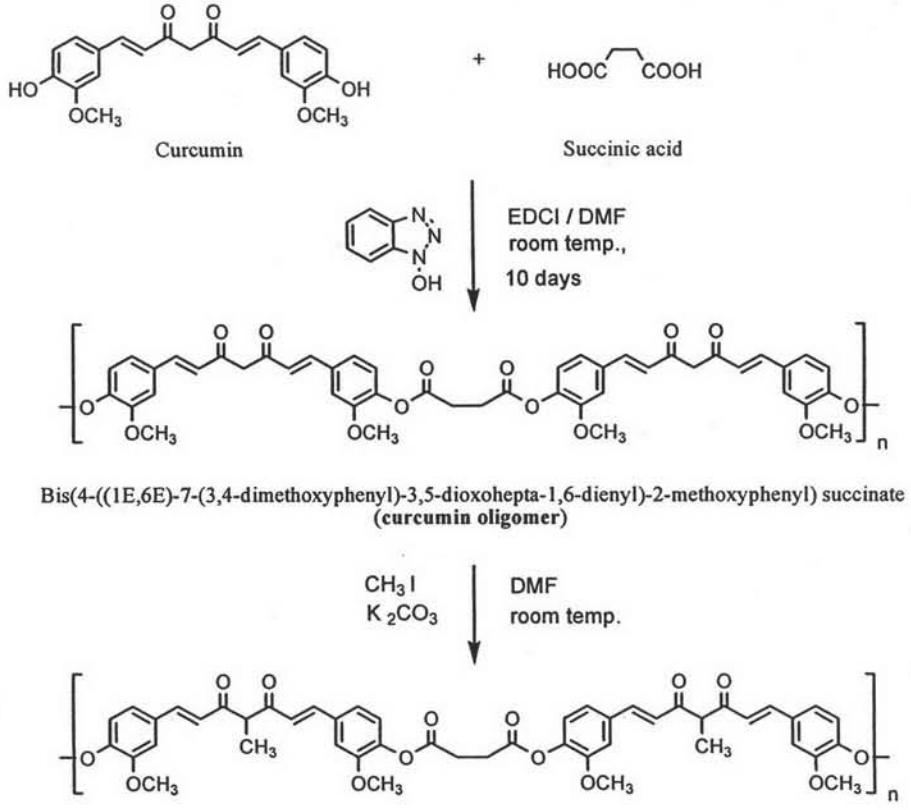
CHAPTER III

RESULTS AND DISCUSSION

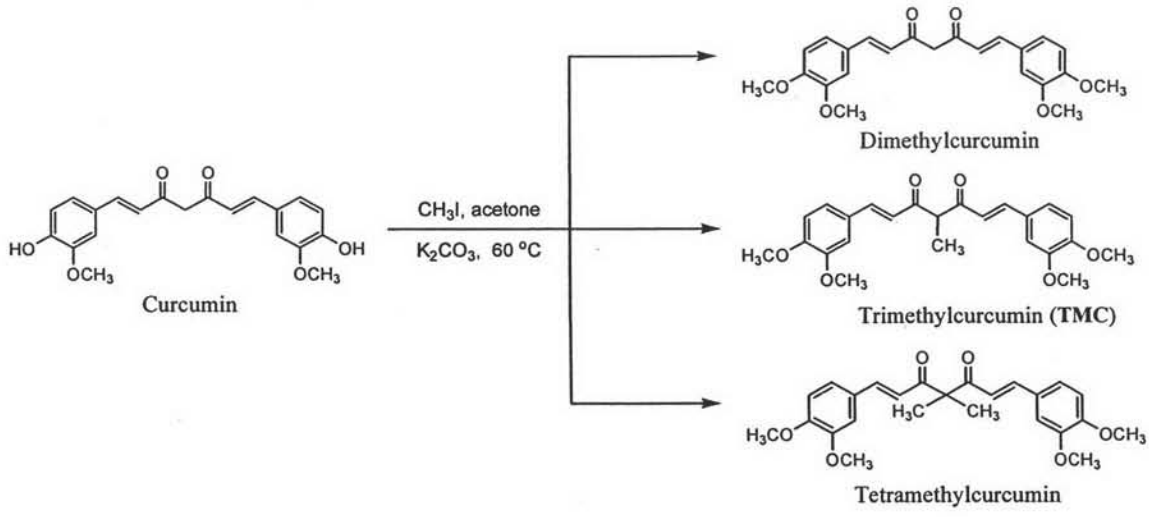
The aim of this work was to modify curcumin through nucleophilic substitution reaction in order to shift its absorption wavelength from the visible region to the UVA region. In the first step, bis(4-((1E,6E)-7-(3,4-dimethoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl) succinate (curcumin oiligomer) was prepared from curcumin and succinic acid. The obtained curcumin oligomer was alkylated with methyl iodide to obtain bis(4-((1E,6E)-7-(3,4-dimethoxyphenyl)-4-methyl-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl) succinate, **MCO** (Scheme 3.1). In addition to the preparation of **MCO** which is an oligomer of curcumin derivative, 1,7-bis-(3,4-dimethoxyphenyl)-4-methyl-1,6-heptadiene-3,5-dione-(trimethylcurcumin, **TMC**) was also synthesized. **TMC** was UVA absorptive curcumin derivative which was used as a standard in stability study of **MCO** in this work.



Scheme 3.1 The route of synthesis of methyl curcumin oligomer (**MCO**)

3.1 Synthesis of 1,7-bis-(3,4-dimethoxyphenyl)-4-methyl-1,6-heptadiene-3,5-dione (trimethylcurcumin, TMC)

Trimethylcurcumin, **TMC** was synthesized from the reaction between curcumin and methyl iodide. The reaction was a nucleophilic substitution reaction (S_N2 reaction, see mechanism in Figure 3.1). Since phenolic O-H has lower pK_a value (~8.54) than the methylene proton between the two carbonyl moieties (pK_a~9.3), methylation at the phenolic moieties was completed while substitution at the methylene protons was not completed. Thus, mixture of 1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione (dimethylcurcumin), 1,7-bis(3,4-dimethoxyphenyl)-4-methyl-1,6-heptadiene-3,5-dione (trimethylcurcumin) and 1,7-bis(3,4-dimethoxyphenyl)-4,4-dimethyl-1,6-heptadiene-3,5-dione (tetramethyl curcumin) were obtained (Scheme 3.2) with **TMC** as the major product. Column chromatography was employed in the separation of **TMC**.



