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) and Sephadex™ LH-20 (Amersham Biosciences AB, Uppsala, Sweden). Molecular weight was determined by gel permeation chromatography; PLgel 10 μm mixed B 2 columns (Water 150-CV) (Water, Germany). For UV irradiation, a Daavlin Psorawand UV-B lamp: PW-UVB-220 was used (Daavlin, OH, U.S.A.).

The FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared spectrophotometer: Impact 410 (Nicolet Instruments Technologies, Inc. WI, U.S.A.) The ^{29}Si-NMR spectra were obtained in deuterated chloroform ($CDCL_3$): JNM-A500 spectrometer (JEOL, Ltd., Japan) which operated at 500.00 MHz for ^{29}Si-NMR. The ^{1}H-NMR and ^{13}C-NMR spectra were obtained in deuterated chloroform ($CDCL_3$) using ACF 200 spectrometer which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, U.S.A.). Ultraviolet absorption spectra were obtained with the aid of HP 8453 UV/VIS spectrophotometer (Agilent Technologies, CA, U.S.A.). The UV absorbance were recorded using a sample in the 1 cm quartz cell.

2.2 Chemicals

Solvents used in syntheses and spectroscopic techniques 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.

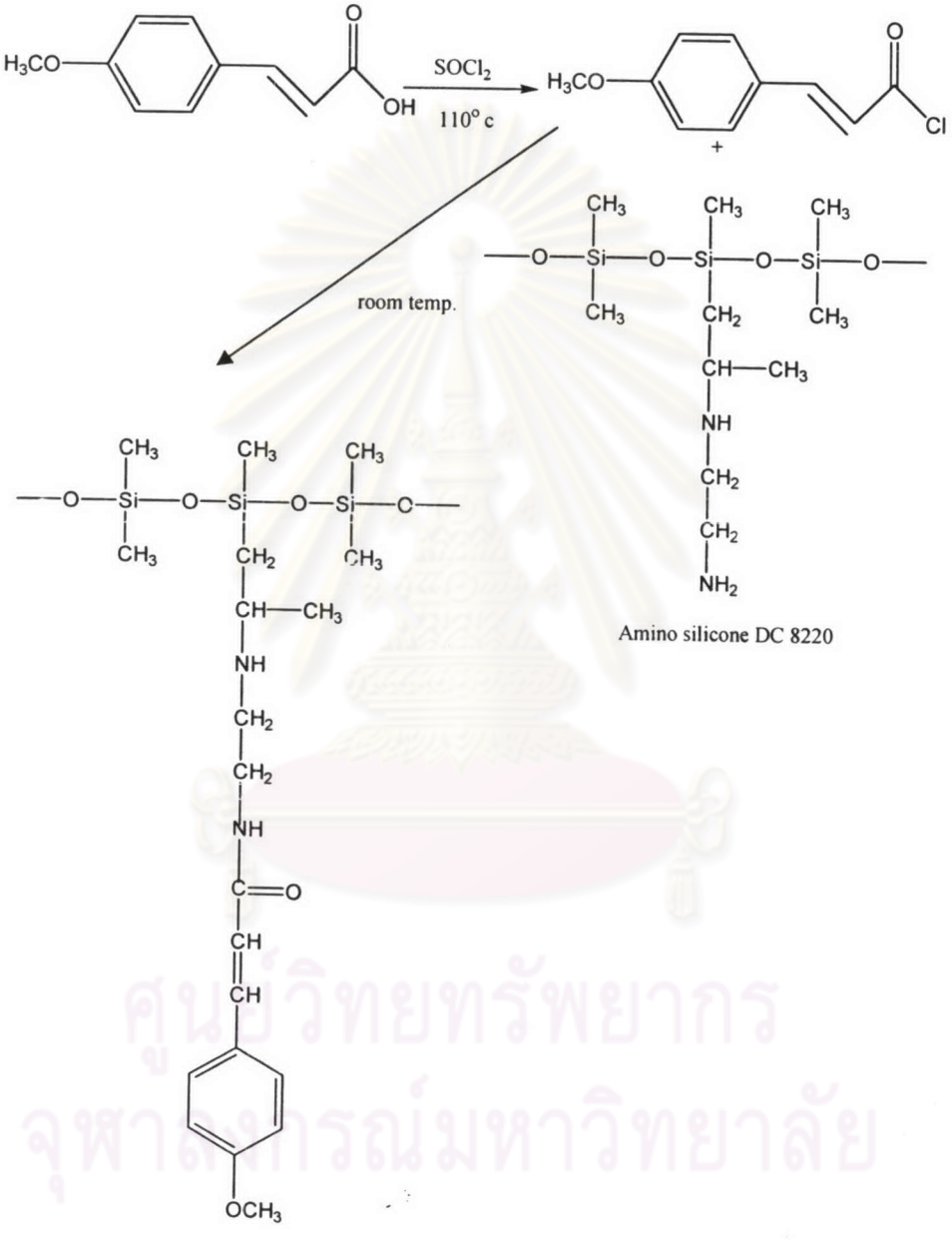
Platin/Aktivkohle: Pt catalyst, poly(methylhydrogensiloxane), octyl methoxycinnamate (OMC, Eucolex 2292), allyl alcohol and 1-octene was purchased from Merck Co. Ltd. (NJ, U.S.A.).

