adsorption spectroscopy (Spectraa 300, Varian). DSC analyses were carried out using a Perkin-Elmer DSC 7 instrument. The samples were heated from 0 ° to 200 °C at a heating rate of 10 °C/min under a N_2 atmosphere with a 10 ml/min flow rate. TG-DTA curve of master batch was collected on a Perkin-Elmer Pyris Diamond TG/TGA instrument. The sample was loaded on the platinum pan and heated from 30° to 700 °C using heating rate at 10 °C/min under in N_2 atmosphere at a flow rate of 50 mL/min. HITACHI S-4800 scanning electron microscope was used to observe the surface morphology and filler dispersion of master batch at 5000X and 30000X magnification. Before analysis, samples were cut in after soaking in liquid nitrogen and then coated with platinum under vacuum. N_2 adsorption-desorption isotherms were obtained at -196 °C on a sorptomatic BET. Samples were

degassed at 300 °C for 17 hour in a vacuum furnace prior to analysis. Surface areas were calculated using the BET equation. The pore size distributions were constructed based on Barrett, Joyner and Halenda (BJH) method, using the adsorption branch of the nitrogen isotherm. The quantity of Ag and Cu that was in the zeolite was measured by X-ray Fluorescent Spectroscopy (AXIOS PW 4400). Furthermore, X-RAY diffraction was used to determine the difference of zeolite crystal and crystallinity of master batch when mixed with filler. Master batch production was carried out by mixing in twin screw extruder, Lab Tech Engineering Company Ltd., (20 centimeters in diameter of screw and 80 centimeters in length of screw). Compression molding, Lab Tech Engineering Company Ltd., was used to compress the master batches into 0.1 mm x 5 x 10 cm film for XRF and bacteria test.

3.3 Methodology

3.3.1 Ion Exchange Process

Zeolite-A was weight at 2 grams and stirred with 50 milliliters of $AgNo_3$ and $Cu(NO_3)_2$ solution for 1 hour. The concentration of Cu was fix at 7200 ppm and vary the concentration of Ag as a ratio of Ag:Cu from 1:1 to 2:1. The blue zeolite was obtained after mixing. The sample was washed with deionized water and dry at 70 °C for 2 days.

3.3.2 Master Batch Production Process

The LDPE and zeolite was heated at 60 °c overnight before mixing. When mixing, the temperature in each zone of twin screw extruder was 130 °c in feeding zone, 150 °c in compression zone and 170 °c in metering zone. The ration of LDPE and zeolite was 50% of zeolite toward LDPE. After extruded, plastic was cut into 3 mm pellet.

3.4 Characterization of Zeolite and Master Batch

3.4.1 Determination of Optimum Ion Exchange Time

Zeolite was stirred in 8000 ppm $AgNO_3$ and $Cu(SO_4)$ solutions separately (20% metal ion weight toward zeolite weight) for 48 hours. During the experiment, the solution was collected at 0, 30 min and 1 hour. After that the solution was collected at every 1 hour until 8 hour, after that the solution at 1 day and 2 day was collected. The Atomic absorption spectroscopy was used to determine the amount of Ag^+ and Cu^{2+} left in the solution in order to plot the graph of the concentration of metal ion at various time.

3.4.2 Equilibrium Ion Exchange Isotherm Study

Ion exchange study was studied in two ions: a) Ag^+-Na^+ system and b) $Cu2^+$-Na^+. The zeolite was stir with the solution at 600 rpm at room temperature. After exchange process finished, the solution was collected. The concentration of each ion left in the solution was determined by Atomic Absorption spectroscopy (spectra 300, varian). In the exchange process, initial concentration of metal was varied between 800 to 20 x 10^4 ppm and varied the weight of zeolite from 0.5 gram to 2 gram. Then the isotherm was determined follow these equations