Scheme 3.2 Synthesis of dimethylcurcumin, trimethylcurcumin and tetramethylcurcumin

Table 3.1 Percent yield of dimethylcurcumin, trimethylcurcumin and tetramethyl curcumin

Compound	%yield
Dimethylcurcumin	~26
Trimethylcurcumin	~32
Tetramethylcurcumin	~38

The obtain product was characterized by ^{1}H-NMR and IR spectroscopy. From ^{1}H-NMR spectrum, an appearance of resonances signal at 1.44 ppm (s, 3H, CO-CH(C**H**$_3$)-CO) and 3.90 ppm (s, 3H, OC**H**$_3$) indicated that methyl groups could replace the methylene protons and phenolic protons of the curcumin structure (see Figure 3.1). IR peak at 2964, 2924 and 2837 cm^{-1} also indicated C-H stretching of methyl groups (see Figure 3.2). As show in Figure 3.3, **TMC** has one absorption band with λ_{max} of 345 nm. This absorption band is in the range of UVA. Comparing with the absorption band of curcumin (λ_{max} = 431 nm), it can be concluded that hypsochromic shift could be successfully induced. Explanation on this is that the presence of the methyl group between the two carbonyl moieties probably caused the disruption of keto-enol tautomerization.

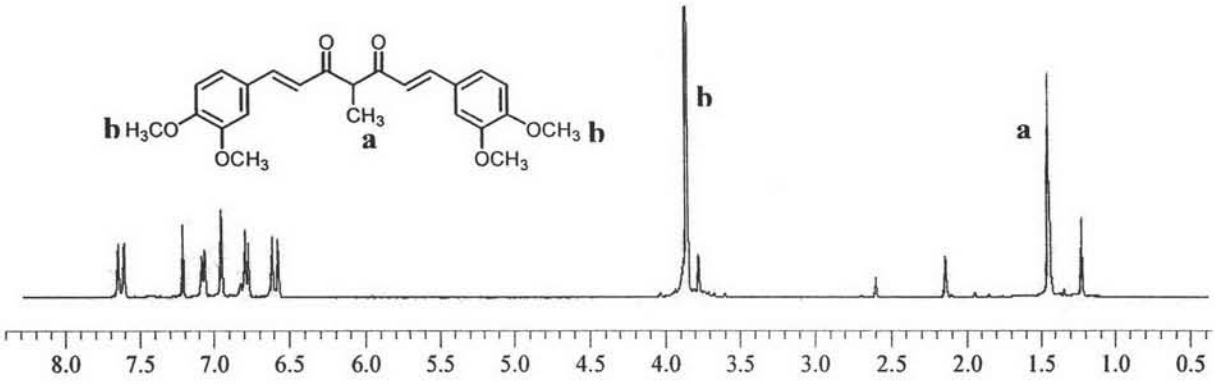


Figure 3.2 ^{1}H-NMR spectrum of trimethylcurcumin.

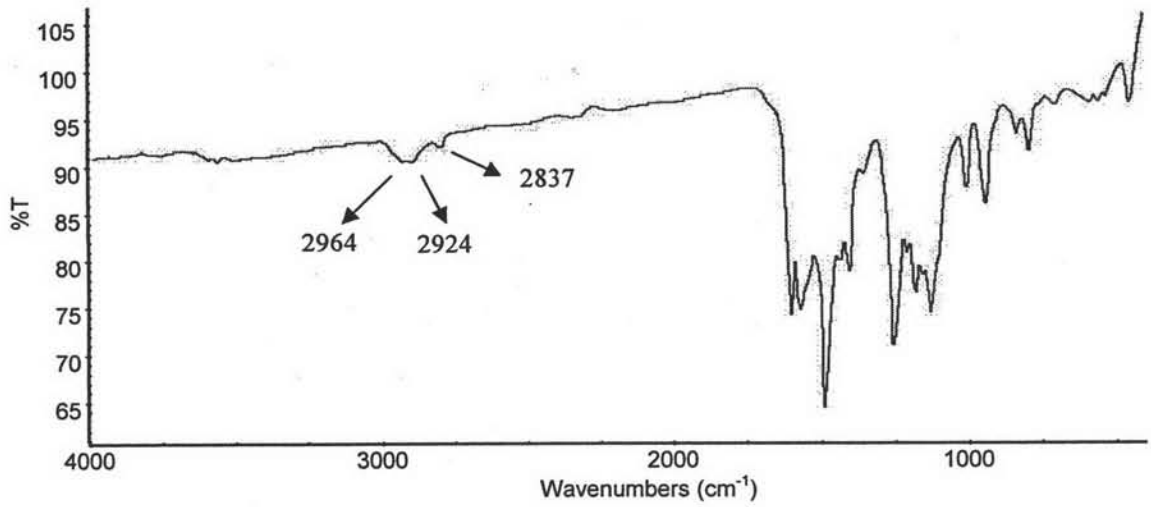


Figure 3.3 IR spectrum of trimethylcurcumin.

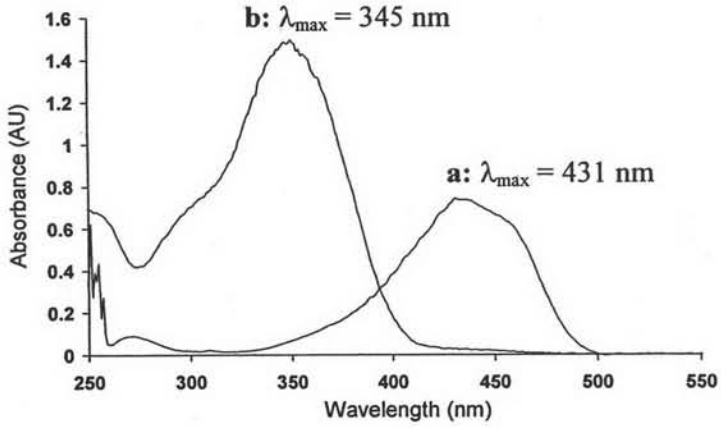


Figure 3.4 UV spectra of a) 10 ppm curcumin solution in DMSO, b) 10 ppm trimethylcurcumin, **TMC** solution in DMSO.