Amino silicone DC8220 and decamethyl cyclopentasiloxane were obtained from Dow Corning Corporation (Michigan, U.S.A.). The (6-7% aminopropylmethylsiloxane)-dimethylsiloxane copolymer was purchased from Gelest Company (Morrisville, PA, U.S.A.). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and N,N'-dicyclohexylcarbodiimide (DCC) were purchased from Acros Organics (New Jersey, U.S.A.).

4-Methoxycinnamic acid was purchased from Fluka Chemical Company (Buchs, Switzerland).

A. Amino silicone

2.3 Grafting of 4-methoxycinnamic acid on [N-(2-aminoethyl)-2-amino-2-methylethyl](methyl) siloxane; (Amino Silicone DC8220)[30]



Preparation of 4-methoxycinnamoyl chloride

A mixture of 1.78 g (0.01 mol) 4-methoxycinnamic acid and 1.77 g (0.015 mol) distillated thionyl chloride was refluxed at 110^0c. To the top of the condenser is attached an exit tube leading to a gas-absorption trap (K_2CO_3). A mixture was heated until no further evolution of

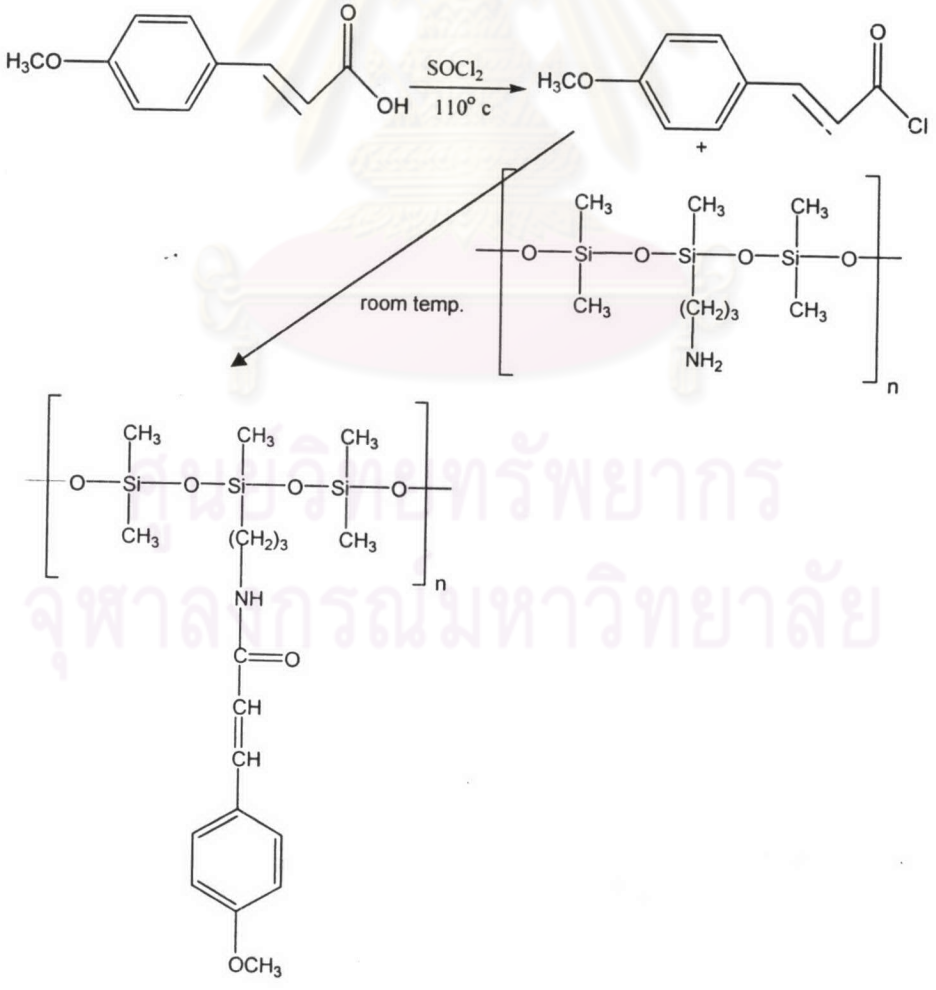
hydrogen chloride (60-90 minutes). An aspirator vacuum was applied to the top of the condenser to remove any remaining thionyl chloride.

