CHAPTER III

RESULT AND DISCUSSION

A. Amino silicone

3.1 Grafting of 4-methoxycinnamic acid on [N-(2-aminoethyl)-2-amino-2-methylethyl] (methyl) siloxane; DC 8220

When 1.78 g (0.01 mol) 4-methoxycinnamic acid were refluxed with 1.77 g (0.015 mol) distilled thionyl chloride at 110^0c for 60-90 minutes, the formation of 4-methoxycinnamoyl chloride was detected by TLC (the spot of acid chloride was only a little upper than that of acid but still can be detectable). When adding about 1 ml of 4-meyhoxycinnamoyl chloride into 15.0 g amino silicone **DC 8220** at room temperature a white sticky rubbery material was observed. The amounts of this white rubber material increased as more 4-meyhoxycinnamoyl chloride were added into the reaction mixture. When only 0.5 mol equivalent (as compared to number of amino groups of the starting material) of 4-meyhoxycinnamoyl chloride was used, although clear yellow liquid product was obtained, NMR analysis revealed almost undetectable grafted chromophores. Since amino silicone DC 8220 has only 2% primary amino groups this may cause a problem in analysis, we, therefore, decided to use amino silicone with more primary amino moieties on the siloxane chain.

3.2 Grafting of 4-methoxycinnamic acid on (6-7% aminopropylmethylsiloxane)-dimethylsiloxane copolymer; AS

3.2.1 Acid Chloride Method

When 0.78 g ($4.38x10^{-3}$ mol) 4-methoxycinnamic acid were refluxed with 1.30 g (0.01 mol) distilled thionyl chloride at 110^0c for 60-90 minutes, the formation of 4-meyhoxycinnamoyl chloride was detected by TLC. When adding about 1 ml of 4-meyhoxycinnamoyl chloride into 5.0 g amino silicone (**AS**) at room temperature, the formation of white rubbery solid could be seen. This white rubbery solid increased as amount of added 4-meyhoxycinnamoyl chloride increased. The reaction seemed to be too vigorous since crosslinking and formation of the solid occurred quickly. We, therefore, decided to switch into a milder method.

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

When (0.78 g, 4.38×10^{-3} mol) 4-methoxy cinnamic acid were mixed with 5.0 g **AS** in the presence of **DCC** (0.90 g, 4.38×10^{-3} mol) and 20 ml dichloromethane, after stirring for 24 hours at room temperature, white precipitate was observed. Attempts were made in order to separate the white precipitate (N,N'-dicyclohexylurea) out from example. Filtering and washing with methanol followed by passing (the evaporized product) through sephadex LH-20 column (using dichloromethane as mobile phase) was performed. The DCU was still contaminated after 4 times column through sephadex LH-20. We, therefore, decide to switch to a coupling agent with less trouble by product.

3.2.3 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) Coupling Method

In this reaction, grafting of 2.314 g (1.5 mol equivalent) 4-methoxycinnamic acid on 15.0 g (1.0 mol equivalent between 4-methoxycinnamic acid and amino groups on **AS**) **AS** was done with 2.52 g (1.5 mol equivalent) of the coupling agent, EDCI. The reaction was stirred at room temperature for 24 hours and the resulting reaction mixture was a pale yellow solution. Formation of amide linkage between 4-methoxycinnamic acid and **AS** was confirmed by IR (see section 3.6). Purification of N-ethyl-N'-[3-(N''-dimethyl)ethyl]urea (by product) was done easily by extraction with 3×100 ml 5% hydrochloric solution. The yellow viscous liquid product was characterized by ^{1}H, ^{13}C-NMR and IR. From NMR data (see section 3.6) it can be concluded that the product was [3-(*p*-methoxycinnamido)propyl](methyl)-dimethylsiloxane copolymer; **G-AS**. Integration of ^{1}H-NMR indicates 6-7% (by mole) 4-methoxycinnamic acid residues on the polymer chain (see Appendix A for calculation). The molecular weight of the product was obtained by gel permeation chromatography technique (see section 3.6) was 5,000-6,000.

Experiments were done to obtain UV absorption properties (λ_{max}, ε) of this product. As shown in Figure 3.1, [3-(*p*-methoxycinnamido)propyl](methyl)-dimethylsiloxane copolymer; **G-AS** has one absorption band; λ_{max} of 288 nm (ε = 20.6255 AU Lg^{-1}) in hexane which correspond to the UVB region. This is correspond to the absorption profile of the 4-methoxycinnamoyl moiety. The value of ε also correspond to the fact that there was about 6-7% (by mole) substitution of 4-

methoxycinnamoyl group on **AS** (see Appendix A for calculation).

