



CHAPTER III EXPERIMENTAL

3.1 Materials

3.1.1 Clay Mineral

Commercial sodium activated bentonite Mac-Gel[®] (GRADE SAC), Na-BTN, with cationic exchange capacity (CEC) of 44.5 meq/100 g clay, was supplied by Thai Nippon Co., Ltd. Thailand.

3.1.2 Surfactant

Dipalmitoylethy Hydroxyethylmonium Methosulfate under trade name Stepantex[™] SP-90 shown in Figure 3.1 was purchased from Sunny World Co., Ltd.

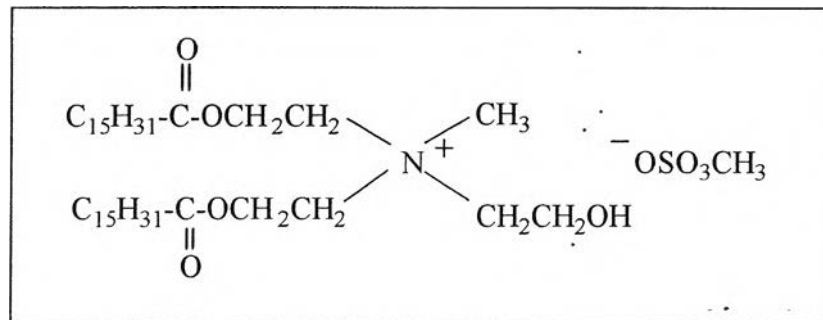


Figure 3.1 Chemical structure of Stepantex[™] SP-90

3.1.3 Polymer

Polypropylene under trade name Polene NK 1126 (MFI 11 dg/min) was purchased from Thai Petrochemical Industry Public Co., Ltd (IRPC Co., Ltd).

Poly(vinyl acetate) emulsion under trade name Adhesive Latex TOA NO.LA-22S was purchased from TOA Chemical Industries Limited.

3.1.4 Monomer

3,4-ethylenedioxythiophene (EDOT) under trade name Baytron M was purchased from Sigma-Aldrich.

3.1.5 Compatibilizer

Sodium-neutralized ethylene-co-methacrylic acid with MFI = 4 dg/min, under the trade name Surlyn[®] PC350, was purchased from DuPont Co Ltd.

3.1.6 Reagent

Copper (II) nitrate ($\text{Cu}(\text{NO}_3)_2$) was purchased from Univar.

Poly(sodium-4-styrenesulfonate) (PSS), M_w 500,000 g mol^{-1} , Iron (III) sulfate hydrate ($\text{Fe}_2(\text{SO}_4)_3$) and Sodium peroxodisulfate ($\text{Na}_2\text{S}_2\text{O}_8$) were purchased from Sigma-Aldrich.

3.1.7 Solvents

Ethanol ($\text{C}_2\text{H}_5\text{OH}$) 98% v/v, hydrochloric acid (HCl) 0.1 M and trichloroacetic acid ($\text{C}_2\text{HCl}_3\text{O}_2$) were commercially purchased from Carlo Erba.

Boric acid (H_3BO_3 , M_w 61.83 g mol^{-1}) was purchased from Merck.

3.1.8 Meat Sample

Tenderloin of chicken was purchased from the same filets and placed in ice container at Samyan market.

3.2 **Equipment**

3.2.1 X-ray Diffractometer (XRD)

The X-ray Diffractometer (XRD) was used to determine the interlayer spacing of pristine bentonite, organomodified bentonite, and to observe the crystal structure of nanocomposites. Wide angle X-ray diffraction (WAXD) patterns were obtained by using a Rigaku Model Dmax 2002 diffractometer with Ni-filtered $\text{Cu K}\alpha$ ($\lambda = 0.154$ nm) radiation operated at 40 kV and a tube current of 30 mA. The organoclay powder samples were measured in the 2θ range of 2-20 degrees with scan speed 2 degree/min and scan step 0.01 degree. For the nanocomposite film samples, the experiment was performed on a 2-40 degrees with scan speed 5 degrees/min and scan step 0.02 degree.