Reaction of 4-methoxycinnamoyl chloride with amino silicone

Under N_2 atmosphere, approximately 2.5 mL (~ 0.015 mol) of freshly prepared 4-methoxycinnamoyl chloride were slowly dropped (1 drop/minute) from addition funnel into 15.0 g (~ 3.9×10^{-3} mol equivalent of primary amino groups) of stirring amino silicone DC8220. After completion, the mixture was kept stirring for 1 more hour (still under N_2 atmosphere) at room temperature. The reaction mixture was then diluted with 20 mL diethyl ether and washed with 100 mL of 5% sodium bicarbonate solution 4 times. Product was then dried with anhydrous Na_2CO_3, removed solvent and analyzed by ^{1}H-NMR and IR spectroscopy.

2.4 Grafting of 4-methoxycinnamic acid on (6-7% aminopropylmethylsiloxane)-dimethylsiloxane copolymer (AS)[30, 31]

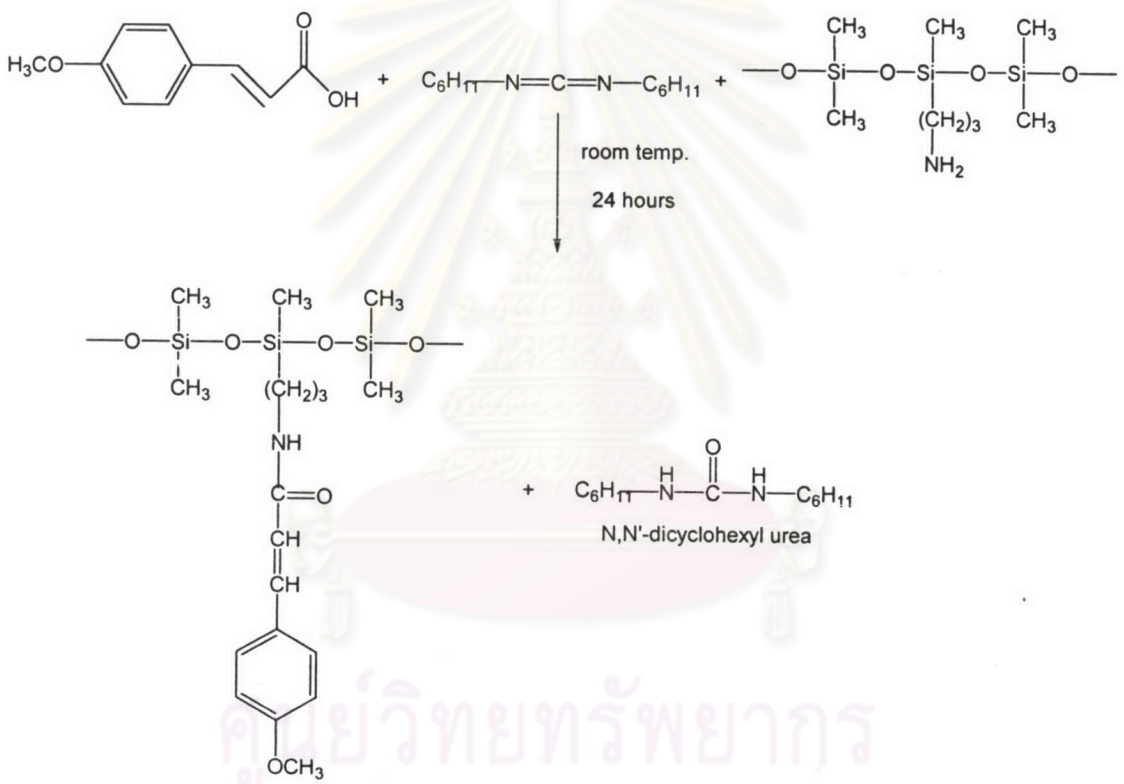
2.4.1 Acid Chloride Method



Reaction of 4-methoxycinnamoyl chloride with **AS**

Under N_2 atmosphere, approximately 1.5 mL (~ 0.01 mol) of freshly prepared 4-methoxycinnamoyl chloride were slowly dropped (1 drop/minute) from addition funnel into 5.0 g (~ 4.4×10^{-3} mol equivalent of amino groups) of stirring **AS**. After completion, the mixture was kept stirring for 1 more hour at room temperature. The reaction mixture was then diluted with 20 mL diethyl ether and washed with 100 mL of methanol 4 times. Product was then dried with anhydrous Na_2CO_3, removed solvent and analyzed by ^{1}H-NMR and IR spectroscopy.

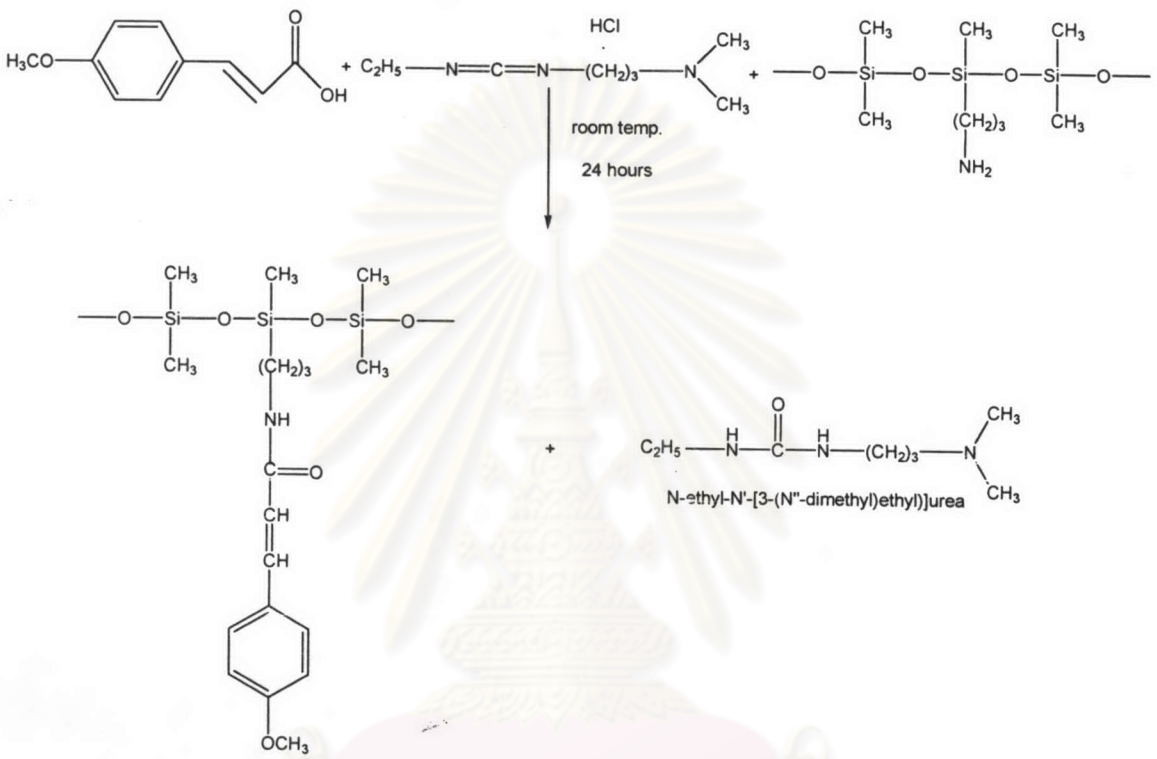
2.4.2 N,N'-Dicyclohexylcarbodiimide (DCC) Coupling Method



