CHAPTER III

RESULTS AND DISCUSSION

3.1 Determination of percutaneous absorption by diffusion cell

3.1.1 Analysis of sunscreen in receptor fluid by UV/VIS spectrophotometry

It can be seen that UV absorption spectrum of each UV filters in the receptor fluid (Figure 3.1 and 3.2) contains characteristic peaks, i.e., at 360 nm for BMDBM, 310 nm for OMC, 300 nm for 4-methyl benzylidene camphor and 310 nm for Eusolex UV pearl OMC. Such peaks were used to quantitate amouts of the sunscreens in the receptor fluids. Calibration curves of the three sunscreens were constructed using absorbances at the λmax of each UV filters. Figure 3.3 shows calibration plots of OMC, BMDBM and 4- methyl benzylidene camphor. It can be seen that all calibration plots are linear. Therefore, quantitation of each sunscreen in the receptor fluids of the following Franz diffusion cell experiment can be done easily using UV absorption spectroscopy.

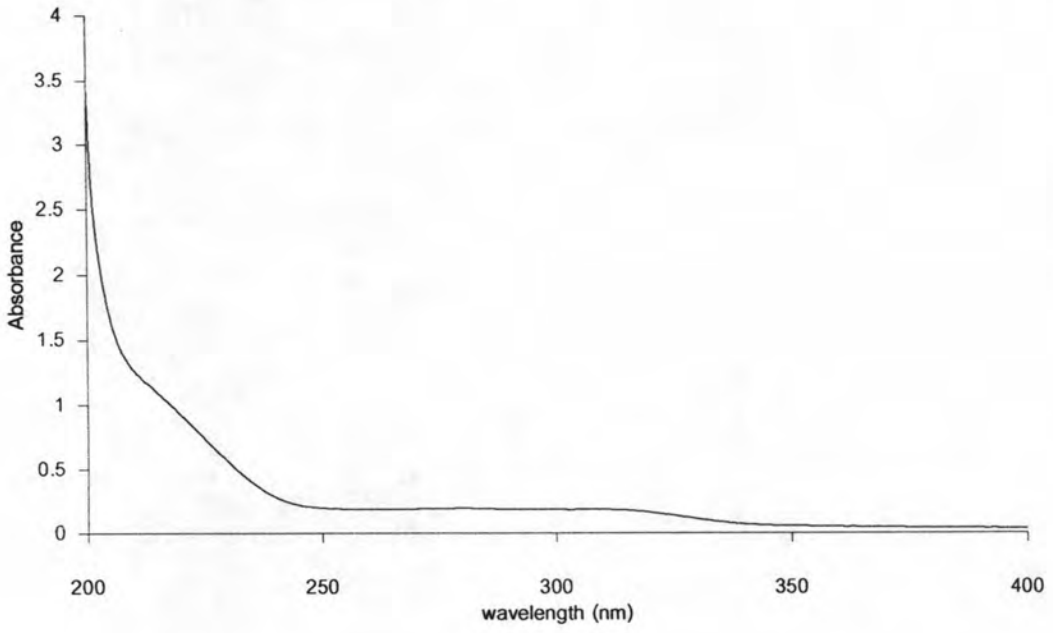
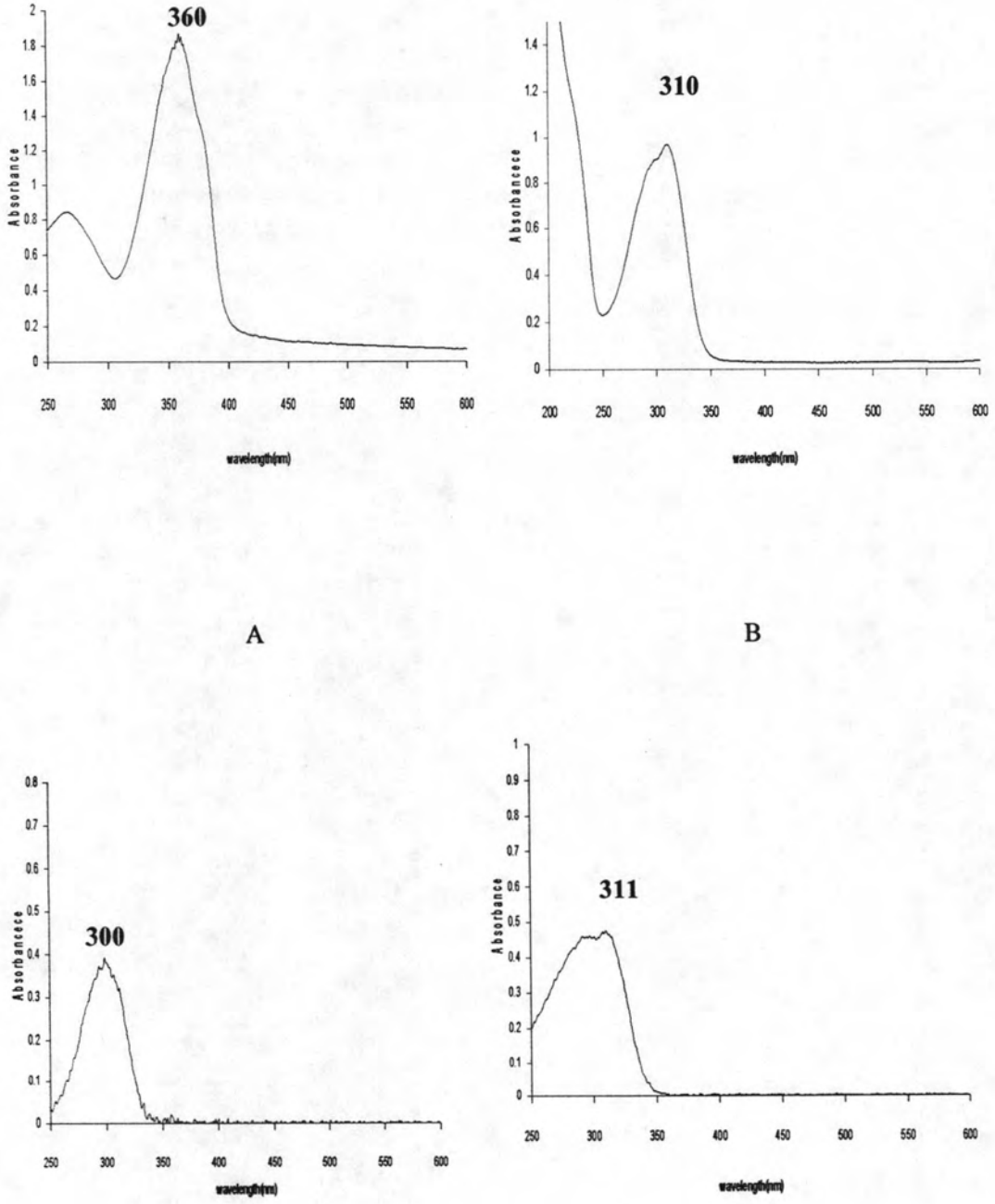