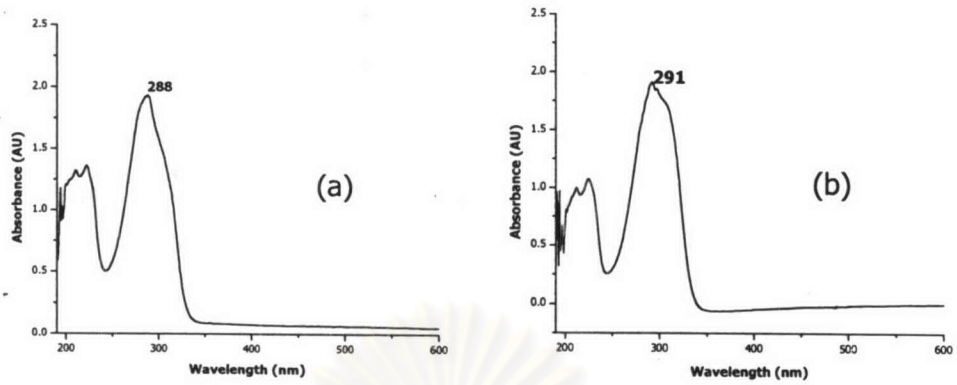


Figure 3.1 UV spectra of 0.1 g/L **G-AS** in hexane (a) and n-butanol (b)

B. Poly (methylhydrogensiloxane); MHS

3.3 Grafting of 10% 2-propylene-4-methoxy cinnamate on MHS

The reaction of 1.526 g ($7.0x10^{-3}$mol) 2-propylene-4-methoxy cinnamate with 4.6 g (2 x 10^{-3}mol) **MHS**♣ was carried out in 20 ml of toluene at 140 ^{0}c with 0.02 g Pt on charcoal (contain $3.5x10^{-6}$ mol of Pt) as catalyst (see page 20). To follow the reaction, the reaction mixture was withdraw (approximately 2-3 drops) at appropriated time and subjected to ^{1}H-NMR analysis using $CDCl_3$ as NMR solvent. The disappearance of vinylic proton of the propylenic moiety at 6.04-5.88 ppm (m, 1H, CH_2=C**H**CH_2-R) and 5.39-5.25 ppm (q, 2H, C**H**$_2$=CH-C**H**$_2$-R) indicated the completeness of hydrosilylation of 2-propylene-4-methoxy cinnamate on **MHS** (see Appendix B). After all the 2-propylene-4-methoxy cinnamate were grafted which usually took about 48 hrs, excess amounts of freshly distilled octene (15.0 g , 0.13 mol) were added and the reaction was kept stirring at the same temperature. Completion of hydrosilylation was checked by withdrawing the reaction mixture (approximately 2-3 drops) and subjected to ^{1}H-NMR analysis using $CDCl_3$ as solvent. The disappearance of proton at 0.00 ppm (s, 1H, Si-**H**) indicated that there was no Si-H left and therefore all hydrides proton on silicon must be replaced by octyl groups (see Appendix B). After this, the reaction was stopped by removing the heat and filtering out the catalyst. After evaporizing out the left over octene and toluene, yellow viscous liquid product was obtained. The product's structure was well characterized using various spectroscopic techniques including ^{1}H, ^{13}C, ^{29}Si-NMR and IR spectroscopy (see section 3.6). It can be concluded that the product was

♣ M.W. of **MHS** is 2280.5. Its molecular formula is $(CH_3)_3SiO[SiH(CH_3)O]_{35}Si(CH_3)_3$. The 4.6 g of **MHS** contain 35 mol equivalent of Si-H funtional groups.

poly[(methyl)(octyl)(methyl)(propyl-4-methoxycinnamatesiloxane]; **G-MHS**. The molecular weight of the product obtained by gel permeation chromatography technique (see section 3.6) was 6,000-7,000. This number agrees with the calculation of 10% 2-propylene-4-methoxy cinnamate and 90% octyl groups on siloxane chains. The reasons that octyl groups were put onto silicone is 1). to make a more hydrophobic environment around cinnamate moieties in order to promote more *E*-configuration.[34] 2). to prevent close encounter between two cinnamate groups which may lead to photocyclization.

Experiments were done to obtain UV absorption properties (λmax, ε) of this **G-MHS** product. As shown in Figure 3.2, poly[(methyl)(octyl)(propyl)-4-methoxycinnamatesiloxane] has one absorption band; λmax of 284 nm (ε = 2.6374 AU Lg^{-1}) in hexane which corresponds to the UVB region.

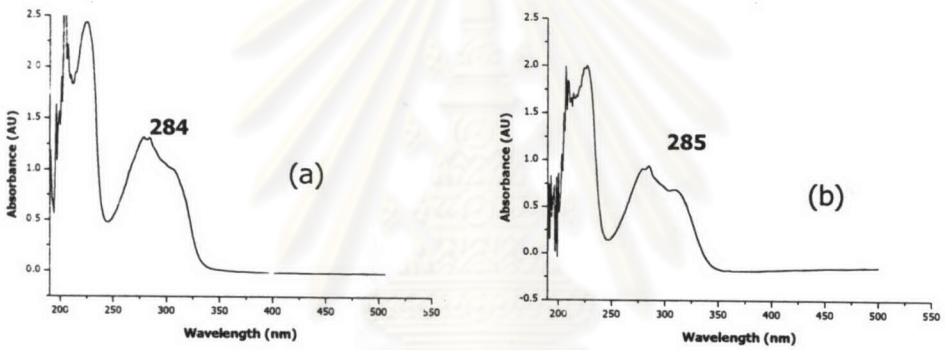


Figure 3.2 UV spectra of 0.6 g/L **G-MHS** in hexane (a) and n-butanol (b).