4-Methoxycinnamic acid (0.78 g, 4.4×10^{-3} mol) and 5.0 g (~ 4.4×10^{-3} mol equivalent of amino groups) **AS** were dissolved together in 20 mL dichloromethane and DCC (0.90 g, 4.38×10^{-3} mol) was added. The mixture was stirred at room temperature for 24 hours. Then the mixture was washed with 100 mL of 5% hydrochloric acid solution for 4 times. The solvent was then removed from the mixture by rotary evaporator. The product was then passed through sephadex™ LH-20 column (1 g product:15 g sephadex LH-20 dry weight) using dichloromethane as mobile

phase. Fractions were collected and analyzed by ^{1}H-NMR spectroscopy. The pooled fractionized product was reapplied to the column 4 times.

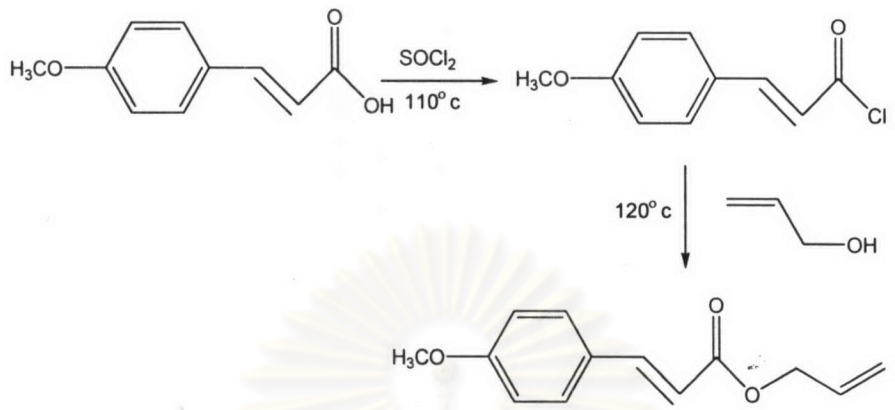
2.4.3 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) Coupling Method



4-Methoxycinnamic acid (2.314 g, 0.013 mol) and 15.0 g (~ 0.013 mol equivalent of amino groups) **AS** were dissolved in 30 mL dichloromethane and EDCI (2.52 g, 0.013 mol) was added. The mixture was stirred at room temperature for 24 hours. After that, the mixture was washed with 4 times of 100 mL of 5% hydrochloric acid solution. The product was then dried with anhydrous Na_2CO_3, removed solvent and analyzed by ^{1}H, ^{13}C-NMR, IR, UV spectroscopy and gel permeation chromatography.

