CHAPTER II

EXPERIMENTAL

2.1 Instruments and Equipments

Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 F_{254}) (Merck KgaA, Darmstadt, Germany). Column chromatography was performed in silica gel (Merck Kieselgel 60 G) (Merck KgaA, Darmstadt, Germany). Melting points were determined with an Elctrothermal 9100 melting point apparatus (American Instrument Exchange, Inc., MA, USA). For UV irradiation, broad band UVA (320-400 nm) was generated by F24T12/BL/HO (PUVA) lamp (National Biological Corporation, Twinsburg, Ohio 44087, USA) and broad band UVB (280-320 nm) was generated by FSX24T12/UVB/HO lamp (National Biological Corporation, Twinsburg, Ohio 44087, USA). UV irradiance was measured using UVA-400C and UVB-500C power meter (National Biological Corporation, Twinsburg, Ohio 44087, USA).

The FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared spectrophotometer: Impact 410 (Nicolet Instruments Technologies, Inc. WI, USA). Solid samples were incorporated into a pellet of potassium bromide. The ^{1}H-NMR and ^{13}C-NMR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) using ACF 200 spectrometer which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, CA, USA). Ultraviolet absorption spectra were obtained with the aid of HP 8453 UV/VIS spectrophotometer (Agilent Technologies, CA, USA). The UV absorbance was recorded using 1 cm quartz cell.

2.2 Chemicals

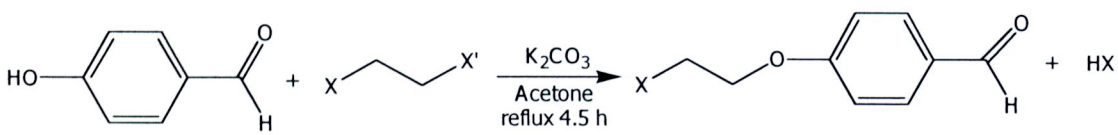
Solvents used in syntheses and spectroscopic analyses were reagent or analytical grades purchased from Labscan (Bangkok, Thailand). Solvents used in column chromatography were purified from commercial grade solvents prior to use by distillation. The reagents used for synthesizing were purchased from

- Acros Organics (New Jersey, USA): 1,5-diaza-bicyclo[4.3.0]non-5-ene (DBN); potassium *tert*-butoxide 98+% and dibenzoyl peroxide 75% remainder water
- Aldrich Chemical Company (Steinheim, Germany): poly(vinyl alcohol) 80% hydrolyzed
- Carlo Erba Reagent (Milan, Italy): sodium carbonate (anhydrous); N,N-dimethylformamide and pyridine
- Fluka Chemical Company (Buchs, Swizerland): 2,4,5 trimethoxy benzaldehyde; malonic acid and potassium carbonate
- Merck Co. Ltd. (Darmstadt, Germany): N,N'- dicyclohexylcarbodiimide (DCC); 4-hydroxybenzaldehyde; diethylmalonate; chloroform-D1; methanol-D4 and dimethylsulfoxide-D6
- Univar (Seven Hills, Australia): sodium sulfate.

Membranes used for dialysis experiments were seamless cellulose tubing, molecular weight cut off range (MWCO) 14,000 Dalton, size 36/32 100 ft (Viskase Companies, Inc., Japan).

2.3 Synthesis of Poly(diethylbenzalmalonate vinyl ether)

2.3.1 Preparation of 4-((2-halo)ethoxy)benzaldehyde and 4-((2-hydroxy)ethoxy)benzaldehyde (33)



When X, X' is halogen (Cl, Br, I) or hydroxy

In a round-bottomed flask equipped with a magnetic bar and a reflux condenser, 1,2-dibromoethane (14.0 mL, 0.16 mol) or 1,2-diiodoethane (45.00 g, 0.16 mol) or 1-bromo-2-chloroethane (13.0 mL, 0.16 mol) or 2-bromoethanol (12.0 mL, 0.16 mol) and excess potassium carbonate 11.04 g (0.08 mol) were dissolved in acetone (30 mL). The mixture was stirred for 10 minutes at room temperature and 4-hydroxybenzaldehyde 2.00 g (0.016 mol) was then added slowly. The mixture was refluxed for 4.5 hours and allowed to cool to room temperature. The mixture was

filtered and the solvent was evaporated under reduced pressure. The resulting residue was dissolved in dichloromethane (60 mL) and washed with (3 x 30 ml) aqueous sodium bicarbonate. The organic phase was separated and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The product was further purified by a silica gel column using dichloromethane as an eluent.