3.2.2 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of organoclay were collected using a Nicolet Nexus 670 FT-IR spectrometer in the frequency range of 4000-400 cm^{-1} with 32

scans at a resolution of 2 cm^{-1} . KBr pellet technique was applied in the preparation of powder samples. The presence of alkylammonium cationic surfactant into silicate clay layers was investigated by FT-IR.

3.2.3 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was used to study thermal stability of organomodified bentonite and PP/organoclay nanocomposites as compared to the pure PP. TG-DTA curves, collected on a Perkin-Elmer Pyris Diamond TG/DTA instrument was carried out from 30°C to 900°C at a heating rate of $10^{\circ}\text{C}/\text{min}$ in a nitrogen atmosphere of $200\text{ ml}/\text{min}$. The degradation temperature, initial degradation temperature, weight loss, and final degradation temperature of the samples were determined. The nanocomposite pellets from twin screw extruder as well as PP pellets were loaded on the platinum pan and heated from 30°C to 900°C at a heating rate of $10^{\circ}\text{C}/\text{min}$ under N_2 flow of $200\text{ ml}/\text{min}$.

3.2.4 Differential Scanning Calorimetry (DSC)

A Differential Scanning Calorimeter (DSC), Perkin-Elmer DSC 7, was used to measure the crystallization and melting behavior of the PP/organoclay nanocomposites. All operations were performed under a nitrogen atmosphere. The samples were first heated from 30°C to 200°C at a heating rate of $10^{\circ}\text{C}/\text{min}$ in order to eliminate the influence of thermal history and then cooled down at a rate of $10^{\circ}\text{C}/\text{min}$ from 200°C to 30°C to observe the melt crystallization behavior. After that the sample was immediately reheated to 200°C at the same heating rate in order to observe the melting behavior. The crystallinity can be calculated with the following formula

$$\% \text{ crystallinity} = \frac{\Delta H_{\text{sample}}}{\Delta H_{\text{PP}}^0} \times 100$$

where, ΔH_{sample} = enthalpy of fusion of the sample (J/g)

ΔH_{PP}^0 = enthalpy of fusion of completely crystalline PP ($\sim 209\text{ J}/\text{g}$)

3.2.5 Lloyd Universal Testing Machine

The mechanical properties of PP/organoclay nanocomposite as well as neat PP films were operated as ASTM D 882 on Lloyd Universal Testing Machine.

The films were prepared by blown film extrusion machine of thickness of 45 μm and were cut in machine direction into rectangular shape with 10x100 mm. The crosshead speed was 50 mm/min and the load cell was 500 N.

PP and nanocomposite films have different thermal history. That is, nanocomposite films were prepared by using twin-screw extruder and blow molding whereas PP film was only processed by blow molding.

3.2.6 Scanning Electron Microscope (SEM)

Scanning electron microscopy was performed on JEOL JSM-5410 LV Model to observe the dispersion of clay particles in the PP matrix. The selected specimens, which were nanocomposite pellets from twin screw extruder as well as PP pellets, were dipped and fractured in liquid nitrogen. Then, the specimens were coated with gold under vacuum to make them electrically conductive before viewing under a scanning electron microscope (SEM) operating at 15 kV.

3.2.7 Colorimeter

The color changes of the intelligent films were observed according to ASTM D65 by using a Chroma Meter, HunterLab[®] with 45°/0° optical geometry, software of Easy Match QC, diffuse/45°, and reported in Hunter system (L , a , and b) values and total color difference (TCD). The target color is (93.13, -0.96, 1.69) corresponding to (L^* , a^* , and b^*) for white standard color in Hunter system. The intelligent films, which were tested with meat at any time, were cut into circular shape with 5 cm in diameter and were measured by reading 3 points at random position over tested area.

3.2.8 Gas Permeability Tester

Gas permeation experiments were investigated according to ASTM D1434 by using Oxygen Permeation Analyser Model 8000, Illinois Instrument Inc. with oxygen flow rate of 50 cm^2/min at 23 °C to determine oxygen permeability constants of neat PP and nanocomposite films. The films were prepared from a compression-molded sheet with the same thickness of 150 μm and were cut into circular shape with 15 cm in diameter. The thickness of the films was measured by using the peacock digital thickness gauge model PDN 12N by reading 15 points at random position over tested area and the results were averaged.