-25% grafting

This grafting reaction was done using the same condition as in 10% grafting reaction. The reaction was also monitored similarly. After evaporizing solvent and the remaining octene by rotary evaporation, yellow viscous liquid was obtained. The product was kept in the light-proof container under N_2. It was observed that as the product started to cool down, viscosity increased rapidly and the product started to gel within about 1 hour after cooling. At last, all of the products transformed into a non-soluble pale yellow gel.

-50% grafting

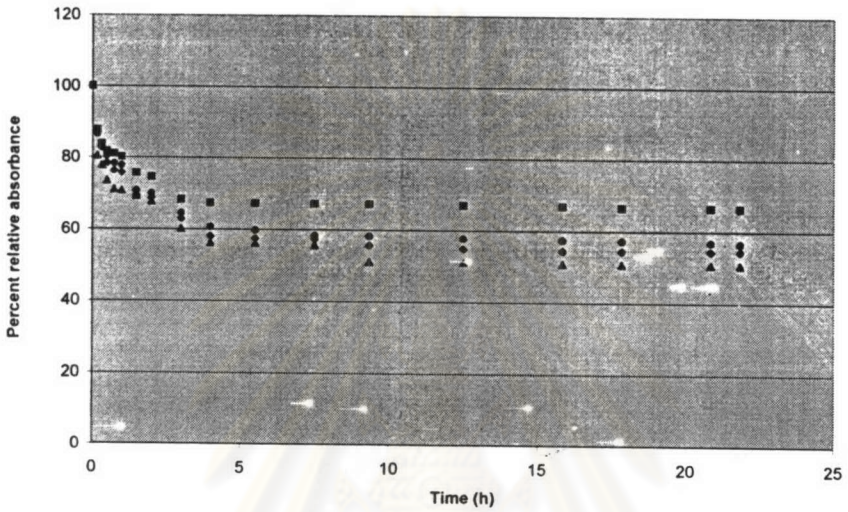
This grafting reaction was done as previously described for 10% grafting. The reaction was also monitored similarly. After evaporizing solvent and the remaining octene by rotary evaporation, the product started to gel immediately. At last, all of the products transformed into non-soluble yellow gel.

3.4 Photostability Test

3.4.1 G-AS

G-AS, OMC+**AS** and OMC were subjected to photostability test in hexane and n-butanol. The tests were done at two different concentrations. Figure 3.3 and 3.4 shows the photo-equilibrium of **G-AS** in hexane and n-butanol respectively.

A)



B)

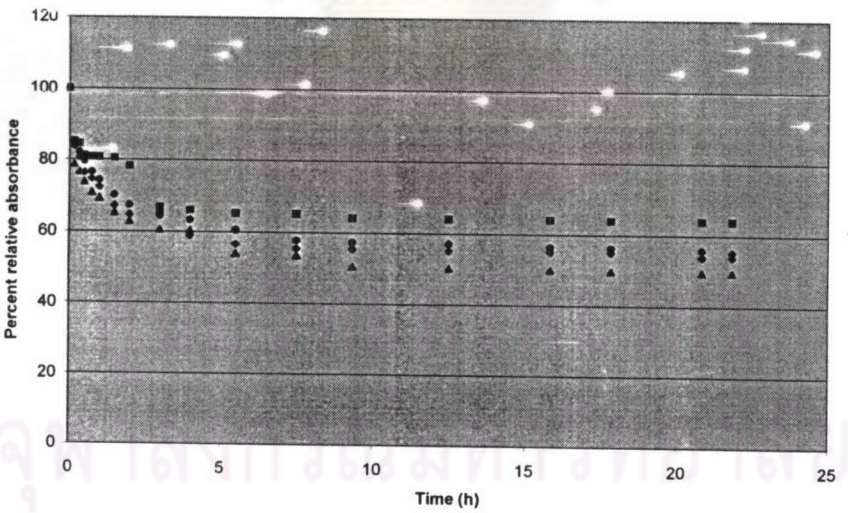
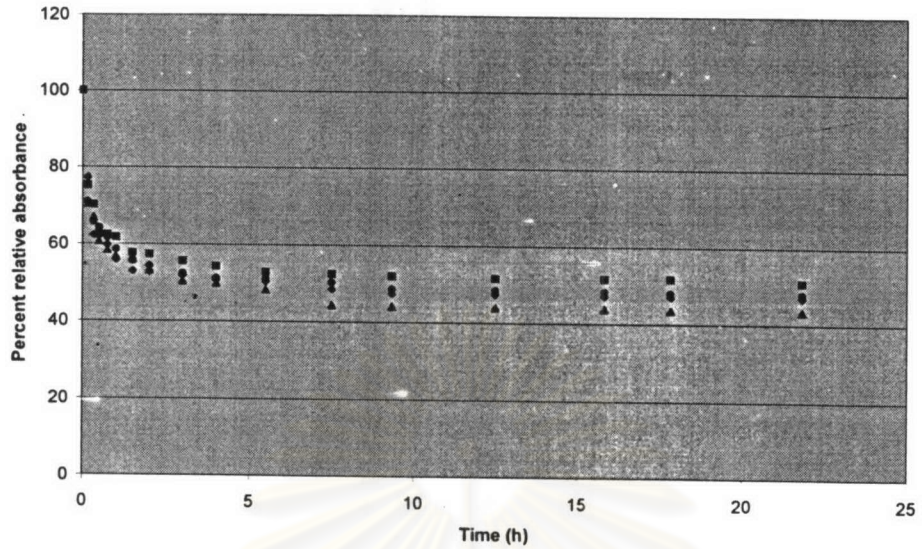


