CHAPTER III
EXPERIMENTAL

3.1 Chemicals and Materials

3.1.1 Materials

PP films were purchased from Somboon Plastic Co., Ltd (Thailand).

3.1.2 Chemicals

(a) $Zn(NO_3)_2$, technical grade, was purchased from Ajax Finechem Pty Ltd.

(b) NaOH anhydrous pellets, analytical grade, were purchased from Ajax Finechem Pty Ltd.

3.1.3 Air Gas for Plasma Treatment

Air zero (high purity) used in the plasma treatment was obtained from Thai Industrial Gas Co., Ltd. (Thailand).

3.2 Equipment

3.2.1 Water Contact Angle Measurement

Water contact angle measurement was carried out at room temperature using the sessile drop technique. The contact angle formed between the 10-µl distilled water droplet and the PP surface was measured by a drop shape analysis system (Krüss, DSA10 Mk2). The reported values were the average of five measurements.

3.2.2 Lloyd Tensile Tester

Mechanical properties in terms of ultimate tensile strength and elongation at break were measured by using a universal testing machine (Lloyd, LRX). The specimen was cut into a dumb bell shape with the dimension of 6 mm × 1 mm with the gauge area of 6 mm × 1 mm. The load cell, the gauge length, and the displacement rate used during the testing were 2500 N, 5 mm, and 100 mm min^{-1}, respectively. Each reported datum was the mean of ten measurements.

3.2.3 ATR-FTIR Spectroscopy

The ATR-FTIR spectroscopy (Thermo Nicolet Nexus, 670) was employed to analyze the surface chemical composition of the PP films both before and after the DBD plasma treatment. All ATR-FTIR spectra were collected using 32 scans in a wavenumber range of 4000 cm^{-1} to 600 cm^{-1} at a resolution of 4 cm^{-1}. All PP specimens subjected to the ATR-FTIR analysis were cut into a precise dimension of 1.0 cm × 7.0 cm.

3.2.4 Scanning Electron Microscopy (SEM)

The surface morphology of the synthesized ZnO and the PP specimens were observed under a SEM microscope (Hitachi, S-4800) operated at 2.0 kV. The samples were placed on a brass stub before coated with a thin layer of gold using an ion sputtering device operated at 120 mA for 2 min. The SEM was also operated in the energy dispersive X-ray (EDX) mode in order to qualitatively determine the deposition of zinc oxide (ZnO) on the surface of PP film.

3.2.5 Atomic Absorption Spectroscopy (AAS)

An AAS spectrophotometer (Varian spectra, 300/400) was used for the quantitative analysis of the deposition of ZnO on the PP film. The samples were examined by using a hollow cathode lamp with a standard wavelength of 213.9 nm. The concentration of the deposited ZnO was calculated from a calibration curve of Zn^{2+} in the concentration range of 0.4 ppm to 2.0 ppm.

3.2.6 Wide Angle X-ray Diffraction (WAXD) Analysis

The crystalline structure of ZnO was characterized by an X-ray diffractometer (Bruker AXS, D8 advance) operated with the use of Cu Kα as the X-ray source. The WAXD analysis was done in a continuous mode with a scan speed of 1° min^{-1} covering the angle (2θ) from 10° to 80°. The WAXD data were also used to calculate the ZnO grain size using the Scherrer formula:

$$D = \frac{\kappa\lambda}{\beta\cos\theta}$$

where D is the grain size, K is 0.89, λ is the X-ray wavelength (0.15 nm), θ is the Bragg diffraction angle, and β is the peak width at half maximum (FWHM).

3.2.7 Surface Probe Microscopy (SPM)

The surface topography of the polymeric substrates was investigated by using a scanning probe microscope (SPM) (Veeco, Nanoscope IV) with the tapping mode in air at room temperature. The obtained micrographs were minimally flattened, and high frequency noise was diminished in order to facilitate data analysis.

3.2.8 X-ray Photoelectron Spectroscopy (XPS)

The chemical composition of treated PP film and ZnO coated PP films were also analized by Kratos Axis Ultra DLD with the active area 2 mm^2. The XPS spectra were excited by the Al Kα X-ray source (1486.6 eV).