3.2.9 Centrifugal Ball Mill

Dried organoclay sediments were ground by FRITSCH Peluerisette 6 Centrifugal Ball Mill with rotational speed of 450 rpm in forward and reverse milling directions for 20 min. The particle size of the powder was less than 44 μm .

3.2.10 Twin Screw Extruder

PP/organoclay nanocomposites were prepared by conventional melt intercalation using a Collin D-8017 Model T-20 co-rotating twin-screw extruder with L/D ratio of 30 and diameter of 25 mm. The operating temperatures of extruder were maintained at 80, 170, 180, 190, 200, and 210 $^{\circ}\text{C}$ from hopper to die respectively screw speed was maintained at 50 rpm. The pellets were analyzed by TGA, DSC, and SEM.

3.2.11 Blow Film Extrusion Machine

PP/organoclay nanocomposites films were prepared by using blow film extrusion machine at Tang Packaging Co., Ltd with screw diameter of 45 mm, die diameter of 150 mm, L/D ratio of 26, rotational speed of screw of 50 rpm, and blowing ratio of 0.65. The barrel and mold temperature were 210 $^{\circ}\text{C}$. Films with the same thickness of 45 μm were used for preparing intelligent film and studying mechanical properties.

3.2.12 Compression Molding Machine

PP/organoclay nanocomposites films were prepared using a Wabash V50H 50 ton compression molding machine. The mold containing the pellets was preheated at 200 $^{\circ}\text{C}$ for 5 min, followed by compressed under 10 tons of force for 5 min. After that the molding was cooled to 50 $^{\circ}\text{C}$ under pressure. Films with the same thickness of 150 μm were used to investigate crystal structure and oxygen permeability constants

3.2.13 UV-Vis Spectrometer

UV-Vis spectrometer (SHIMADZU model UV-2550) with a wavelength spectrum 200-900 nm at medium scan rate and sampling the data every 1 nm was used to determine the leakage of sensor in the packaging.

3.3 Methodology

3.3.1 Preparation of Organomodified Bentonite

In a container, 350 g of Na-bentonite, Na-BTN, was swollen in 1.05 liters of distilled water for 24 h, followed by heated at 80°C for 30 min. In another container, a cationic surfactant with 2.0 equivalents of CEC was dissolved in 100 ml of ethanol at 80°C for 30 min. After that, the solution of both containers were mixed and vigorously stirred at 80°C for 2 h and homogenized at the same temperature for 1 h. The resulting organomodified bentonite, denoted as OBTN was filtered and washed with hot water several times to remove the excess surfactant. Then, it was dried in vacuum oven at 100°C overnight, ground, and screened through a 325 mesh sieve.

3.3.2 Characterizations of Organomodified Bentonite

X-ray diffractometer (XRD) was used to observe interlayer spacing of pristine bentonite and organomodified bentonite.

Thermogravimetric analysis (TGA) was used to study thermal stability of organomodified bentonite as compared to unmodified bentonite.

FTIR spectrometer was used to investigate the presence of the cationic surfactant into silicate clay layers.

3.3.3 Preparation of PP/Organoclay Nanocomposites

PP/organoclay nanocomposites were prepared by conventional melt intercalation a Collin D-8017 Model T-20 co-rotating twin-screw extruder with L/D=30 and D=25 mm. The operating temperatures of extruder were maintained at 80, 170, 180, 190, 200, and 210 °C from hopper to die respectively. The screw speed was maintained at 50 rpm. The master batches containing 50 wt% organomodified clays with compatibilizer, was added to PP and Surlyn[®], fixed at 6 wt%, in appropriate amounts to obtain nominal contents of 1, 3 and 5 wt% clays in the nanocomposites. Each composition was premixed by a tumble mixer for 10 min before introducing into the twin-screw extruder and extruded through a single strand die. The yielded single strands of the PP/clay nanocomposites were obtained and

solidified by cool water, palletized and then dried in a vacuum oven at 80°C before characterizations by SEM, TGA, and DSC.

PP and nanocomposite pellets have different thermal history. That is, nanocomposite pellets were prepared by using twin-screw extruder whereas PP pellets were not processed by twin-screw extruder.