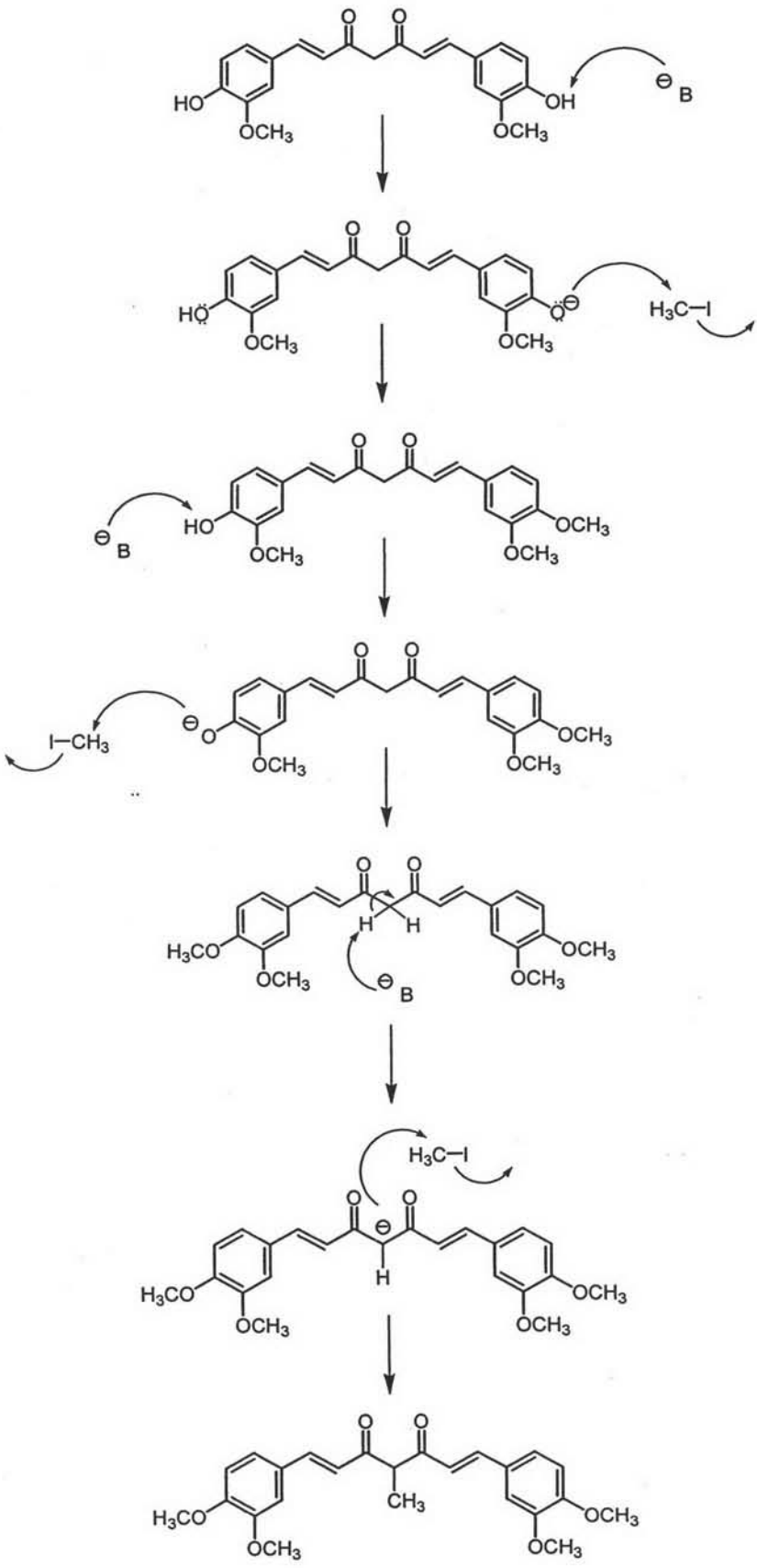
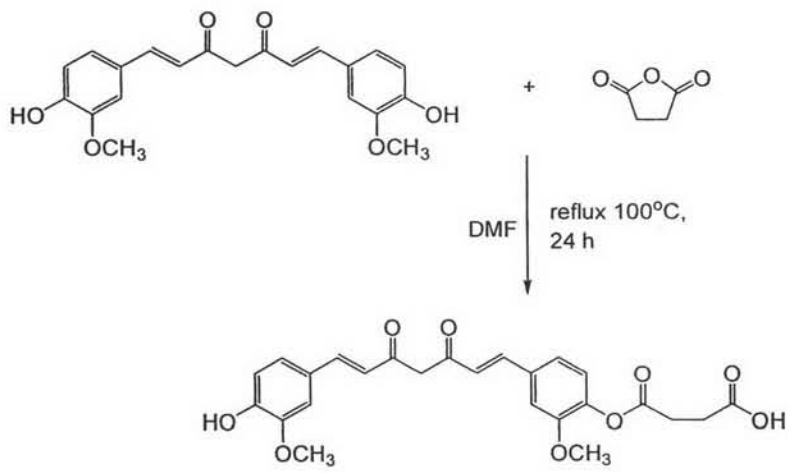


Figure 3.1 The methylation mechanism of curcumin with methyliodide.

3.2 Synthesis of bis(4-((1E,6E)-7-(2-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl) succinate (curcumin oligomer)

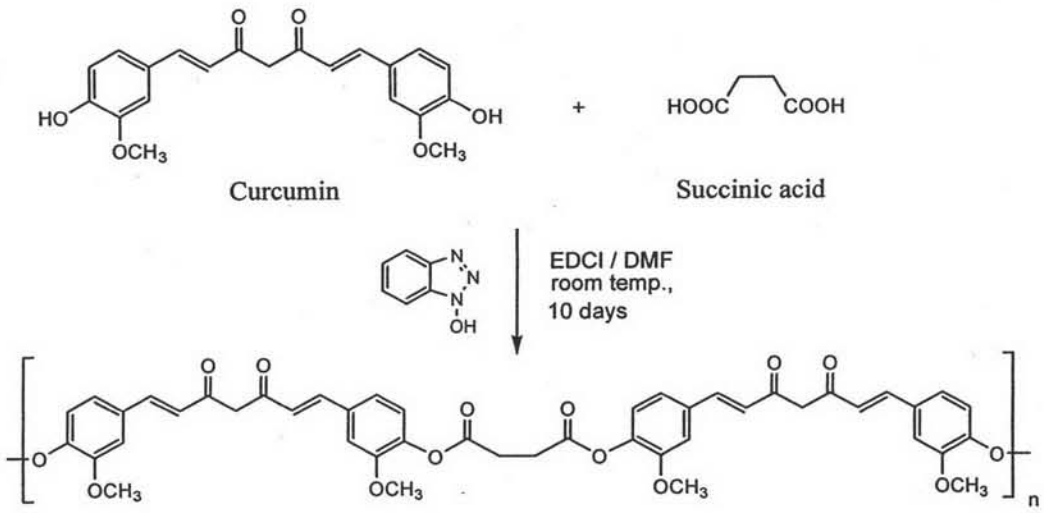
Curcumin oligomer was prepared as a curcumin derivative with increasing molecular weight. It was speculated that the higher molecular weight of the material might decrease the transdermal penetration of the compound. In the first step, monomer was prepared through condensation between one mole equivalent of 1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione (curcumin) and one mole equivalent of succinic anhydride (Scheme 3.3).



Scheme 3.3 Synthesis of curcumin oligomer

The formation of bis(4-((1E,6E)-7-(2-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl) succinate could be monitored by TLC (R_f 0.31, 75% EtOAc/Hex).

Bis(4-((1E,6E)-7-(2-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxy phenyl) succinate (curcumin oligomer) was prepared from 1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione (curcumin) and succinic acid through esterification using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 1-hydroxy-benzotriazole (HOBt) (Scheme 3.4).