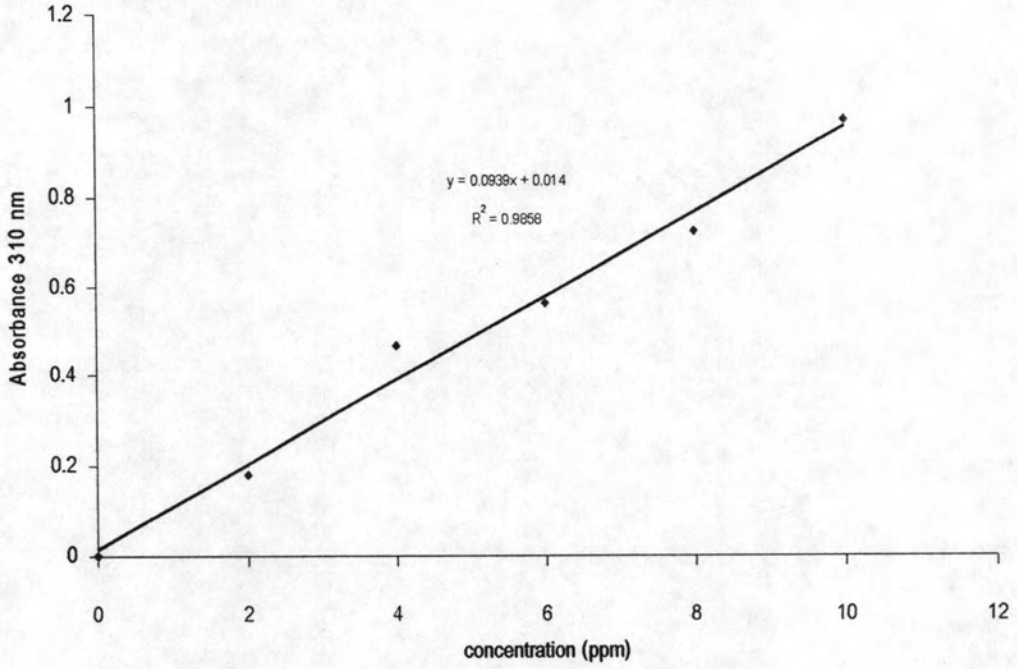
Figure 3.1 : UV spectrum of receptor fluid