3.3.4 Preparation of Nanocomposites Films

The nanocomposites films were prepared by blown film extrusion machine. The nanocomposites pellets were dried in vacuum oven at 80°C for 12 h prior to blowing. Following extrusion conditions were employed, the rotational speed of screw of 50 rpm and blowing ratio of 1.52. The barrel and mold temperature were 210 °C. The thickness of the films was controlled to about 45 µm.

PP and nanocomposite films have different thermal history. That is, nanocomposite films were prepared by using twin-screw extruder and blow molding whereas PP film was only processed by blow molding.

3.3.5 Characterizations of PP/Organoclay Nanocomposite Films

X-ray diffractometer (XRD) was used to investigate the crystal structure of neat PP and nanocomposites.

Thermogravimetric analysis (TGA) was used to study thermal behavior of pure PP and nanocomposites.

Differential Scanning Calorimeter (DSC) was used to observe crystallization and melting behavior of virgin PP and nanocomposites.

Scanning electron microscopy (SEM) was used to investigate the dispersion of organoclay in polymer matrix

Gas Permeability Tester was used to determine oxygen gas permeability constants of pristine PP and nanocomposite films according to ASTM D1434 by using Oxygen Permeation Analyser Model 8000, Illinois Instrument Inc.

Lloyd universal testing machine according to ASTM D882 was used to study the mechanical properties of neat PP and nanocomposite films. The films were prepared by blow molding of thickness of 45 µm and were cut in machine direction into rectangular shape with 10x100 mm. The data were taken at room

temperature without preconditioning of the samples and were reported as the average of the data taken from 5 specimens.

3.3.6 Synthesis of PEDOT:PSS Sensor

Synthesis of PEDOT:PSS sensor was carried out by following the experiment by Louwet *et al.*, (2002).

PEDOT:PSS was prepared by mixing of 10.65 g (75 mmol) of 3,4-ethylenedioxythiophene (EDOT), 439 g of 5.99 wt% PSS solution and 21.4 g (104 mmol) $\text{Na}_2\text{S}_2\text{O}_8$ in 2062 ml of distilled water. After initial stirring at room temperature for 10 min, 187 mg of $\text{Fe}_2(\text{SO}_4)_3$ was added and the mixture was stirred vigorously for 24 h resulting in the aqueous PEDOT:PSS.

PEDOT which was synthesized from this procedure was in the doped state which has clear blue color. PSS acts as counter ion for charge compensation of PEDOT.

3.3.7 Characterization of PEDOT:PSS Sensor

FT-IR spectrometer was used to investigate the PEDOT:PSS polymerized in this work.

3.3.8 Preparation of Intelligent Films

The sensor fabrication was carried out by following the experiment established by Pacquit *et al.*, (2006).

A typical sensor solution contained a binder, poly(vinyl acetate) and a sensor, PEDOT:PSS (1, 3, 5% w/w) and $\text{Cu}(\text{NO}_3)_2$ (1, 3, 5, 10% w/w). The mixture was vigorously stirred for 30 min and degas in an ultra sonic bath for 30 min to remove the bubbles. A sensor solution was then spin-coated at 1000, 2000, 3000 rpm onto nanocomposite film substrate discs by using an automatic pipette and depositing 0.5 ml of the sensor solution. The obtained intelligent films with circular shape of 7 cm diameter were heated at 60 °C for 10 min or left at room temperature overnight to dry. After that, each intelligent film was laminated at 120 °C by lamination machine to improve adhesion between nanocomposite film and sensor. The thickness of sensor was measured by using the peacock digital thickness gauge model PDN 12N.

In the present work, the effect of the following parameters on the color change of PEDOT:PSS and Cu^{2+} sensors were investigated.

- 1) Effect of concentration of sensor
 - 1, 3, and 5 %w/w for PEDOT:PSS sensor
 - 1, 3, 5, and 10 %w/w for Cu^{2+} sensor
- 2) Effect of sensor thickness by controlling spin-coating speed
 - 1000, 2000, and 3000 rpm
- 3) Effect of clay content in nanocomposite film
 - 1, 3, 5 %clay
- 4) Effect of the amount of meat tissues
 - 25, 50, 100, and 150 g
- 5) Effect of type of sensor
 - PEDOT:PSS sensor
 - Cu^{2+} sensor

3.3.9 Meat Spoilage Trial

Tenderloin of chicken was purchased from the same filets, under the same aseptic conditions at Samyan market. Meat spoilage trial was carried out by following the experiment established by Byrne *et al.*, (2002).