Figure 3.3 Photo-equilibrium of **G-AS** in hexane;

A) ◆ **G-AS** 0.1 g/L (7.8×10^{-5}mol of chromophore/L), ■ OMC+**AS** 0.11 g/L (8.6×10^{-5}M OMC), ● OMC+dimethicone 0.11 g/L (8.6×10^{-5}M OMC) , ▲ OMC 6.9×10^{-5} M

B) ◆ **G-AS** 0.04g/L (3.1×10^{-5}mol of chromophore /L), ■ OMC+**AS** 0.05 g/L (3.9×10^{-5}M OMC), ● OMC+dimethicone 0.05 g/L (3.9×10^{-5}M OMC), ▲ OMC 2.81×0^{-5} M

A)



B)

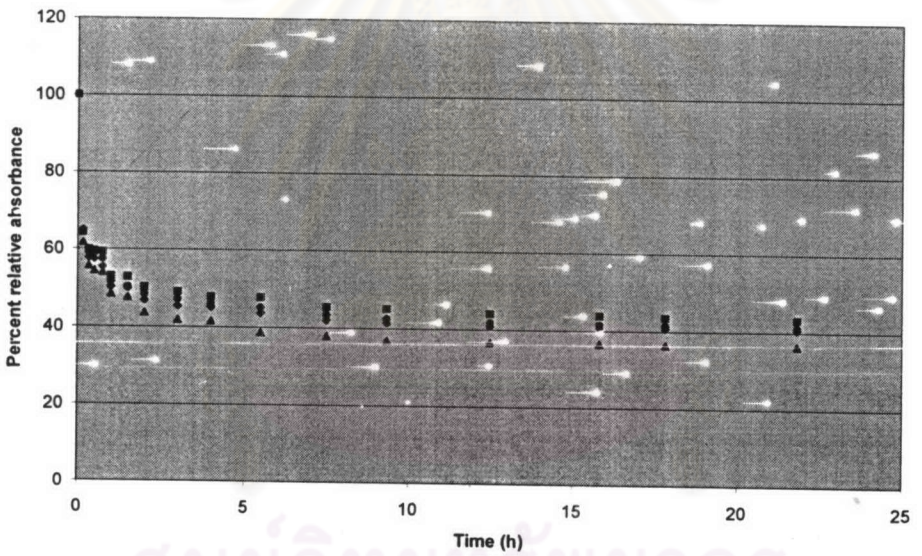


Figure 3.4 Photo-equilibrium of **G-AS** in n-butanol;

A) ◆ **G-AS** 0.1 g/L (7.8×10^{-5} mol of chromophore/L), ■ OMC+**AS** 0.11 g/L (8.6×10^{-5}M OMC), ● OMC+dimethicone 0.11 g/L (8.6×10^{-5}M OMC) , ▲ OMC 6.9×10^{-5} M

B) ◆ **G-AS** 0.04g/L (3.1×10^{-5} mol of chromophore /L), ■ OMC+**AS** 0.05 g/L (3.9×10^{-5}M OMC), ● OMC+dimethicone 0.05 g/L (3.9×10^{-5}M OMC), ▲ OMC 2.81×0^{-5} M

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From Figure. 3.3 and 3.4, it can be shown that the product **G-AS** was more photostable than free OMC. The reason on this may due to the clustering of hydrophobic silicone around the grafted chromophore. However, this **G-AS** product was less stable than OMC+**AS.** The reason that OMC+**AS** was more stable than **G-AS** may stem from possible hydrogen bonding between primary amino groups of the amino silicone **AS** and oxygen at carbonyl groups of the OMC molecules (Figure. 3.5) hence hinder the configurational change and stabilize the clustering of silicone around the chromophore.