Bis(4-((1E,6E)-7-(3,4-dimethoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl) succinate
(curcumin oligomer)

Scheme 3.4 Synthesis of curcumin oligomer by coupling agent method

This reaction could also be monitored by TLC, the appearance of new spot (R_f 0.46, 75% EtOAc/Hex) together with the disappearance of curcumin spot (R_f 0.56, 75% EtOAc/Hex) indicated product formation. Although precipitate was formed easily after the addition of water into the reaction mixture, suction filtration or centrifugation could not be used to separate out the product because of the very fine particle. As a result, dialysis technique was used to get rid of DMF. The structure of orange brown solid product was characterized using various spectroscopic techniques including ^{1}H-NMR and IR spectroscopy. From ^{1}H-NMR spectrum, an appearance of resonances signal at 3.0 ppm (s, 2H, CO-C**H$_2$**-CH$_2$) indicated product formation (see Figure 3.5). IR peak at 1762 cm^{-1} also indicated ester functionality (see Figure 3.6).

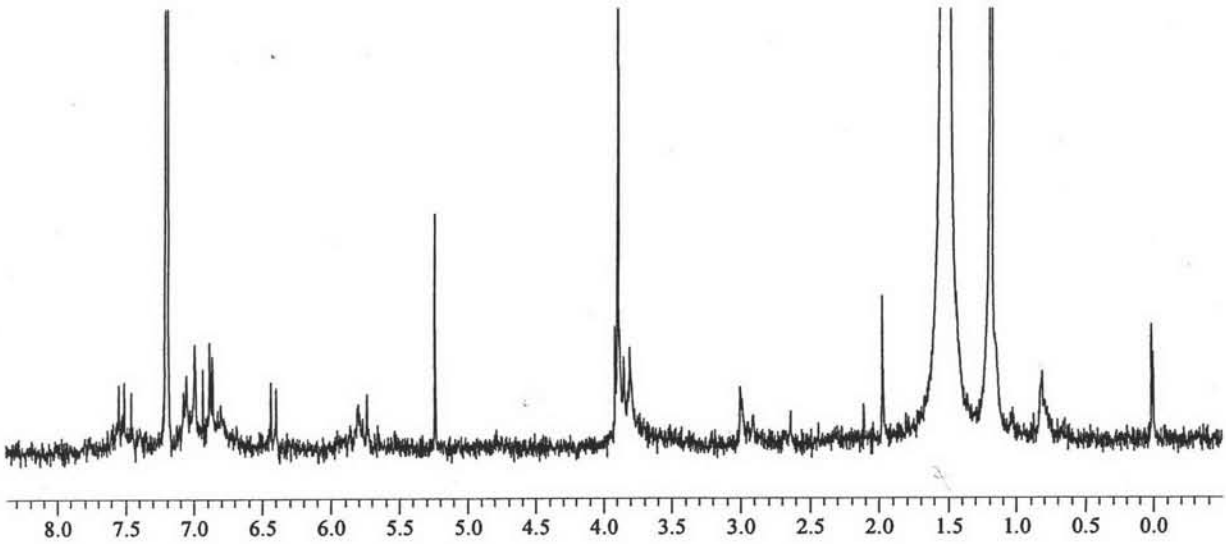


Figure 3.5 ^{1}H-NMR spectrum of curcumin oligomer.

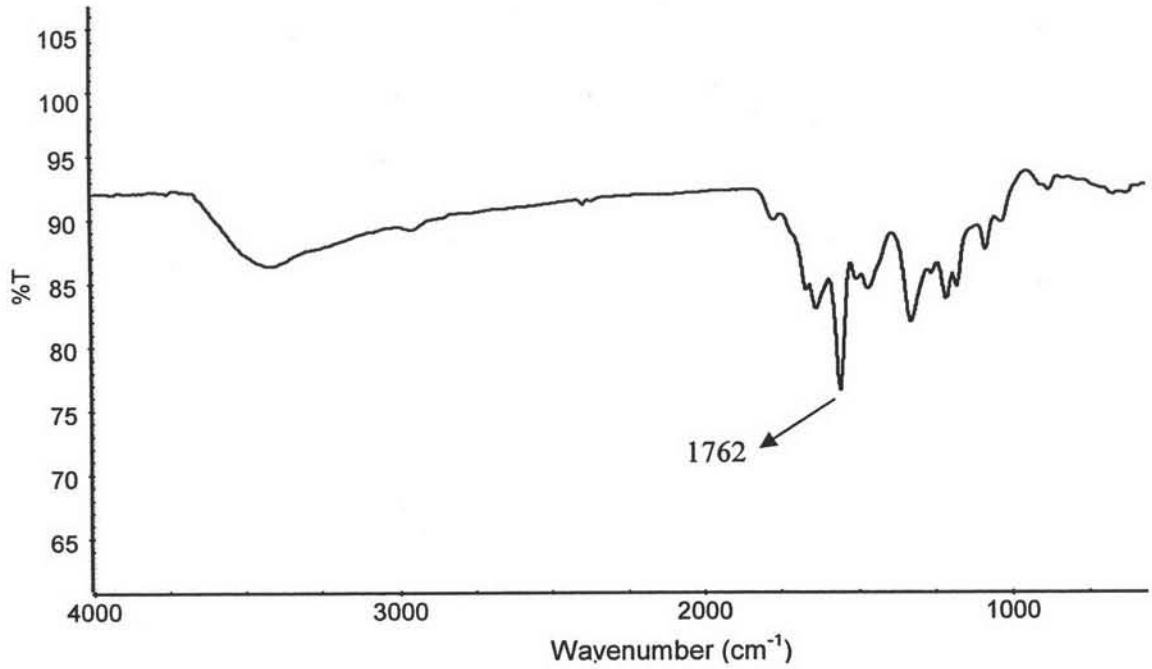


Figure 3.6 IR spectrum of curcumin oligomer.

The GPC analysis of this product gave weight average molecular weight (Mw) of 1255 with the polydispersion value of 1.00. The product could be detected by electrospray ionization mass spectroscopy (ESI-MS, see Figure 3.7 and Table 3.2).