4-((2-bromo)ethoxy)benzaldehyde: White needle (66%), ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.93 (s, 1H, ArC<u>H</u>O), 7.89 (d, 2H, Ar<u>H</u>, *J* = 8.6 Hz), 7.05 (d, 2H, Ar<u>H</u>, *J* = 8.6 Hz), 4.42 (t, 2H, C<u>H</u>$_2$OAr, *J* = 6.2 Hz), 3.71 (t, 2H, C<u>H</u>$_2$Br, *J* = 6.2 Hz)

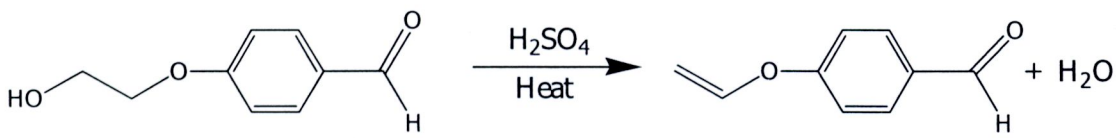
4-((2-chloro)ethoxy)benzaldehyde: White needle (65%), ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.93 (s, 1H, ArC<u>H</u>O), 7.88 (d, 2H, Ar<u>H</u>, *J* = 8.6 Hz), 7.06 (d, 2H, Ar<u>H</u>, *J* = 8.6 Hz), 4.35 (t, 2H, C<u>H</u>$_2$OAr, *J* = 6.0 Hz), 3.88 (t, 2H, C<u>H</u>$_2$Cl, *J* = 6.0 Hz)

4-((2-iodo)ethoxy)benzaldehyde: Only trace amount was found.

4-((2-hydroxy)ethoxy)benzaldehyde: White needle (65%), ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.82 (s, 1H, ArC<u>H</u>O), 7.77 (d, 2H, Ar<u>H</u>, *J* = 8.6 Hz), 6.95 (d, 2H, Ar<u>H</u>, *J* = 8.6 Hz), 4.15 (t, 2H, C<u>H</u>$_2$OAr, *J* = 6.2 Hz), 3.82 (t, 2H, C<u>H</u>$_2$OH, *J* = 6.2 Hz)

2.3.2 Preparation of 4-Vinyloxybenzaldehyde

I. Dehydration (34)

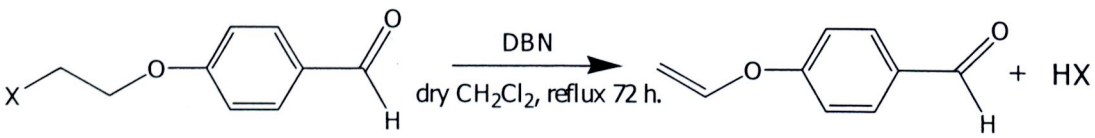


In a round-bottomed flask equipped with a reflux condenser was placed 1.66 g (0.01 mol) of 4-((2-hydroxy)ethoxy)benzaldehyde and added slowly a mixture of 15 mL of water and 15 mL of concentrated sulfuric acid. The mixture was heated in an oil bath kept at temperature of 120-130 °C for 10-12 hours. The solution was poured into a separatory funnel with 25 mL of diethyl ether, and organic layer was separated and dried over sodium sulfate. The drying agent was separated and rinsed with 25 mL

of ether. The diethyl ether was removed, and the product was distilled under reduced pressure.