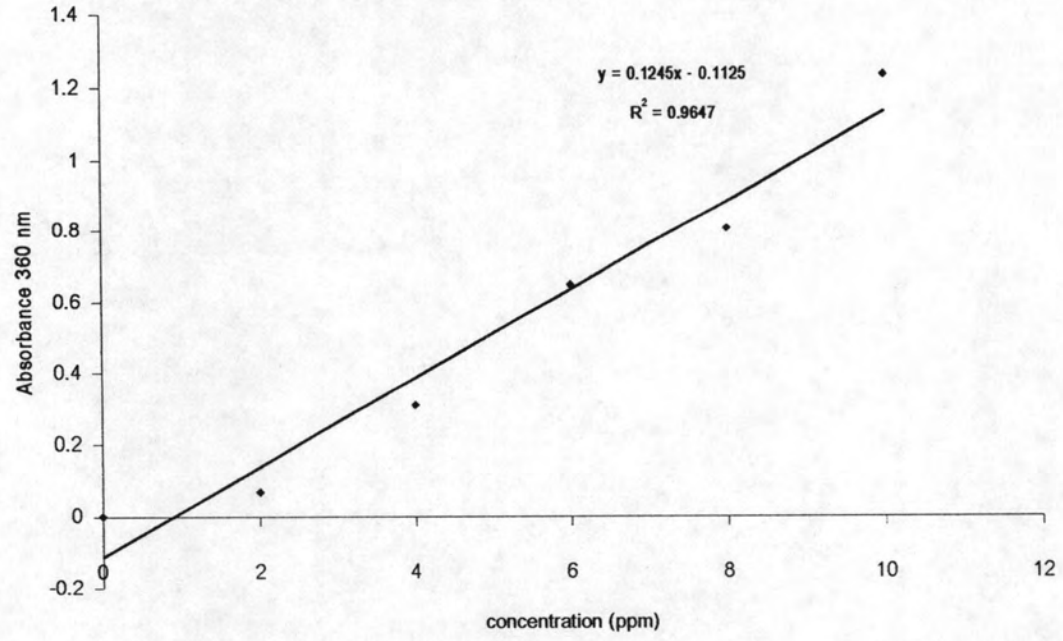


A B

C D

Figure 3.2 UV absorption spectra of A) BMDBM, B) OMC, C) 4-methyl benzylidene camphor and D) Eusolex UV pearl OMC in the receptor fluid


y = 0.0939x + 0.014
R2 = 0.9858
Absorbance 310 nm
concentration (ppm)
0
0.2
0.4
0.6
0.8
1
1.2
0
2
4
6
8
10
12


y = 0.1245x - 0.1125
R2 = 0.9647
Absorbance 360 nm
concentration (ppm)
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
2
4
6
8
10
12