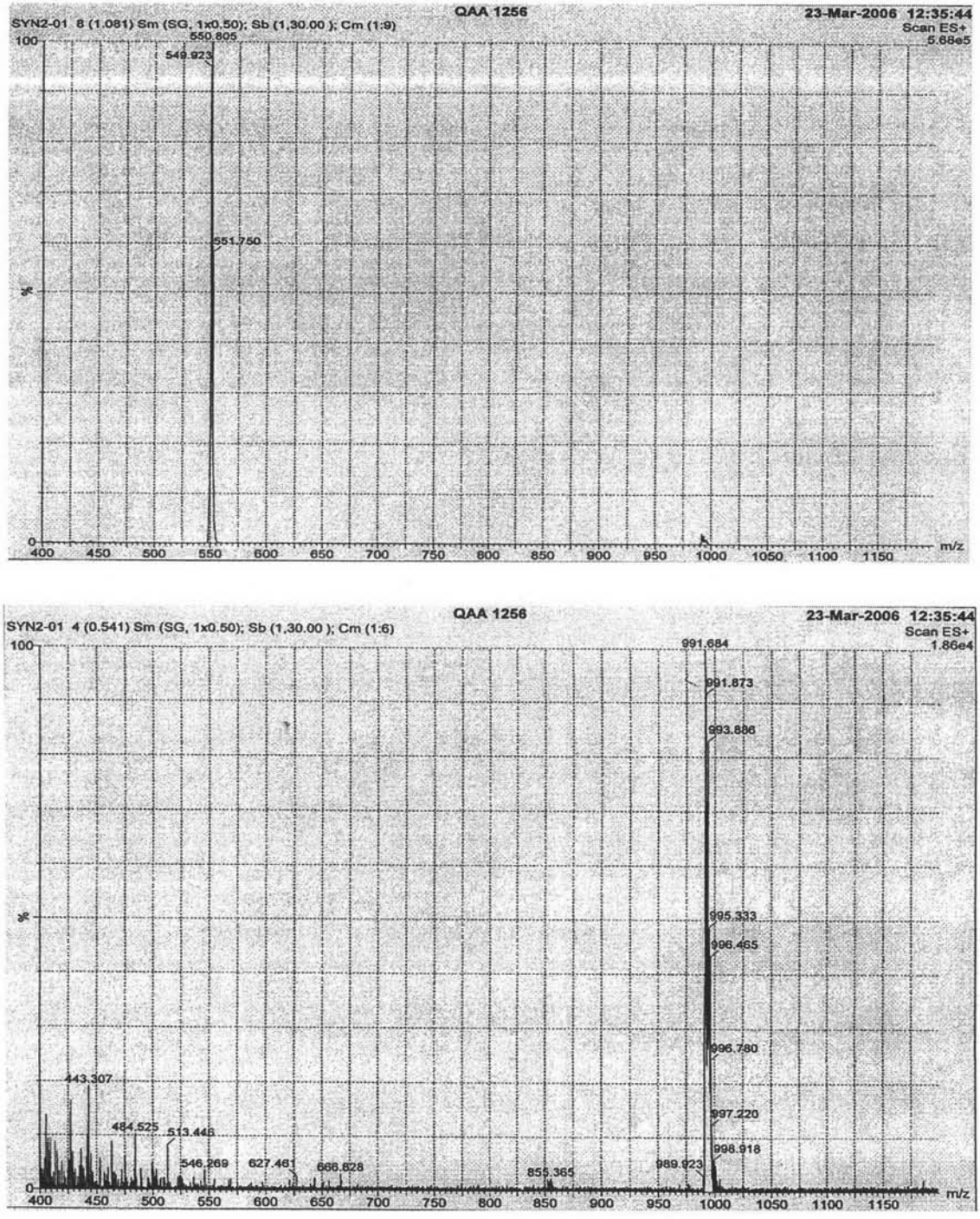
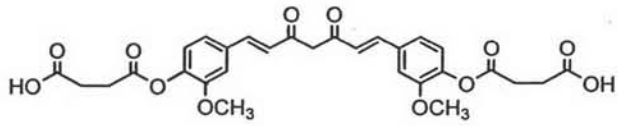
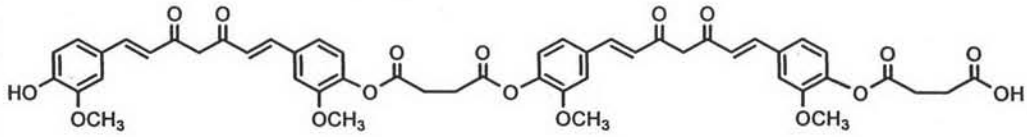


Figure 3.7 ESI-MS spectrum of curcumin oligomer.

Table 3.2 The proposed structure of curcumin oligomer by ESI-MS

m/z	Structure
550	$C_{29}H_{26}O_{11}$: one unit of curcumin with two units of succinic acid 
991 or 996	$C_{50}H_{46}O_{17}$-MeOH-CH_3CN or $C_{50}H_{46}O_{17}$-DMSO: two units of curcumin with two units of succinic acid which had a presence of MeOH and CH_3CN 

However, It should be noted here that higher molecular weight than 1200 could not be detected because of the limitation of the analyzer. Experiments using MALDI-TOFF with various matrixes failed to give any information. As a result, molecular weight from GPC was used.

As show in Figure 3.8, curcumin oligomer has one absorption band with λ_{max} of 431 nm. This absorption band is similar to that of curcumin.

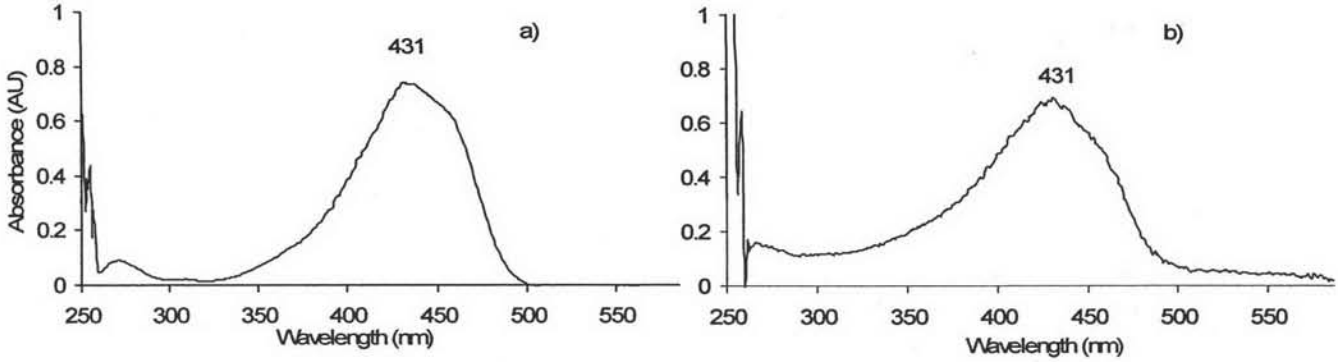
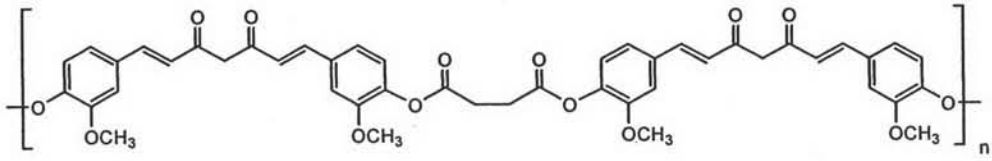
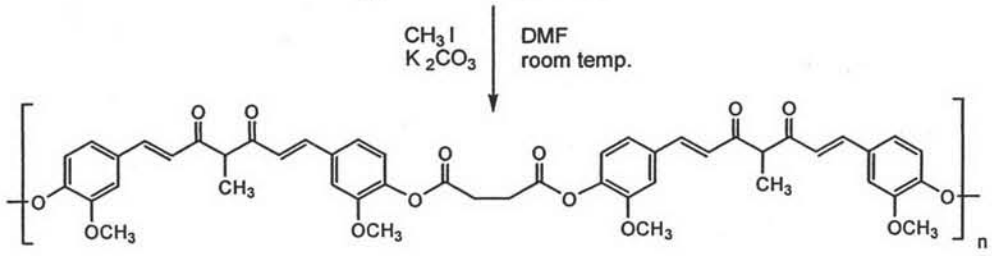


Figure 3.8 UV spectra of a) 10 ppm curcumin solution in DMSO, b) 10 ppm curcumin oligomer solution in DMSO.