3.3 Methodology

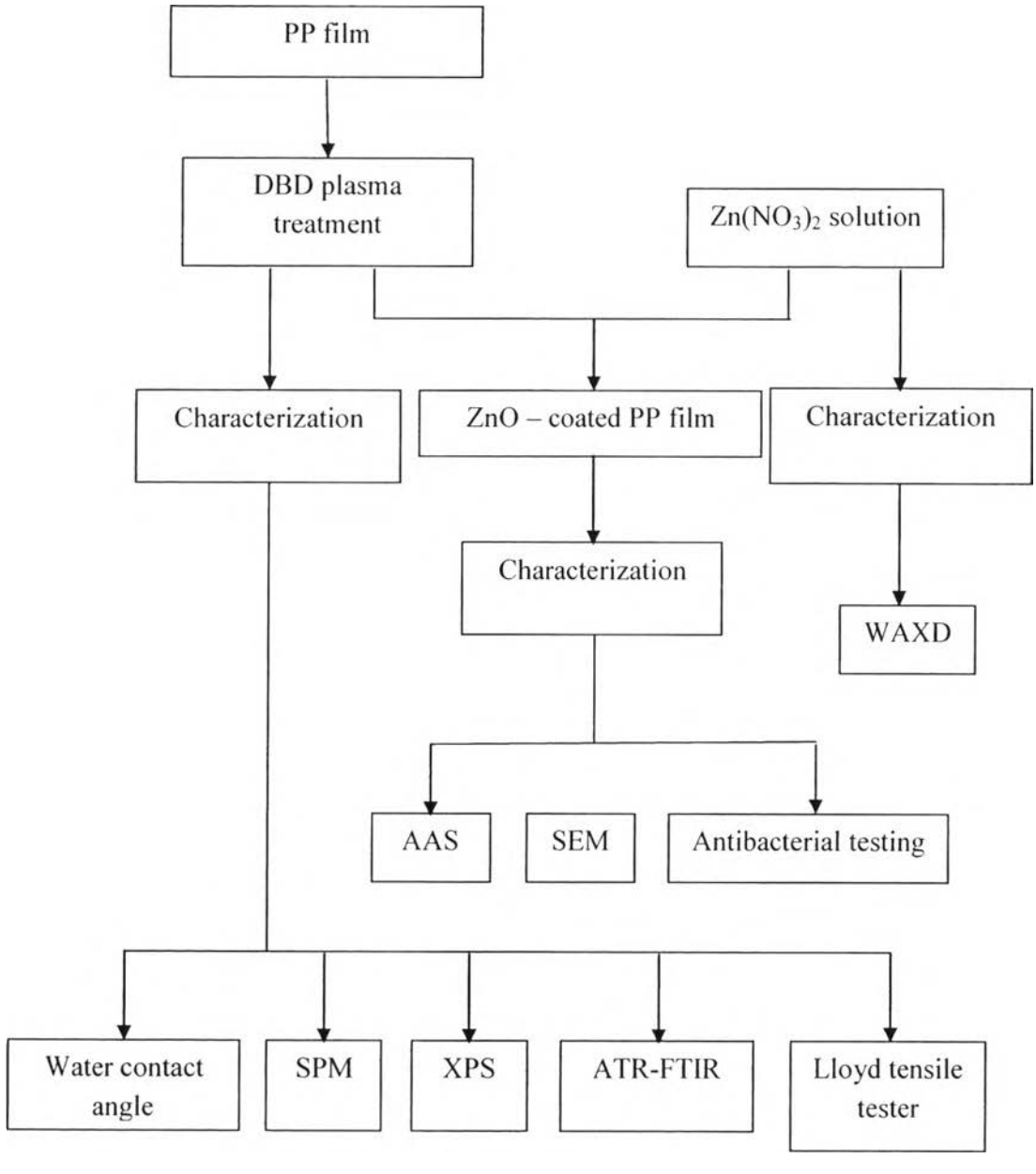

Figure 3.1 Flow chart of the entire experimental procedure.

3.4 Plasma Treatment and Sample Preparation

The PP-films were cut into a square shape with the dimension of 2 cm× 2 cm before treated with the DBD plasma. The optimum operating conditions for the DBD plasma treatment were selected at the voltage of 50 kV, the frequency of 325 Hz, and the electrode gap of 4 mm under air environment (Onsuratoom *et al.*, 2009). After that, the treated PP-film was dipped into an aqueous $Zn(NO_3)_2$ solution at different concentrations, followed by a drop wise addition of a 2.5 M NaOH solution. Next, the sample was washed with an excess amount of deionized water and dried in air at the room temperature.