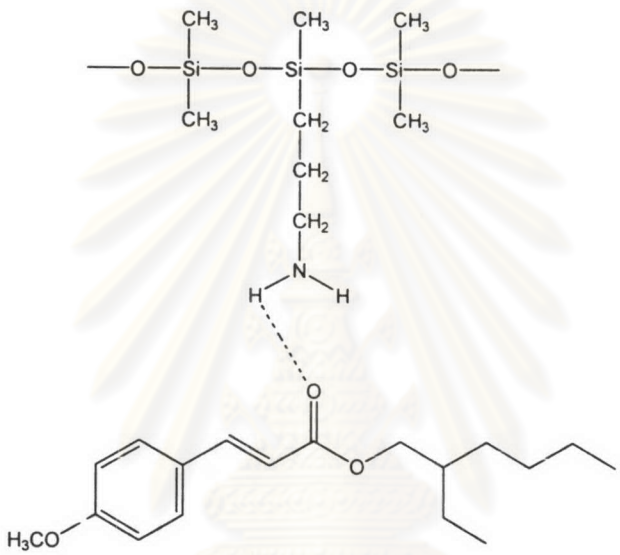


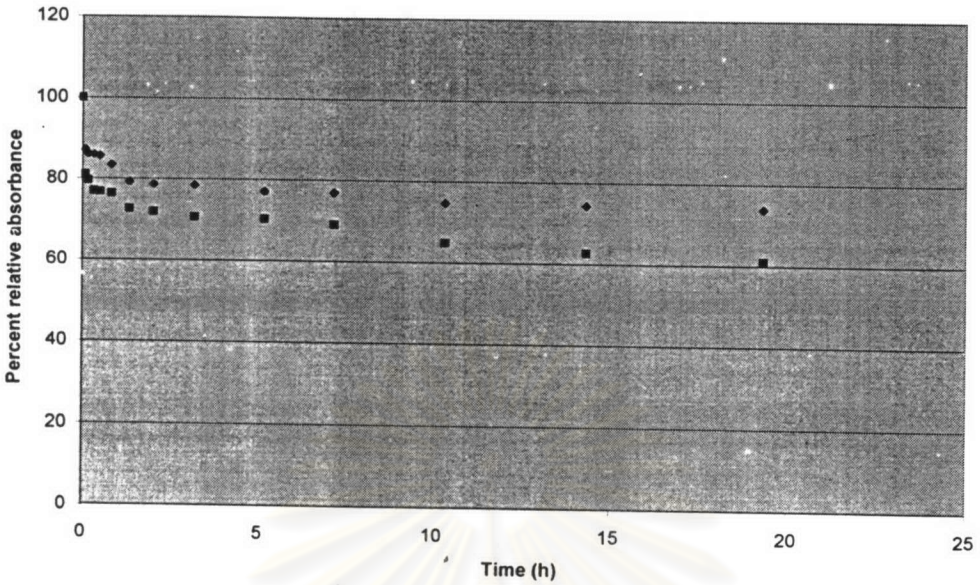
Figure 3.5 The hydrogen bonding between amino silicone **AS** and OMC

The photostability of all the four cases (**G-AS**, OMC+**AS**, OMC+dimethicone, free OMC) in hexane and butanol gave the same trend as discussed above. Moreover, all cases show more photostability in hexane than in butanol. This result agrees with previous study which indicated that free OMC is more stale in nonpolar solvent than in polar solvent.[26]

3.4.2 **G-MHS**

G-MHS and OMC were subjected to the similar photostability test in both hexane and n-butanol. The tests were done at two different concentrations. Figure 3.6 and 3.7 show the photo-equilibrium of **G-MHS** in hexane and n-butanol respectively.

A)



B)