3.3 Synthesis of bis(4-((1E,6E)-7-(3,4-dimethoxyphenyl)-4-methyl-3,5-dioxohepta-1,6-dienyl)-2-methoxy phenyl) succinate (MCO)



Bis(4-((1E,6E)-7-(3,4-dimethoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl) succinate
(curcumin oligomer)



Bis(4-((1E,6E)-7-(3,4-dimethoxyphenyl)-4-methyl-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl) succinate
(methyl curcumin oligomer, MCO)

Scheme 3.5 Synthesis of **MCO**

The obtained curcumin oligomer was further alkylated with methyl idodide (Scheme 3.6) in order to induce hypsochromic shift of its absorption profile. Reaction was carried out at room temperature using excess methyl iodide and potassium carbonate. After stirring for 24 h in nitrogen atmosphere, the product was extracted by dichloromethane and washed with water. The dark orange solid product was characterized by various spectroscopic techniques including ^{1}H-NMR, ^{13}C-NMR, IR and UV-Vis spectroscopy. From ^{1}H-NMR spectrum, the disappearance of keto-enol proton resonance at 5.74 ppm (s, 2H, CO-C**H$_2$**-CO) together with a new signal at 1.41 ppm (s, 3H, CO-CH(C**H$_3$**)-CO) indicated methylation at the methylene group between the two carbonyl moieties (see Figure 3.9). Appearance of resonances at 21.1 ppm (CO-**C**H-(CH$_3$)-CO), 39.0 ppm (CO-**C**H$_2$-**C**H$_2$-CO), 170 and 172 ppm (HC-**C**O-CH$_2$-**C**O-CH) in the ^{13}C-NMR spectrum indicated formation of methyl substitutents (see Figure 3.10). IR spectrum showed absorption band around 2924 and 2837 cm^{-1} which corresponded to C-H stretching of the substituted CH$_3$ between diketone moieties. The disappearance of O-H stretching vibrations of alcohol also indicated the methylation at the hydroxyl groups (see Figure 3.11). The weight average molecular

weight (Mw) of the product obtained by GPC was 978, with the polydispersity of 1.11. **MCO** could be detected by ESI-MS, the result showed that the obtained product had various molecular ion (m/z) so the obtained product was the oligomers of various sizes.

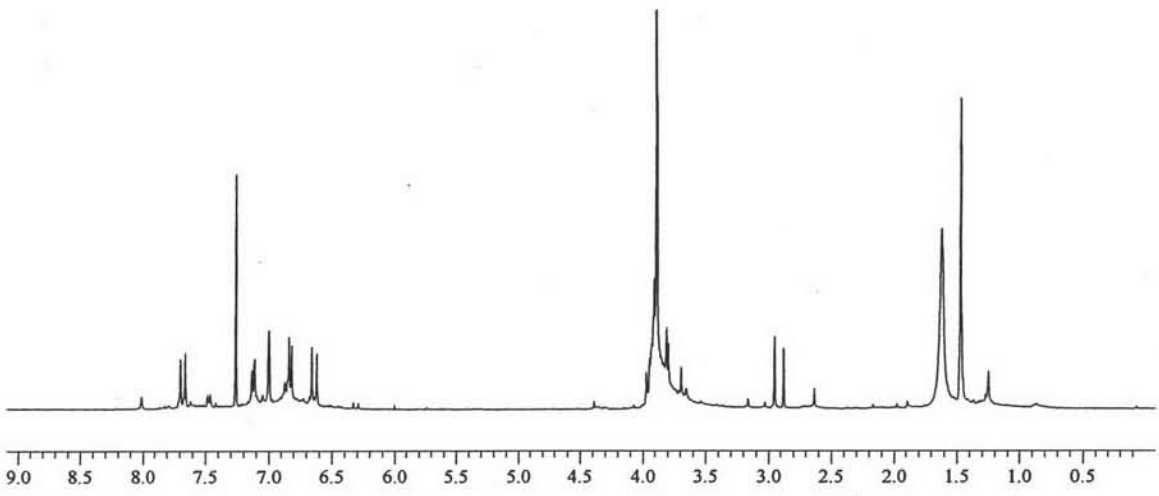


Figure 3.9 ^{1}H-NMR spectrum of **MCO**.

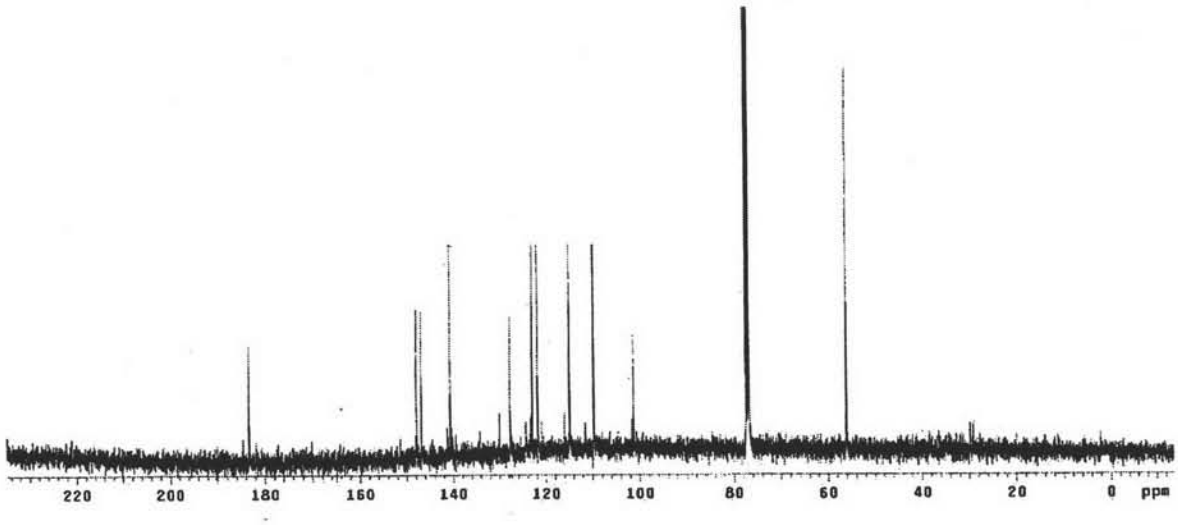


Figure 3.10 ^{13}C-NMR spectrum of **MCO**.