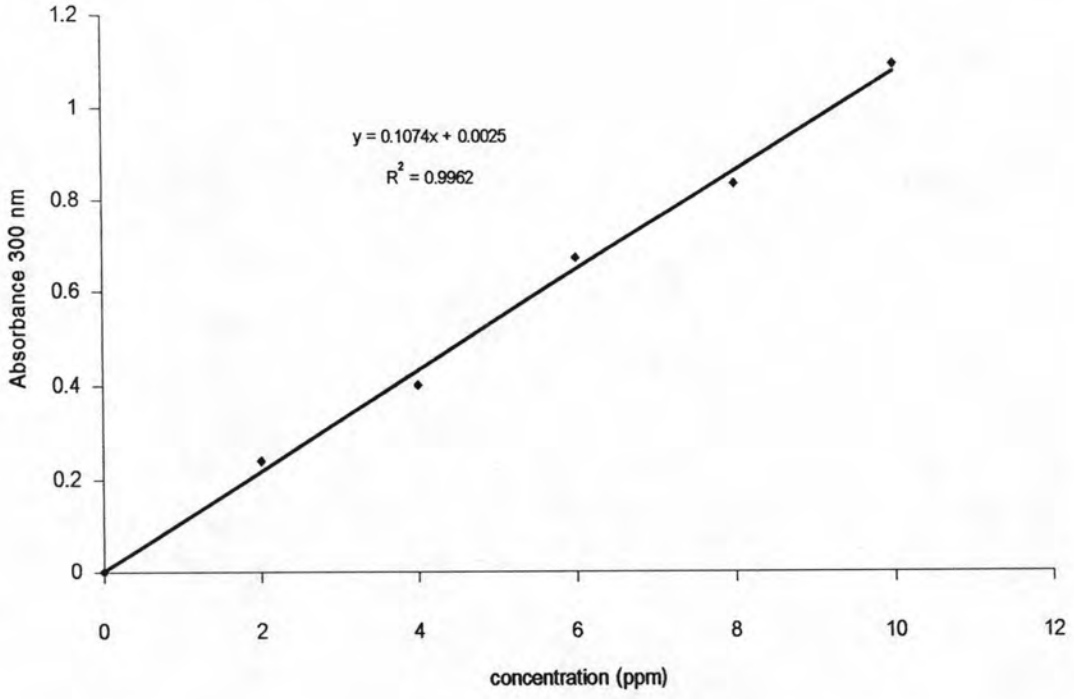


Figure 3.3 Calibration curves of OMC, BMDBM and 4-methyl benzylidene camphor in receptor fluid.

3.1.2 Comparing permeability of fresh skin, -20°C frozen skin and -80°C frozen skin.

Since the use of fresh abdominal mouse skin is not convenient, experiments were set up to test for the differences between fresh skin, skin kept at -80°C and skin kept at -20°C. In this test, penetrations of OMC through the three skins were compared. Since the skin from one mouse was not big enough to be divided into three pieces, skin from the same mouse was divided into two pieces. Penetration of OMC through fresh mouse skin and skin kept at -20°C were done using skin from the same mouse. Penetration of OMC through fresh mouse skin and skin kept at -80°C were also done using skin from the same mouse. Comparing penetration rate using skin (from the same mouse) kept at different condition could, therefore, revealed the effect of the storage condition on the skin permeability.