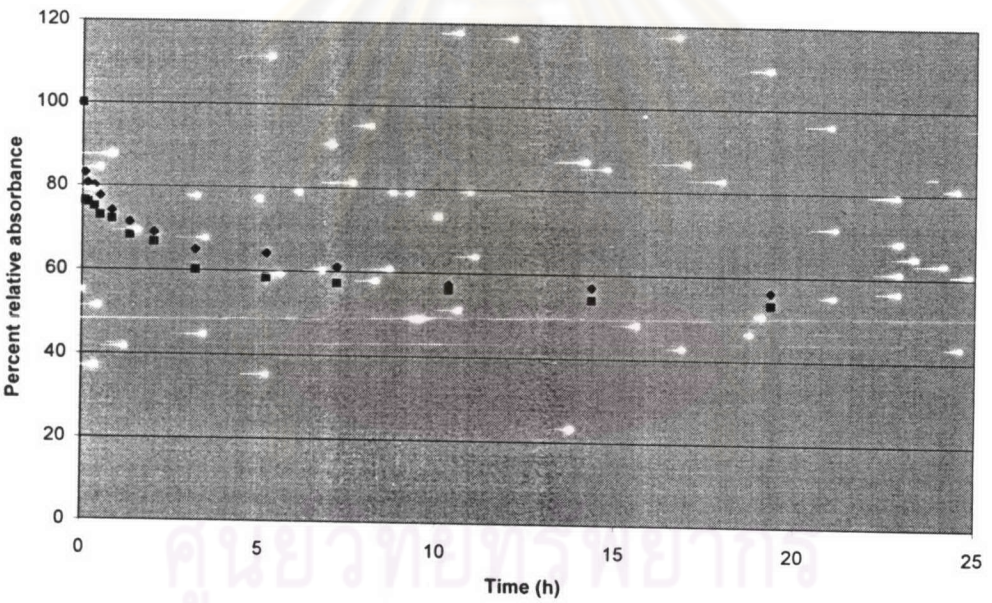


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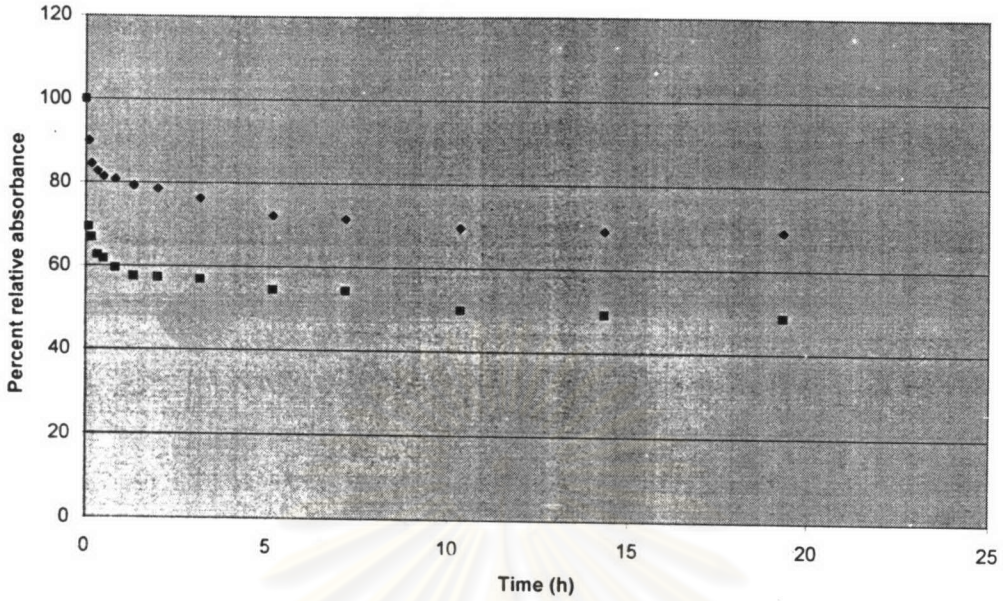
Figure 3.6 Photo-equilibrium of **G-MHS** in hexane;

A) ◆ **G-MHS** 0.6 g/L (2.9×10^{-4}mol of chromophore/L), ■ OMC 1.0×10^{-4}M

B) ◆ **G-MHS** 0.2 g/L (9.6×10^{-5}mol of chromophore/L), ■ OMC 2.8×10^{-5}M

A)



B)

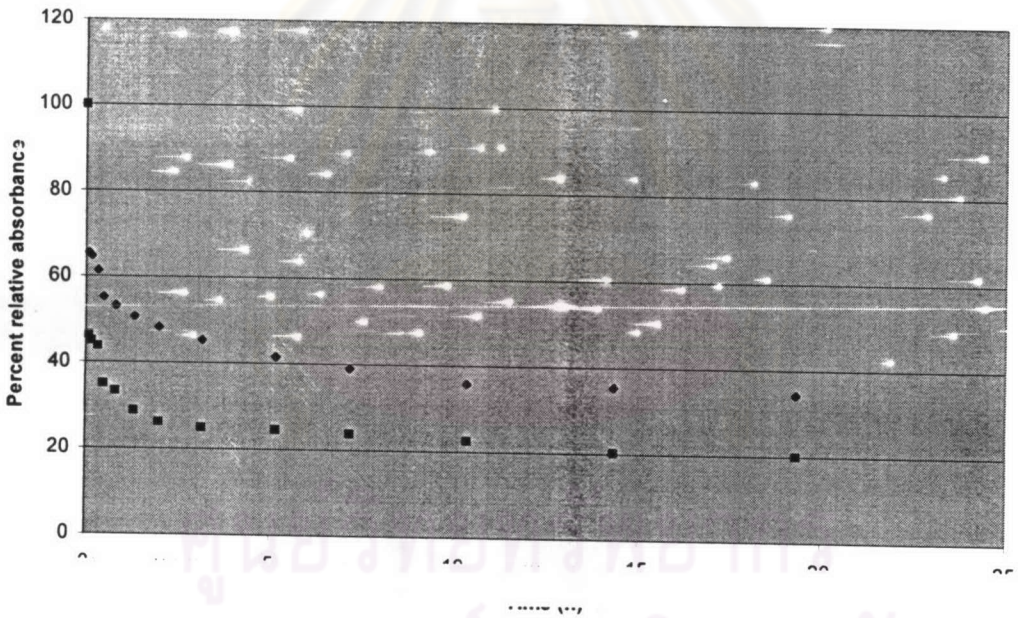


Figure 3.7 Photo-equilibrium of **G-MHS** in n-butanol;

A) ◆ **G-MHS** 0.6 g/L (2.9×10^{-4}mol of chromophore/L), ■ OMC 1.0×10^{-4}M

B) ◆ **G-MHS** 0.2 g/L (9.6×10^{-5}mol of chromophore/L), ■ OMC 2.8×10^{-5}M

From Figure. 3.6 and 3.7, it can be seen that **G-MHS** product was more photostable than OMC. The reason on this may due to the clustering of hydrophobic silicone around the grafted chromophore. Both **G-MHS** and OMC were also more photostable in hexane than in n-butanol.