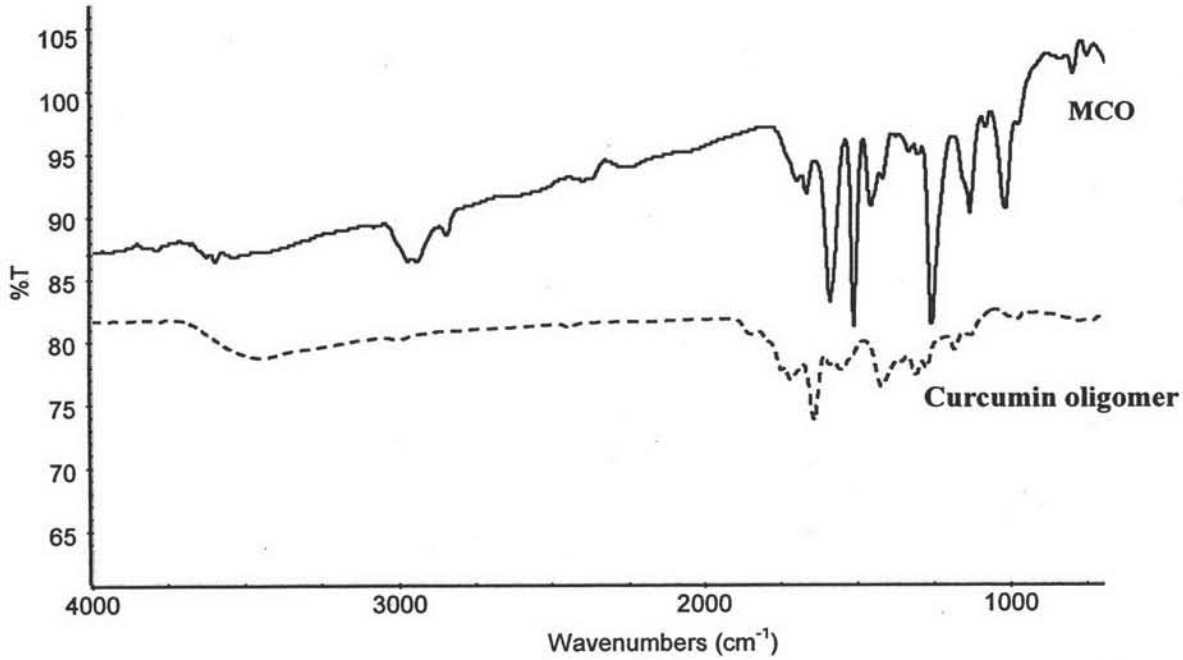


Figure 3.11 IR spectrum of **MCO** comparing with curcumin oligomer.

Table 3.3 Solubility of curcumin, curcumin oligomer and **MCO**

Type of solvent	Solubility of curcumin	Solubility of curcumin oligomer	Solubility of MCO
Hexane	- -/+ -[a]	- -/- -[a]	- -/+ -[a]
Diethyl Ether	+ +	+ -/+ -[a]	+ +
Dichloromethane	+ +	+ -/+ -[a]	+ +
Chloroform	+ +	+ -/+ -[a]	+ +
Ethyl Acetate	+ +	+ -/+ -[a]	+ -/+ +[a]
Acetone	+ +	+ -/+ -[a]	+ +
Methanol	+ +	+ -/+ -[a]	+ -/+ +[a]
Ethanol	+ +	+ -/+ -[a]	+ -/+ +[a]
Acetonitrile	+ +	+ -/+ -[a]	+ +
THF	+ +	+ +	+ +
DMF	+ +	+ +	+ +
DMSO	+ +	+ +	+ +
Water	- -/- -[a]	- -/+ -[a]	- -/- -[a]

[a] Heating

- - insoluble

+ - partial soluble (2 mg of sample was dissolved in 2 mL of a solvent)

+ + soluble (10 mg of sample was dissolved in 2 mL of a solvent)

Table 3.3 shows that curcumin can be dissolved in various organic solvents. High molecular weight of curcumin oligomer was probably responsible for the decrease in its solubility. **MCO** can be dissolved in various organic solvents better than curcumin oligomer. This agrees well with the fact that **MCO** molecule is more hydrophobic than curcumin oligomer.

Experiment was carried out to obtain UV absorption property of **MCO**. As shown in Figure 3.12, **MCO** showed absorption band at λ_{max} of 351 nm, corresponding to the UVA region. This blue shift from curcumin and curcumin oligomer, is probably a result of less resonance in the **MCO** structure caused by a presence of the grafted methyl group. This implies that the keto-enol might not be fully interchangeable in the **MCO**. Its maximum absorption at 351 nm indicates that the compound is more likely to be in the keto-state. This result agrees well with the keto-predominated structure of **TMC** discussed earlier. The hypothesis is confirmed by the fact that UV absorption of the **MCO** is pH independent (Figure 3.13).

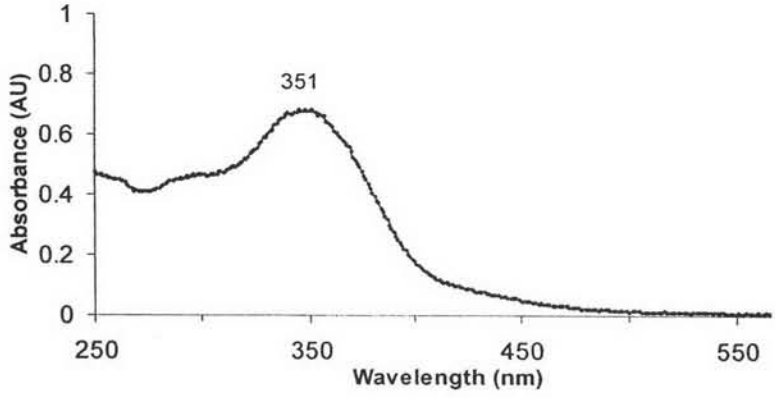


Figure 3.12 UV spectrum of 10 ppm **MCO** solution in dichloromethane.

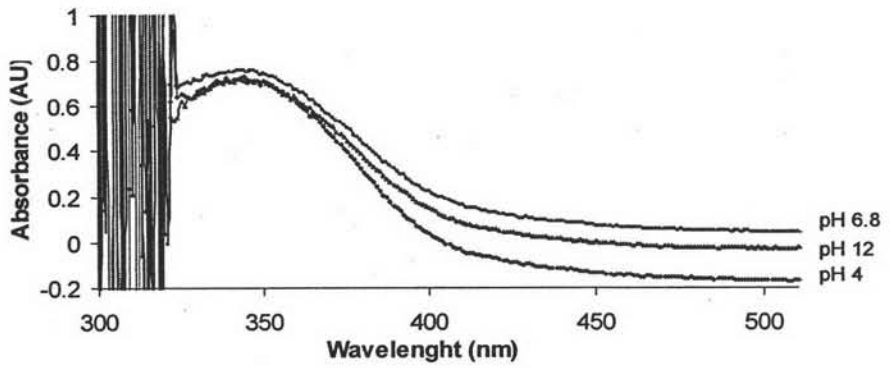


Figure 3.13 UV spectrum of 10 ppm **MCO** solution in methanol/0.05 M phosphate buffer (90:10 v/v) pH 4, 6.8 and 12.