II. Dehydrohalogenation

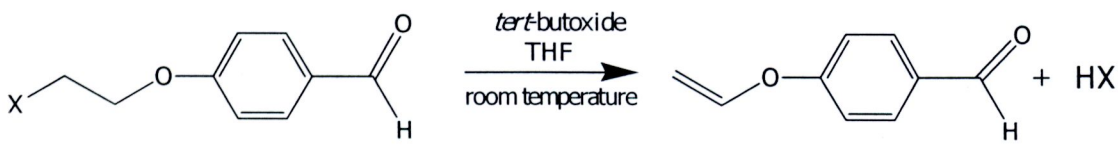
- *1,5-Diaza-bicyclo[4.3.0]non-5-ene (DBN)* (35)



When X is halogen (Cl, Br)

A three-necked, round-bottomed flask fitted with a reflux condenser, an addition funnel containing 15.0 mL (0.1 mol) 1,5-diaza-bicyclo[4.3.0]non-5-ene, DBN, a magnetic bar, a drying tube and a nitrogen inlet, was purged with nitrogen gas. The reaction was started by dropping DBN slowly at such a rate that the temperature of the reaction mixture did not exceed 40 °C. After the DBN was added the reaction mixture was maintained at about 40 °C for 24 hours. Thirty mL of 1.0 M ammonium hydroxide solution were added to the reaction mixture, and the reaction mixture was transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with 20 mL of dichloromethane. The organic fractions were combined, washed with water (30 mL) and dried with sodium sulfate. After filtration the solvent was evaporated under reduced pressure.

- *Potassium tert-butoxide* (36)



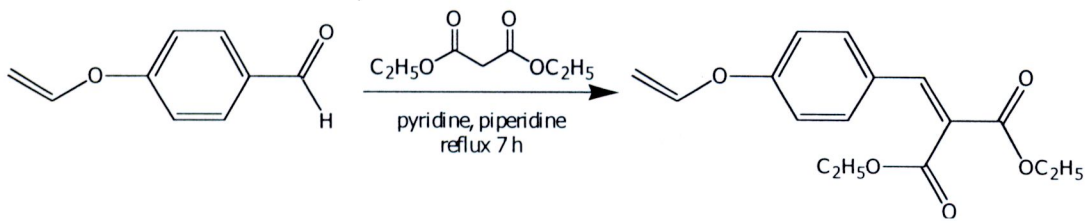
When X is halogen (Cl, Br)

In a two-necked, round-bottomed flask fitted with drying tube and nitrogen inlet, 2.29 g (0.01 mol) 4-((2-bromo)ethoxy)benzaldehyde or 1.85 g (0.01 mol) 4-((2-chloro)ethoxy)benzaldehyde and *tert*-butoxide 0.15 g (0.01 mol) were dissolved in 40 mL of freshly distilled tetrahydrofuran. Under nitrogen atmosphere, the mixture

was stirred at room temperature for 2 hours. The reaction was quenched by adding 30 mL of water and the organic part was extracted with 20 mL of diethyl ether. The organic part was washed again with another 30 mL water before it was dried with anhydrous sodium sulfate. The solvent was then removed by rotary evaporation. The product was further purified by a silica gel column using 15:85 (v/v) hexane:dichloromethane as an eluent. The yield was about 60%.

4-vinyloxybenzaldehyde: Colorless oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.97 (s, 1H, ArC<u>H</u>O), 7.91 (d, 2H, Ar<u>H</u>, J = 8.6 Hz), 7.15 (d, 2H, Ar<u>H</u>, J = 8.6 Hz), 6.73 (dd, 1H, C<u>H</u>OAr, J = 13.26, 6.24 Hz), 4.99 (dd, 1H, C<u>H</u>$_2$CHO, J = 13.26, 0-1 Hz) and 4.67 (dd, 1H, C<u>H</u>$_2$CHO, J = 6.24, 0-1 Hz).

2.3.3 Preparation of diethylbenzalmalonate vinyl ether monomer (37)



Diethyl malonate 0.16 g (0.001 mol) was dissolved in 10 mL (0.12 mol) of pyridine and 0.15 g (0.001 mol) 4-vinyloxybenzaldehyde and 0.50 mL (5.05 mmol) piperidine were added. The mixture was refluxed for 7 hours at 70-75 °C. After the mixture had been cooled, the solution was washed with two 20 mL portions of water, with two 15 mL portions of 15% aqueous hydrochloric acid solution, and then with 15 mL of saturated sodium bicarbonate solution. The organic solution was dried with anhydrous sodium sulfate. The product was further purified by a silica gel column using 30:70 (v/v) hexane:dichloromethane as an eluent. Percent yield was about 75%.