B. Poly (methylhydrogensiloxane); MHS

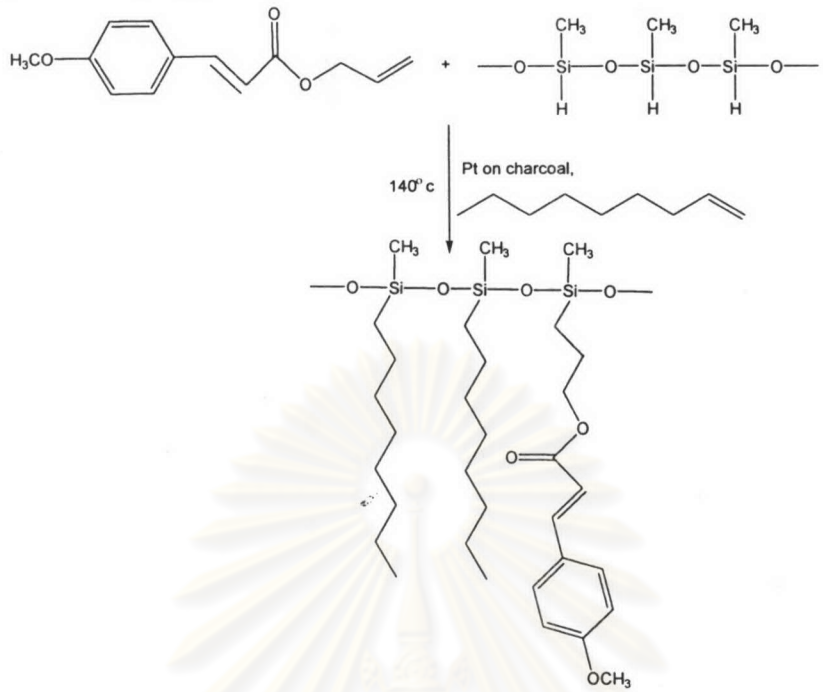
2.5 Synthesis of 2-propylene-4-methoxy cinnamate[31, 32]



A mixture of 17.8 g (0.1 mol) of 4-methoxycinnamic acid and 17.7 g (0.15 mol) freshly distilled thionyl chloride was refluxed at 110^0c. To the top of the condenser is attached on exit tube leading to a gas-absorption trap (K_2CO_3). A mixture was heated until no further evolution of hydrogen chloride (60-90 minutes). An aspirator vacuum was applied to the top of the condenser to remove any remaining thionyl chloride before 0.1 mol of freshly distilled allyl alcohol (6.0 mL) was added. The mixture was again refluxed on a sand bath at 120^0c for 2 hours. After the reaction was completed (checked by TLC), the reaction mixture was cooled and washed with 200 ml of 5% sodium bicarbonate solution. The solvent was removed by rotary evaporator. The product was purified by passing through silica gel column and was analyzed with ^{1}H-NMR.

2-propylene-4-methoxy cinnamate: pale red oil (83%), R_f 0.65 (30% EtOAc/Hex), IR (neat,cm^{-1}): 825, 934, 1026, 1364, 1456, 1511, 1636, 1707, 2932; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.69-7.65 (d, *J*=16.0 Hz, 1H, Ar-C**H**=), 7.49-7.47 (d, *J*=8.0 Hz, 2H, Ar-**H**), 6.91-6.89 (d, *J*=8.0 Hz, 2H, Ar-**H**), 6.36-6.32 (d, *J*=16.0 Hz, 1H, =C**H**COOR$_1$), 6.04-5.88 (m, 1H, CH_2C**H**CH_2), 5.39-5.25 (q, 2H, CH=C**H**$_2$), 4.71-4.70 (d, *J*=4.0 Mz, 2H, OC**H**$_2$R) and 3.83 (s, 3H, OC**H**$_3$)

2.6 Grafting of 10% 2-propylene-4-methoxy cinnamate on MHS [10]



To the 50 mL 2 necks round bottom flask, 1.526 g (7.0×10^{-3}mol) of 2-propylene-4-methoxy cinnamate and 4.6 g (2×10^{-3}mol, 7.0×10^{-2} mole equivalent of Si-H) **MHS** were added. Then 0.02 g (1.03×10^{-4}mol) of Pt catalyst was added to the reaction mixture. The mixture was refluxed at 140^{0}c under N_2 gas until all 2-propylene-4-methoxy cinnamates were completely grafted on **MHS** (no 2-propylene-4-methoxy cinnamate left, checked by ^{1}H-NMR). Then access amount of distilled octene (~ 21 mL, 15.0 g , 0.13 mol) was added while the mixture was still being refluxed at the same condition. The reaction was carried on until no Si-H can be detected (complete disappearance of silyl hydride proton at 0.0 ppm (^{1}H-NMR) together with the complete disappearance of Si-H at 2176 cm^{-1} in IR).