$$A_z = \frac{(C_0 - C)}{C_0} \qquad \text{and} \qquad A_s = \frac{C}{CEC}$$

Az = Ion in the zeolite

As = Ion in the solution

C_0 = Initial concentration of metal

C = Concentration of metal after exchange process

C_0-C = Concentration of metal in zeolite

CEC = cation exchange capacity

3.4.3 Minimal Inhibition Concentration (MIC) Determination

The minimum concentration of zeolite that was be able to kill bacteria was determined in two strains of bacteria: a) *Escherichia coli* as gram negative bacteria and b) *Staphylococcus aureus* as gram positive bacteria. The concentration of zeolite at each formula was varied between 250 μg/ml and 4000 μg/ml. First of all, bacteria was needed to activate overnight and dilute the bacteria concentration have the number of cell at 10^8 CFU(colony forming unit) by adding 200 μl of *E. coli*(UV absorption at 230 nm 0.130±0.010)and 300 μl of *S. aureus* (UV absorption at 230 nm 0.150±0.010) into the 9 ml of saline solution (0.85% NaCl). The sample at each concentration was dispersed in an agar plate and dropped the 50 μl of bacteria on the agar. After 24 hours of inoculation, the turbidity of bacteria was observed.

3.4.4 Antibacterial Activity Test

The antibacterial activity test of master batch against *Escherichia coli*, gram-negative (*E. coli*) bacteria and *Staphylococcus aureus*, gram-positive bacteria (*S. aureus*, ATCC) was carried out according to shake flask method. The master batches were compressed into film by compression molding (Lab Tech Engineering Company Ltd.). The condition used to compress the master batches is preheating 10 minutes, compress at 150 kg/cm^2 for 5 minutes and cooling 7 minutes. Then the film were cut into 15 mm circular disks and sterilized under UV lamps for 2 hours. After activation of bacteria, the bacteria were diluted into 10^8 CFU (colony forming unit)/ml of cells (absorption at 230 nm is 0.130 ±0.010 for *E. coli* and 0.150 ± 0.010 for *S.aureus*) in the 100 ml Triptic soy bloth in order to obtain the stock of bacteria solution and dilute again into 10^5 CFU in 100 ml alkaline peptone water. Each disk of master batches was drop

into each peptone water flask. The sample was shaken at 150 rpm in the incubator 37°C for 24 hours. Then 1 ml of the solution was diluted in 9 ml of saline (0.85% NaCl) for 5 times in order to dilute for 0.1x each time of dilution. Then the solution was spread in the plate and incubated at 37°C. After incubation for 18 hours, the number of cell was counted and calculated with dilution factor. Then the activity of antibacterial could be compared.

3.4.5 Moisture Content and Filler Amount Study

Moisture content and filler that can be added into the plastic could be determined by using TG/DTA. Temperature profile that was carried out was 30 °C to 700 °C at 10 °C heating rate in N_2 atmosphere (50ml/min of N_2 flow rate). The moisture content was determined by calculation of Y-different value from 100 °C to 150°C. Quantity of filler was determined by determine the final weight of the measurement. In addition Decomposition (T_d) was also calculated.

3.4.6 Crystallinity Change Study

Crystallinity change of LDPE when fill was added was calculated by using the DSC. The condition used in this experiment was 0°C to 200 °C at 10 °C/min of heating rate in the N_2 atmosphere (30 ml/min of N_2 flow rate). The enthalpy in normalized value was calculated for neat LDPE and LDPE with filler at all condition. The crystallinity can be calculated by using the equation.

$$X_C = \frac{\Delta H_f(T_m)}{\Delta H_f^0(T_m^0)}$$

Where Xc is The degree of crystallinity. ΔH_f is The enthalpy of fusion measure at melting point and ΔH_f^o is The enthalpy of fusion of totally crystalline polymer measured at the equilibrium melting point.

3.4.7 Metal Content in Zeolite and in Master Batch Measurement

The metal content in zeolite and in master batch was measure by X-RAY fluorescence spectroscopy. Before analysis, sample was prepare by grinding the sample onto XRF pan filled with 6 grams boric acid (B_2O_3) and then pressed the sample pan by hydraulic pressure (carver) at 10 ton for 2 minutes. The program used to analyze the sample is Super Q manager. For qualitative analysis, IQ+ was used to measure the element in the sample.

3.4.8 Morphology and Filler Dispersion of Master Batch Study

The morphology and filler dispersion of zeolite was observed by Scanning Electron Microscope (HITACHI S-4800) at 5000x and 30000x magnification.