Aseptic techniques such as the use of disposable gloves, bactericide built-in cutting board and flame sterilized scalpel were used to avoid sample contamination.

For a set of sensors, fifteen replicate meat samples were placed in separate individual polypropylene cups. Each intelligent film was sealed on the top of PP cup that contains meat tissue inside with fast cure epoxy to create a permanent gas-tight seal and to prevent leakage of amines. Samples were at no time indirect contact with the sensors (i.e. only headspace was sampled). The sensors responses were monitored every 3 h with a Chroma Meter.

In this study, PEDOT:PSS sensors displayed a color change from clear blue to deep blue and Cu^{2+} sensors change color from blue to brown after receiving some electrons.

Experimental design for meat spoilage monitoring (based on Byrne *et al.*, 2002) is shown in Figure 3.2 below.

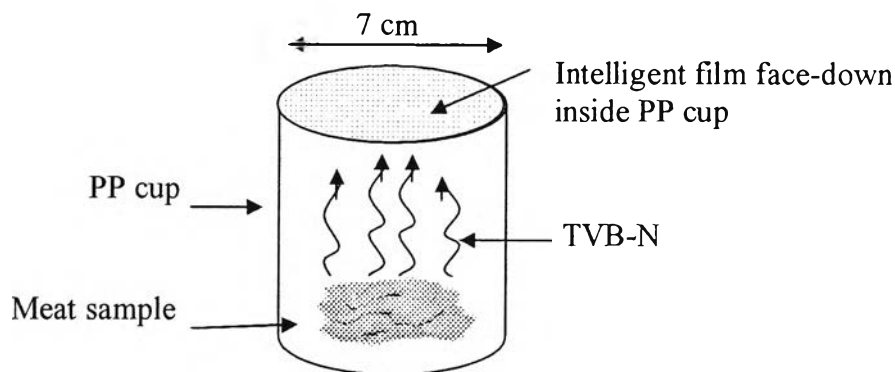


Figure 3.2 Experimental design for meat spoilage monitoring.

3.3.10 Evaluation of Intelligent Films

The responses of meat samples, allowed to spoil at room temperature, to different sensors of various thicknesses (1000, 2000, and 3000 rpm), sensor concentrations (1, 3, 5, and 10 %w/w for Cu^{2+} and 1, 3, and 5%w/w for PEDOT:PSS), clay contents (1, 3, 5 %wt) in the nanocomposite substrates, the amount of meat tissues (25, 50, 100, and 150 g), and type of sensors (PEDOT:PSS and Cu^{2+} ion) were investigated.

Color changes of the intelligent films were measured through the lid with a Chroma Meter and reported in Hunter system (L^* , a^* , and b^*) values and total color difference (TCD). The TCD value (ΔE) was calculated by the following equation (Francis, 1983):

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2 \right]^{1/2}$$

Here, ΔL is the brightness difference between sample and target, Δa the greenness-redness difference between sample and target, and Δb is the blueness-yellowness difference between sample and target. The target color is (93.13, -0.96, 1.69) corresponding to (L^* , a^* , and b^*) for white standard color in Hunter system.

3.3.11 Microbial Analysis of Meat Samples

Microbial analysis can be used to determine the degree of meat spoilage. The number of microorganism, which is more than 10^7 cfu/g is considered as spoilage (Mielmann A., 2006).

Microbial analysis was carried out by following the experiment established by Pacquit *et al.*, (2007).

Samples of approximately 50 g was removed from the same meat filets, under the same aseptic conditions, and placed in zip lock bags. The samples were then allowed to spoil at room temperature under the same conditions and every 3 h, along with every sensor response measurement, a sample was transferred into a freezer. Aerobic Plate Count (APC) or Total Viable Count (TVC) was determined, using the pour plate method, on plate count agar. Plates were counted after 48 h incubation at 30 °C and results were correlated with the sensor response.

Aseptic techniques such as the use of disposable gloves, bactericide built-in cutting board and flame sterilized scalpel were used to avoid sample contamination.