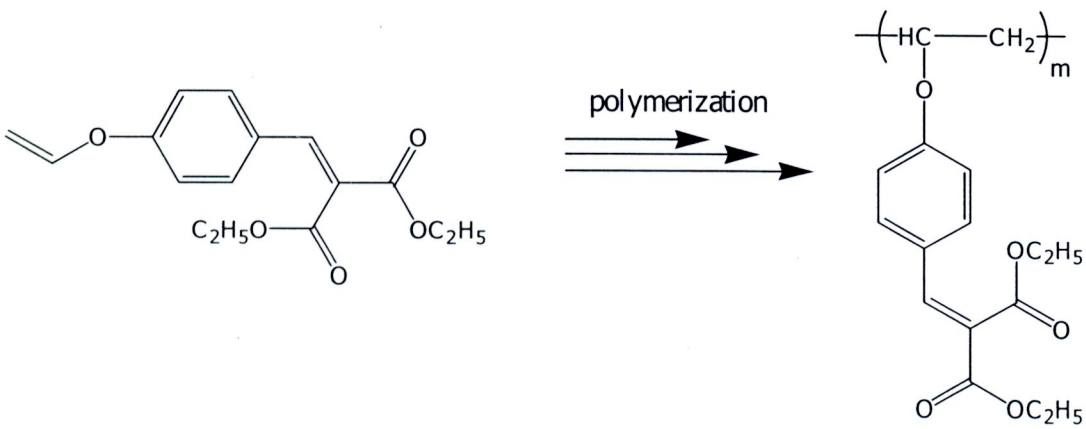
diethylbenzalmalonate vinyl ether monomer: Pale yellowish oil, b.p. 210-212 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (s, 1H, ArC<u>H</u>=), 7.48 (d, 2H, Ar<u>H</u>, J = 8.6 Hz), 7.02 (d, 2H, Ar<u>H</u>, J = 8.6Hz), 6.68 (dd, 1H, C<u>H</u>OAr, J = 14.04, 6.24 Hz), 4.90 (dd, 1H, C<u>H</u>$_{2a}$CHO, J = 14.04, 0-1 Hz), 4.58 (dd, 1H, C<u>H</u>$_2$bCHO, J = 6.24, 0-1 Hz), 4.36 (q, 4H, 2xOC<u>H</u>$_2$), 1.35 (t, 6H, 2xC<u>H</u>$_3$).

2.3.4 Synthesis of Poly(diethylbenzalmalonate vinyl ether)

2.3.4.1 Recrystallization of an initiator; dibezoyl peroxide

Dibenzoyl peroxide was dissolved in ethanol (the temperature of ethanol was maintained at approximately 30 °C). The clear solution was placed on an ice bath for crystallization process to take place. The white crystal was separated by suction filtration.

2.3.4.2 Polymerization of diethylbenzalmalonate vinyl ether monomer (38)

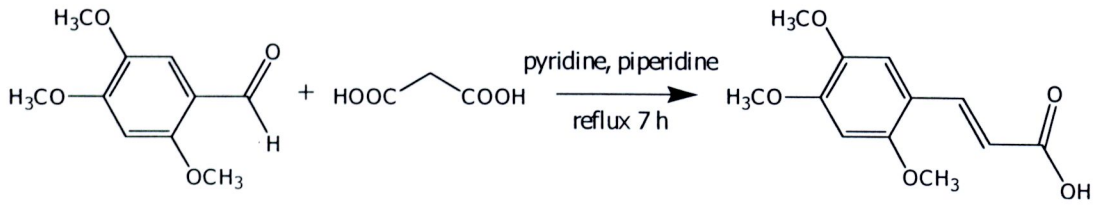


Poly(diethylbenzalmalonate vinyl ether) was prepared by bulk polymerization at 80°C using benzoyl peroxide as an initiator. Dibenzoyl peroxide (3.50 mg, 1.44 x 10^{-5} mol) and diethylbenzalmalonate vinyl ether monomer (0.09 g, 3.1 x 10^{-4} mol) were added to a small pear shaped flask with a small magnetic stir bar. A neck of the reaction vessel was sealed with rubber septa and subsequently purged with nitrogen gas. A few drops of acetonitrile were added to the mixture to reduce the viscosity of the mixture. The reaction mixture was then placed on a sand bath and maintained at 80°C. The product was analyzed by ^{1}H-NMR analysis.