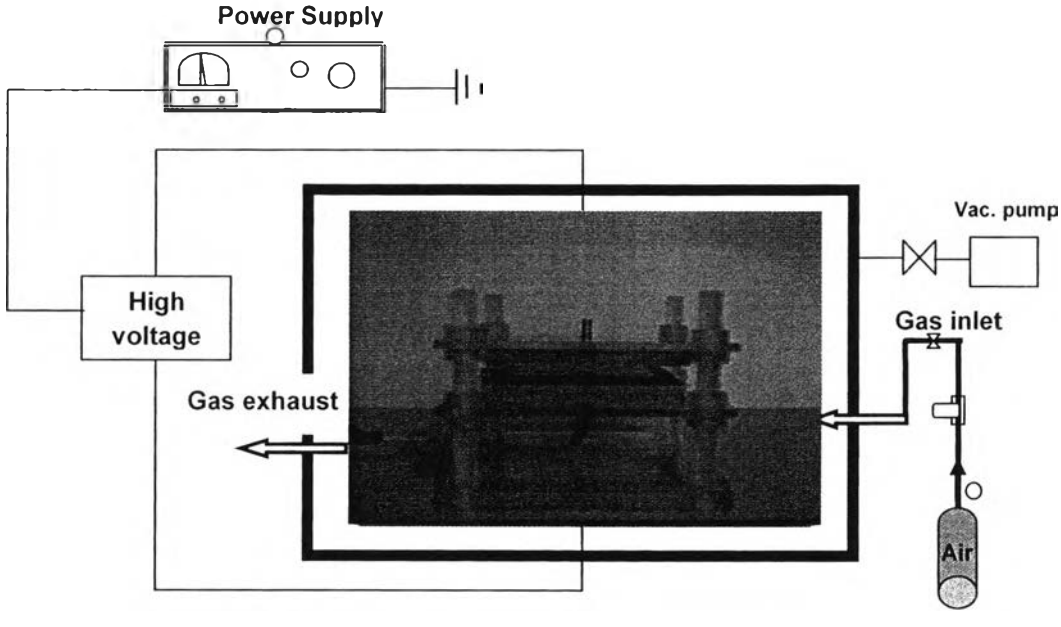

Figure 3.2 Experimental setup of DBD plasma instrument used in the present work.

3.5 Antibacterial Activity Testing

The *E. coli* and *S. aureus* were selected as representatives of Gram-negative and Gram-positive bacteria, respectively. The antimicrobial activities of ZnO nanoparticle-coated DBD plasma-treated PP films were determined based on the two methods.

Antibacterial properties of ZnO-coated PP film were evaluated based on the colony count method using ASTM E 2149-01. First, a culture medium was prepared by mixing 0.3 g of beef extract with 0.5 g of peptone in 100 ml water. The bacterial inoculums were prepared by transferring one colony of each microorganism into 20 ml of a culture medium. After that, the mixture was incubated in a shaking incubator at 150 rpm and 37 °C for 24 h. About 1 ml of the as-prepared inoculums was added into several vials of 9 ml of 0.85% sterile NaCl aqueous solution. Standard serial dilution method was used to obtain an appropriate bacterial concentration, i.e., 10^{-6} for *S. aureus* and 10^{-5} for *E. coli*. Next, the test sample was cut into square shape with the dimension of 2.0 cm × 2.0 cm before added to the bacterial suspension. The suspension mixture was then incubated in a shaking incubator at 150 rpm and 37 °C. After the contact time interval of 3 h, 100 µl of the suspension was withdrawn and subsequently spread on the sterilized agar plate. Bacterial growth was visualized after an overnight incubation at 37 °C for 24 h (Watthanaphanit *et al.*, 2010). The percentage of bacterial reduction was determined by using the following equation:

$$\text{Bacterial reduction} = \frac{(\text{Viable cell count at 0 h} - \text{Viable cell count at 24 h})}{\text{Viable cell count at 0 h}} \times 100$$