By plotting a graph between absorbance (at λ_{max}) and concentrations of **MCO**, a linear relationship was obtained with its slope representing molar absorption coefficient value (ε) of **MCO**. The molar absorptivity of **MCO** was compared to that of the **TMC**, a UVA absorption curcumin derivative prepared earlier. The molar absorptivity values of 62,955 and 59,058 $M^{-1}cm^{-1}$ (calculated using molar concentration of monomeric unit) were obtained for **TMC** and **MCO**, respectively.

3.4 Franz cell absorption test

Penetration through skin was measured in Franz-type diffusion cells using full thickness baby mouse skin as the membrane. 2-Ethylhexyl-4-methoxycinnamate (EHMC) solution was used as a standard. The receptor phase was phosphate buffered saline (pH 7.4). The penetration of EHMC was compared with that of **TMC**, curcumin oligomer and **MCO** (see Table 3.3).

Franz-type glass diffusion cells (~2.27 cm^2 and receptor volume ~13 mL, accurately measured for each cell) were used (Figure 3.14). Full thickness baby mice skin was placed on the lower halves of the cells, the stratum corneum facing the donor chamber. The upper halves of the cell were added and the assembly clamped together. The cell was heat in constant temperature water bath at 37 °C throughout the experiment. Receptor chamber contents were continuously stirred by submersible magnetic stirrers. The receptor phase was phosphate buffered saline (pH 7.4).

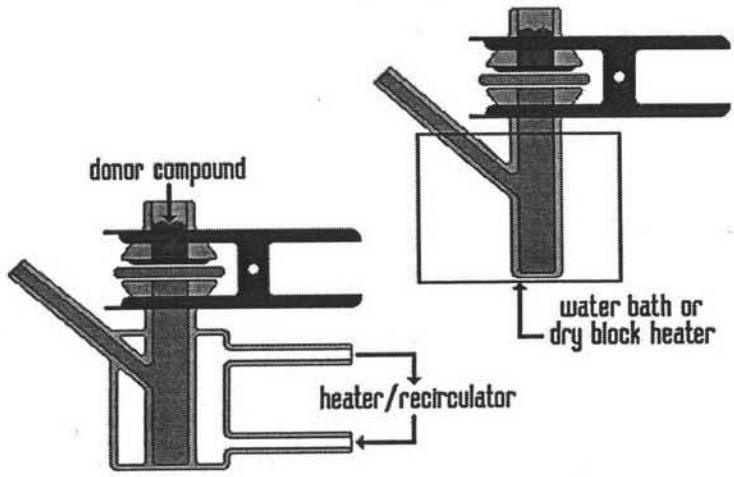


Figure 3.14 Franz-type glass diffusion cells.

Table 3.4 Percentage of penetration of EHMC, **TMC**, curcumin oligomer, **MCO** and curcumin

Times (h)	EHMC (MW = 290)	TMC (MW = 442)	Curcumin oligomer (Mw~1000-1200)[a]	MCO (Mw~1000-1200)[a]	Curcumin (MW = 368)
0	n	n	n	n	n
1	8.27	5.76	0	n	6.69
2	14.23	8.81	0.05	n	5.50
4	20.99	14.79	0	n	5.38
24	59.28	19.80	0.05	n	4.63

[a] Mw of curcumin oligomer and **MCO** were investigated by GPC (see Appendix B.12 and B.13)

n = not detected

It was obvious that **TMC** could penetrate the baby mouse skin much more effectively than curcumin. This agrees well with the fact that **TMC** molecule was more hydrophobic than curcumin. Curcumin and EHMC show obvious penetration. Curcumin oligomer and **MCO** showed no transdermal absorption. High molecular weight of the compound probably helps retarding the transdermal penetration. The result indicated excellent skin accumulation of **MCO**. Skin accumulation is required for good UV filtering agent.

3.5 Photostability test

Preliminary UVA photostability study (Figure 3.15) showed that the trimethyl curcumin (**TMC**) in dichloromethane was unstable upon being exposed to UVA and UVB radiations at 3.0 mW/cm^2 and 0.19 mW/cm^2, respectively. **MCO** was more stable than **TMC** (comparing Figure 3.6b and 3.6a). UV absorption of **TMC** decreased to a level of insignificance after 10 minutes (10.8 and 0.684 J of UVA and UVB, respectively) while **MCO** maintained a reading of 50% after being exposed to 10.8 and 0.684 joules of UVA and UVB radiation, respectively.

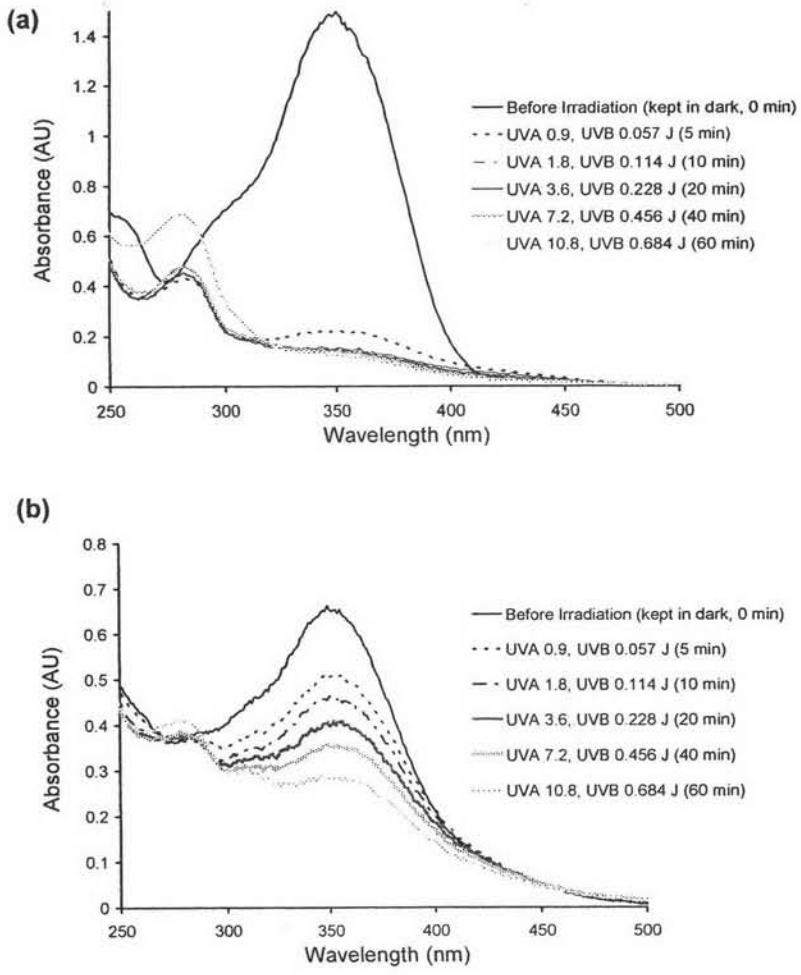


Figure 3.15 UV spectra of (a) **TMC** and (b) **MCO** in dichloromethane before and after being exposed to various doses of UVA and UVB radiations.