3.5 Skin absorption test

This study was done in order to compare the degree of absorption through human skin among grafted products, free OMC and free OMC mixed with silicone. The tests were done on 5 volunteers by applying exact amount of test sample on the upper arm skin. After 5 hours the sample was recovered by whipping with cotton pad moisted with hexane. When all amounts of samples were completely recovered, the cotton pads were soaked in hexane for 24 hours. After hexane was removed by rotary evaporation, samples were dissolved in $CDCl_3$ spiked with known amounts of acetone ($CDCl_3$:acetone = 10 ml: 10 μl). Acetone was used as internal standard because it gives ^{1}H-NMR peak well resolved from peaks of our samples. Integration of $\mathbf{CH_3}C{=}O\mathbf{CH_3}$ protons at 2.1 ppm was then used as reference in the calculation.

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

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

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

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.

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

Example of calculation.

Figure 3.8 shows the positions of acetone protons at 2.1 ppm and Ar-H peak of sample at 7.38-7.36 ppm in ^{1}H-NMR spectrum of a sample.

Total sample

integration of ^{1}H-NMR peak at 2.1ppm (from acetone internal standard) = 1.9

integration of ^{1}H-NMR peak at 7.38-7.36 ppm (from 4-methoxy cinnamate) = 0.9

amounts of sample used = 0.9/1.9 = 0.4737

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

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

Testing sample

integration of ^{1}H-NMR peak at 2.1ppm (from acetone internal standard) = 1.2

integration of ^{1}H-NMR peak at 7.38-7.36 ppm (from 4-methoxy cinnamate) = 0.3

amounts of sample recovered = 0.3/1.2 = 0.25

Percent of recovery = (RS/TS) × 100 = (0.25/0.4737) x 100 = 52.78%

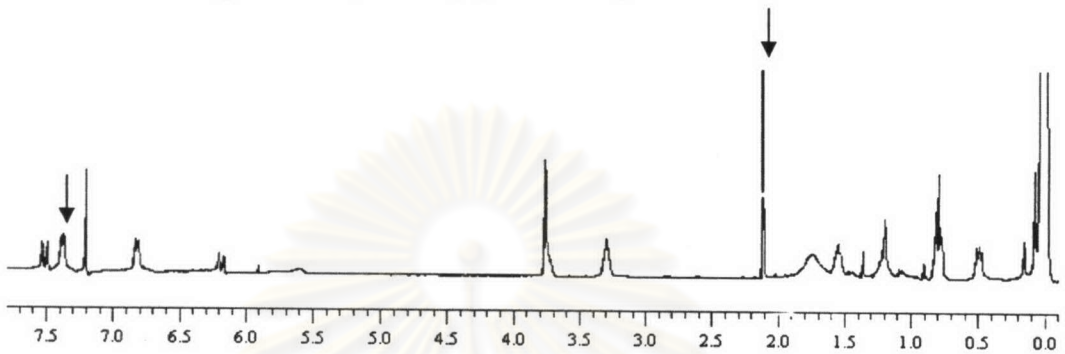

Figure 3.8 ^{1}H-NMR of a sample in absorption test

Table 3.1 and 3.2 summarized the calculations from the results of skin absorption tests on 5 volunteers.

Table 3.1 Results of skin absorption tests of **G-AS** on 5 volunteers

Volunteers	percents recovery			
	OMC	OMC+**AS**	**G-AS**	**AS**
1	54.45	73.28	92.96	96.44
2	53.17	76.54	92.51	
3	53.40	73.76	94.28	
4	52.48	73.28	93.18	
5	53.81	75.48	90.39	

Table 3.2 Results of skin absorption tests of **G-MHS** on 5 volunteers

Volunteers	percents recovery			
	OMC	OMC+dimethicone	**G-MHS**	dimethicone
1	56.46	69.17	86.48	97.98
2	58.23	72.46	89.80	
3	57.78	71.77	87.39	
4	57.99	71.89	88.54	
5	57.85	71.29	88.92	

Absorption tests of both **G-AS** and **G-MHS** gave similar results. All results on volunteers showed moderate lost of free OMC, (only about 54% OMC was recovered) presumably absorbed through skin. While mixing of free OMC with amino silicone **AS** reduce lost of OMC from about 46% to about 26%. Grafting of 4-methoxycinnamic acid on amino silicone **AS** further reduce lost to about 7%. This partially supports the fact that silicone polymer cannot penetrate skin easily, therefore covalently linked the cinnamate groups on silicone could render the cinnamate with silicone to stay on the skin surface. The same explanation can also be applied with the results from **G-MHS** tests.

From the above results, the grafting of UV absorptive chromophore should applicable to others polymer and silicones with other functionalities. It is possible to make polysiloxane with both pendant UV absorptive chromophores and other functional groups thus UV absorptive silicone with more appealing formulation properties can be obtained. More importantly, skin permeation problem of sunscreen can be solved by covalently link the UV absorptive chromophore onto appropriate polymeric materials.