Poly(diethylbenzalmalonate vinyl ether): Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.72 (s, 1H, ArC<u>H</u>=), 7.48 (d, 2H, Ar<u>H</u>, *J* = 8.6 Hz), 7.03 (d, 2H, Ar<u>H</u>, *J* = 8.6Hz), 6.68 (dd, 1H, C<u>H</u>OAr, *J* = 14.04, 6.24 Hz), 4.92 (dd, 1H, C<u>H$_{2a}$</u>CHO, *J* = 14.04, 0-1 Hz), 4.58 (dd, 1H, C<u>H$_{2b}$</u>CHO, *J* = 6.24, 0-1 Hz), 4.36 (q, 4H, 2xOC<u>H$_2$</u>), 1.36 (t, 6H, 2xC<u>H$_3$</u>).

2.4 Grafting of 2,4,5-Trimethoxycinnmic Acid on Poly(Vinyl Alcohol)

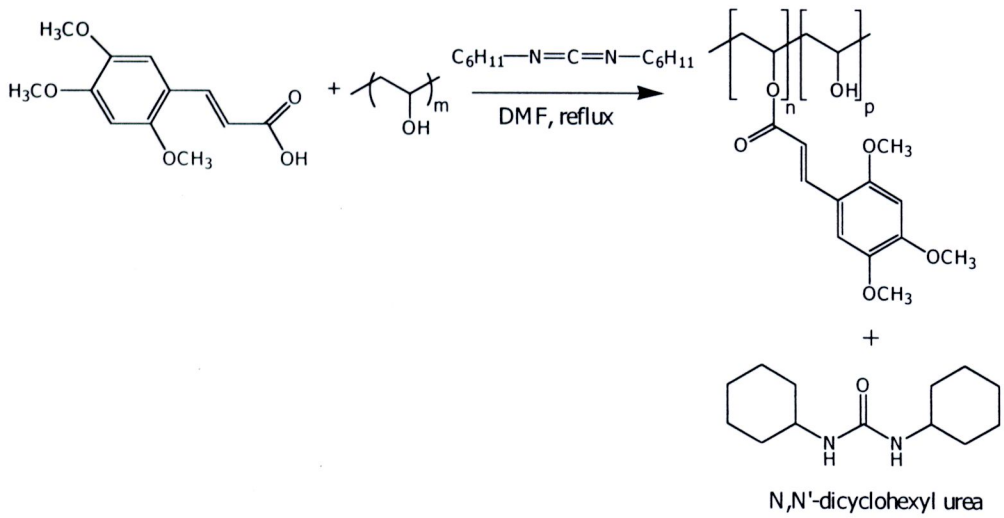
2.4.1 Preparation of trans-2,4,5-trimethoxycinnamic acid (37)



Malonic acid (1.06 g, 0.01 mol) was dissolved in 10 mL (0.12 mol) of pyridine and 2.38 g (0.01 mol) 2,4,5-trimethoxybenzaldehyde and 0.50 mL (5.05 mmol) piperidine were added. The mixture was refluxed for 7 hours at 70-75 °C. After being cooled the reaction mixture was poured into a beaker containing 75 mL of cold water. The mixture was acidified by slowly adding with 15 ml of concentrated hydrochloric acid. The solid was separated by suction filtration, washed with cold water and recrystallized with ethanol.