Twenty five and fifty percents 2-propylene-4-methoxy cinnamate grafting were done as previously described but amounts of starting material added were as followed:

<u>25% grafting</u>

- 2-propylene-4-methoxy cinnamate: 3.815 g ($1.75\text{x}10^{-2}$mol)
- octene: 17 mL (11.8 g, 0.11mol)
- **MHS**: 4.6 g ($2\text{ x }10^{-3}$mol, $7.0\text{x}10^{-2}$ mole equivalent of Si-H)

<u>50% grafting</u>

- 2-propylene-4-methoxy cinnamate: 7.63 g (0.035 mol)
- octene: 12 mL (7.85 g, 0.07 mol)
- **MHS**: 4.6 g ($2\text{ x }10^{-3}$mol, $7.0\text{x}10^{-2}$ mole equivalent of Si-H)

2.7 General Procedure for Molar Absorptivity Measurements[33, 34]

Tested compounds were dissolved in hexane or butanol to the concentration of 1 g/L. The resulting stock solution was then diluted to selected concentrations using corresponding solvents. The UV absorbance of each final dilution was recorded by scanning wavelengths between 200 and 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)

c is the concentration of the absorbing species in mol per litre

2.8 General Procedure for Photostability Test[34]

The photostability tests for the UV filters were performed in butanol and hexane. Stock solution of each compound was prepared in a 100 ml volumetric flask. 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 UV lamp at room temperature (irradiated sample). Then UV absorption profile of each sample was acquired using UV/VIS spectrometer. The absorbance of irradiated sample at various irradiant times were compared to those of dark samples.

The calculation of percent relative absorbance of each irradiated sample is given by:

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

2.9 General Procedure for Absorption Test[35]

The skin absorption tests were performed on volunteers by applying exact amount of the tested sample on the upper arm skin. After 5 hours, the sample was recovered by whipping with cotton pad moisted with hexane. Attention was paid to recover all the remaining compounds on the area which means that most of the dead cell epidermis should be rubbed off. The whipped cotton pads were soaked in hexane for 24 hours. After hexane was removed by rotary evaporation, the recovered remaining compounds was then dissolved in exact amount of $CDCl_3$ mixed with known amount of acetone ($CDCl_3$:acetone = 10 ml:10 μl). Attention was paid to

deliver spiked $CDCl_3$ to each sample equally. Controls were also prepared by dissolving the same amounts of tested compounds (as those applied to the skin) into the same amount of spiked $CDCl_3$.

The calculation of percent recovery of each testing sample was done as follow;

$$RS = \frac{\text{integration of Ar-H of sample at 7.38-7.36 ppm}^{viii}}{\text{integration of acetone protons at 2.1 ppm}}$$

$$TS = \frac{\text{integration of Ar-H of sample at 7.38-7.36 ppm}^{ix}}{\text{integration of acetone protons at 2.1 ppm}}$$

RS is an amount of recovered sample relative to acetone internal standard.

TS is an amount of total sample applied on the skin relative to acetone internal standard.

$$\text{percent of recovery} = \left(\frac{RS}{TS}\right) \times 100$$

Sample Preparation

G-AS absorption test

Amounts of samples applied on skin were as follow;

a). 0.5296 g (1.83×10^{-3} mol) OMC + 2 ml ethanol; syringe 15 μl of the solution and apply to skin

b). 0.5279 g (1.82×10^{-3} mol) OMC + 2 ml **AS** + 4 ml hexane; syringe 45 μl of the solution and apply to skin

c). 2.313 g (1.8×10^{-3} mol equivalent of cinnamate) **G-AS** + 4 ml hexane; syringe 45 μl of the solution and apply to skin

G-HS absorption test

Samples were prepared to give equal molar concentration of chromophoric groups. Amounts of tested compounds which applied on skin were follow;

[viii] From NMR spectrum of sample recovered from skin after 5 hours application.

[ix] From NMR spectrum of control sample (same amount as I but no application on skin).

a). 0.2140 g (7.40×10^{-4} mol) OMC + 1 ml ethanol; syringe 15 μl of the solution and apply to skin

b). 0.2118 g (7.30×10^{-4} mol) OMC + 1 ml decamethyl cyclopentasiloxane + 3 ml hexane; syringe 60 μl of the solution and apply to skin

c). 1.5057 g (7.30×10^{-4} mol equivalent of cinnamate) **G-MHS** + 3 ml hexane; syringe 45 μl of the solution and apply to skin