3.6 Spectroscopic data of all grafted products

The structure of all grafted products were well characterized using various spectroscopic techniques including ^{1}H, ^{13}C-NMR, IR and GPC (gel permeation chromatography). ^{29}Si-NMR was used to confirm the structure of **MHS** and the product; **G-MHS**. Spectroscopic spectra of all compound are shown in Appendix A.

3.6.1 Infrared spectroscopy

Both **G-AS** and **G-MHS** products display the characteristic of common functional groups in IR spectra. For **G-AS**, N-H stretching vibrations of amine and amide are represented at 3270 cm^{-1} (br). C-H stretching of aromatic ring can be seen at 3067 cm^{-1}. The absorption band around 2960-2900 cm^{-1} corresponds to C-H stretching of aliphatic hydrocarbons. The C=O stretching vibration of the amide is at 1660 cm^{-1}. The C=C (next to aromatic ring) stretching vibration at 1552 cm^{-1} and C=C ring stretching at 1600-1400 cm^{-1} are also detected. The C-N stretching vibrations are shown at 1265 cm^{-1}.

In the case of **G-MHS**, C=O stretching vibrations were detected at 1717 cm^{-1}. The C=C (next to aromatic ring) and C=C ring stretching were detected at 1600-1465 cm^{-1}.

3.6.2 NMR spectroscopy

^{1}H-NMR

For NMR spectroscopy, $CDCl_3$ was used as solvents for all compounds. The ^{1}H-NMR spectrum of **G-AS** shows two doublet signals with each of 1H integration at 7.488-7.527 and 6.163-6.202 ppm (J=16.00 Hz) which correspond to Ar-CH=C**H**-COONHR and Ar-C**H**=CH-COONHR respectively. The coupling constant absolutely indicates *trans*-geometry in the molecule. Signals that were detected at 7.363-7.382 ppm correspond to Ar-**H** at C-2 and C-6 while 6.803-6.822 ppm correspond to Ar-**H** at C-3 and C-5 (J=8.00 Hz). Signals at 5.588 and 3.756 ppm were assigned for amine (RN-**H**) and methoxy protons ($OC\mathbf{H}_3$) respectively. The spectra display signal at 0.016 ppm which correspond to Si-$C\mathbf{H}_3$ protons while signals at 0.489, 1.583, 3.284 ppm were assigned for the 3 consecutive methylene on CH_3-Si(O-)(O-)($C\mathbf{H}_2$)$_3$-NH-.

Similarly, the ^{1}H-NMR spectrum of **G-MHS**, displays two doublet signals at 7.437-7.461 ppm and 6.278-6.308 ppm which correspond to Ar-C**H**=CH-COOR and Ar-CH=C**H**-COOR respectively. Signals at 7.113-7.135 ppm correspond to Ar-**H** at C-2 and C-6 while 6.815-6.836 ppm correspond to Ar-**H** at C-3 and C-5 (J=8.00 Hz) and these peaks are shifted from the usual ppm, (around 7.310-7.332 and 6.735-6.756 ppm respectively) possibly because of the hydrophobic environment from silicone.[36-37] The singlet signal at 3.75 ppm corresponds to methoxy protons ($OC\mathbf{H}_3$). Moreover, signals at 0.044 ppm were assigned to -O-Si($C\mathbf{H}_3$)$_3$ and 0.001 ppm were assigned for -O-Si($C\mathbf{H}_3$)$_2$-O-.

^{13}C-NMR

The ^{13}C-NMR spectrum of **G-AS** displays two signals belonging to olefinic carbon at 140.05 and 119.72 ppm. The signals of aromatic carbons were detected at 129.56 and 114.13 ppm respectively. The ^{13}C-NMR exhibit signals of methoxy carbon (OCH_3) at 56.23 ppm and alkyl carbon around 16.08-44.75 ppm. Moreover, signals at -0.5 ppm were assigned to methyl carbon on silicon while signals at 13.70, 22.40 and 41.50 ppm were assigned to the 3 consecutive methylene carbons.

In the case of **G-MHS**, the two signals of olefinic carbons can be seen at 132.47 and 129.80 ppm. Signals at 129.55 and 114.02 ppm can be assigned for aromatic carbons. The spectrum also shows signals of methoxy carbon (OCH_3) at 55.77 ppm and alkyl

carbons around 14.26-33.98 ppm. Moreover, signals at 0.10 ppm were assigned for methyl carbon on silicon at the end of silicone chain [-O-Si($\mathbf{C}H_3)_3$] and -0.05 ppm were assigned for methyl carbon on silicon -O-Si($\mathbf{C}H_3)_2$-O-.

^{29}Si-NMR

The ^{29}Si-NMR spectrum of **MHS** silicone displays doublet signals belonging to H-**Si**-CH_3 around 34.66-34.73 ppm while the ^{29}Si-NMR spectrum of product **G-MHS** shows singlet signal of R-**Si**-CH_3 at 22.96 ppm.

3.6.3 Gel permeation chromatography

The molecular weight (M.W.) range of both products were determined by gel permeation chromatography (GPC). Table 3.3 summarized the results of the M.W. range of each product.

Table 3.3 M.W. ranges of products

Compounds	M.W. range
G-AS	5,000-6,000
G-MHS	6,000-7,000

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