3.3.12 Determination of Total Volatile Basic Nitrogen (TVB-N)

Volatile amine, such as trimethylamine (TMA), dimethylamine (DMA), and ammonia are products of microbial degradation and are collectively known as total volatile basic nitrogen (TVB-N). TVB-N can be also used to determine the degree of meat spoilage. A level of 30-40 mg TVB-N/100g of meat sample is usually regarded as spoiled (Milanes M., 2004)

Determination of TVB-N was carried out by following the experiment by Fish Inspection and Quality Control Division (FIQD), the Department of Fisheries (DOF), Thailand.

3.3.12.1 Sample Extraction

The meat sample was ground by homogenizer. After that 3 g of homogenized sample was placed into a centrifuge tube followed by adding 12 ml of 4% Trichloroacetic acid (TCA) solution. The tube was sealed and vigorously shaken to obtain good mixing. The sample was then left at room temperature for 30 min with stirring from time to time. The sample mixture was filtered using Whatman paper

No.1. When prepared samples were not used within a day, the filtered solution must be kept at -18 °C in vials.

3.3.12.2 Measuring of TVB-N

Sealing agent, Vaseline, was first applied to the top edge of a Conway's unit. The inner ring solution (1% boric acid mixed with 1 ml of indicator) was pipette and placed into the inner ring of Conway's unit (volatile compounds from sample extract would diffuse into boric acid salt and these salts would be reduced to HCl-salts by strong HCl during titration). After that 1 ml of filtered sample extract was pipette into the outer ring of the Conway's unit. 1 ml of saturated K₂CO₃ solution was then pipette into the outer ring of the Conway's unit and placed on the opposite side of the sample (to make sample extract into alkaline condition similar to that of volatile compound). The Conway's unit was immediately covered and shaken gently to dissolve and mix the extract samples with K₂CO₃ solution without contaminating the inner ring of Conway's unit (triplicate for each sample). Then, the samples were kept at room temperature for 3 hr. After the color of boric acid solution changed from pink to green, following the generating of volatile base, this sample was then titrated with 0.01 N HCl containing in a micro-burette until the color changed back to pink.

Note: Blank test was carried out using 1ml of 4%TCA instead of sample extraction

3.3.12.3 Calculation of TVB-N

TVB-N value was calculated by the following equation

$$\text{TVB-N (mg/100g)} = \frac{(V_S - V_B) \times (N_{\text{HCl}} \times A_N) \times [W_S \times (M/100) + V_F] \times 100}{W_S}$$

Where, V_S = Titration volume of 0.01 N HCl for sample extract (ml)
 V_B = Titration volume of 0.01 N HCl for blank (ml)
 N_{HCl} = Normality of HCl (= 0.01 N × factor of HCl)
 A_N = Atomic weight of nitrogen (14.00)
 W_S = Weight of tissue sample (g)

- M = Percentage moisture of tissue sample (Assume 80%)
 V_E = Volume of 4% TCA used in extraction

3.3.13 Volume Electrical Conductivity of Sensor to Meat Spoilage

The volume electrical conductivity (σ_{dc}) was measured via two-point probe technique using Keithley 8009 Resistivity Test Fixture with dc voltage from 1 to 100 V. The sensors, which were tested with meat any time, were peeled off from nanocomposite films before testing. The sensor films were placed in the Keithley 8009 test fixture. The voltage was applied across the sample, and the current was read by the electrometer. Then, the volume electrical conductivity was calculated via the following equation:

$$R = \frac{V}{I}$$

$$\rho_v = \frac{22.9V}{tI}$$

$$\sigma_{dc} = \frac{1}{\rho_v},$$

Where, R is the resistance (ohm), V is the voltage (volts), I is the current (amperes), ρ_v is the volume resistivity (ohm centimeters), t is the sample thickness (centimeters) and σ_{dc} is the volume conductivity (siemens per centimeter).

3.3.13 Leaching Studies

The Cu²⁺ powders were dissolved in water to prepare the standard solution of 1, 5, 10, 20, and 40 ppm. Intelligent films were cut into rectangular shape with 4x4 cm and were soaked with 10 ml of water for approximately 48 h, after which a wavelength spectrum of the water (200-900 nm) was carried out to detect the presence of sensor by using UV-Vis spectroscopy (Shimadzu model UV-2550) with medium scan rate and sampling the data every 1 nm.