Trans-2,4,5-trimethoxycinnamic acid: Yellow solid (85%), m.p. 165-167 °C (lit. (39) 164-166°C), R_f 0.38 (65% EtOAc/Hex), IR (KBr, cm^{-1}) 3584-3363, 3308, 2936, 2823, 1683, 1596, 1519, 1463, 1432, 1406, 1293 and 1206; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.12 (d, 1H, ArC<u>H</u>=, *J* = 15.6 Hz), 7.06, 6.54 (s, 2H, Ar<u>H</u>) , 6.43 (d, 1H, =C<u>H</u>COOH, *J* = 15.6 Hz) and 3.98, 3.93 and 3.91 (s, 9H, 3xOC<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.147 (-<u>C</u>OOH), 154.26, 152.56, 143.25, 114.56, 110.93 and 96.69 (aromatic carbons), 141.86 (Ar<u>C</u>H=), 114.70 (=<u>C</u>HCOOH), 56.43-56.09 (3xO<u>C</u>H$_3$).

2.4.2 Grafting of trans-2,4,5-trimethoxycinnamic acid onto poly(vinyl alcohol) (40)



Poly(vinyl alcohol) (0.13 g, 3 mmol equivalent of hydroxyl groups) were dissolved in 12 mL of heated anhydrous DMF. 2,4,5-Trimethoxycinnamic acid (0.72 g, 3 mmol) and N,N'-dicyclohexylcarbodiimide (DCC) (0.62 g, 3 mmol) were added to this DMF solution of poly(vinyl alcohol). The mixture was stirred at 90°C for 60 hours. As the reaction proceeded, the color of the solution changed from brown to yellow. After being cooled, placed without agitation to precipitate out N,N'-dicyclohexyl urea (DCU) and the solution was then filtered. The liquid part was mixed with 300 mL of 15 % (v/v) cool aqueous hydrochloric acid solution under vigorous stirring. The precipitated product was separated by suction filtration, washed with cold water and recrystallized with hexane. Further purification of the product was carried out by dialysis.

2.4.3 Purification the grafted product by dialysis

Dialysis cellulose membrane was soaked for 10 minutes in water prior to use. The membrane was sealed at one end and 100 mL of the grafted product solution (0.43 g products in 100 mL of ethanol) was poured into the membrane. The top end of the membrane was then sealed by allowing an additional dead space approximately equal to the volume taken up by the sample. The bag was then placed into a beaker containing 800 mL of methanol. Dialysis was performed overnight with 3 changes of 800 mL of methanol.

The grafted product: Pale yellow solid, ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.81 (d, 1H, ArC<u>H</u>=, *J* = 15.6 Hz), 7.00, 6.53 (s, 2H, Ar<u>H</u>) , 6.40 (d, *J* = 15.6 Hz, 1H, =C<u>H</u>COOH), 3.94, 3.89 and 3.88 (s, 9H, 3xOC<u>H</u>$_3$) and 2.05-1.11 ppm [m,-C<u>H</u>$_2$-CH-].

2.5 General Procedure for Molar Absorptivity Measurements (41)

A stock solution of each compound was prepared using 100 mL volumetric flask using ethanol as a solvent. The resulting stock was then diluted to three concentrations. The UV absorbance of each final dilution was recorded by scanning wavelengths from 200 to 800 nm. The molar absorptivity (ε) at the wavelength of maximum absorbance (λ_{max}) was calculated using Beer's law:

$$A = \varepsilon bc$$

where *A* is absorbance

b is the cell path length (1 cm)

and *c* is the molar equivalent of the grafted chromophore

2.6 General Procedure for Photostability Test (41)

The photostability tests for the UV filters were performed in ethanol. Stock solution of each compound was prepared in a 100 ml volumetric flask using ethanol as a solvent. The resulting solutions were divided into two parts. One part was kept away from light (covered with foil) at room temperature (dark sample) while the other part was irradiated by artificial broad band UVA/UVB lamps at room temperature (irradiated sample). Then UV absorption profile of each sample was acquired using UV/VIS spectrometer. The absorbances of the irradiated sample at various irradiant times were compared to those of the dark samples. In each experiment, UV light irradiances were measured using UVA-400C power meter for UVA (320-400 nm) and UVB-500C power meter for UVB (280-320 nm).

The calculation of percent relative absorbance of each irradiated sample was done using the following equation:

$$\text{Percent of relative absorbance} = \left(\frac{\text{Absorbance of irradiated sample at time X}}{\text{Absorbance of dark sample(starting time)}} \right) \times 100$$