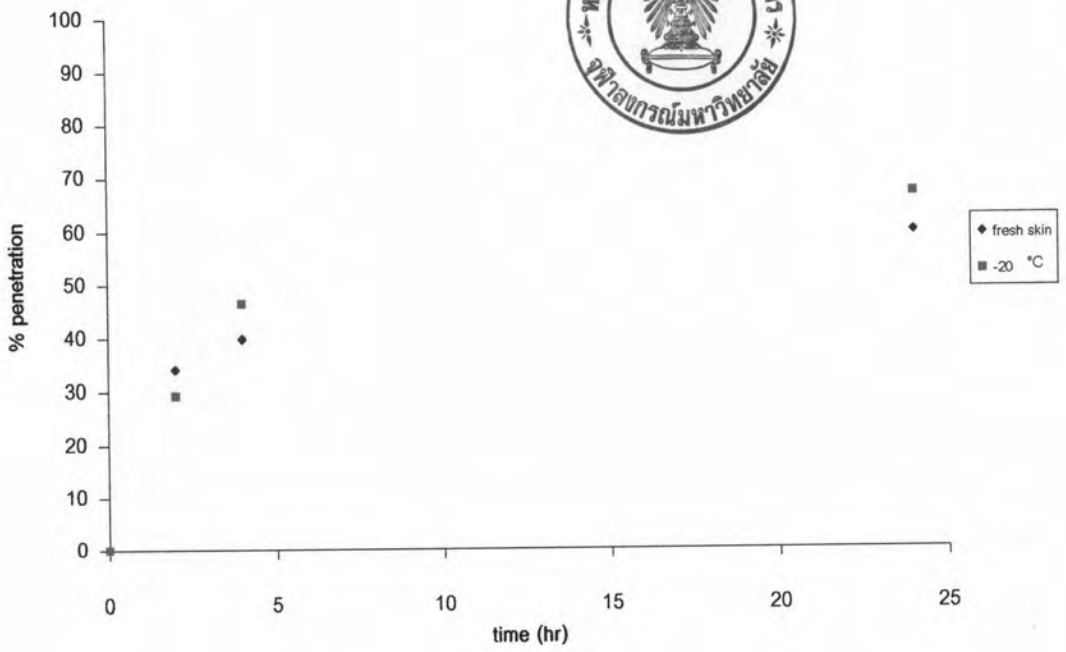


Figure 3.4 percent penetration of OMC in fresh skin (◆) and skin at -20°C (■) by Franz-diffusion cell

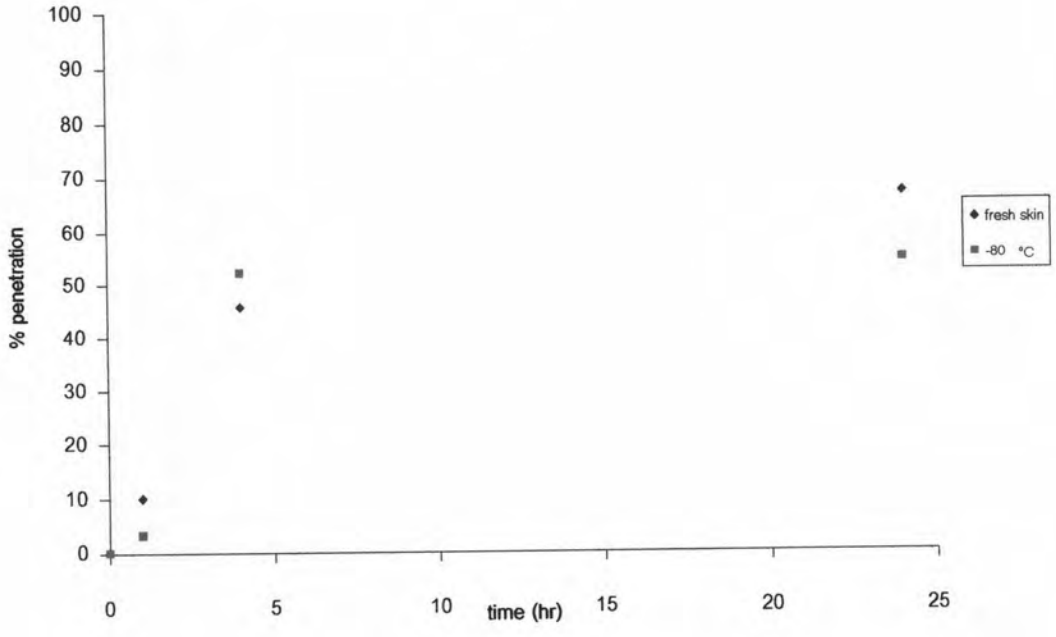


Figure 3.5 percent penetration of OMC in fresh skin (◆) and skin at -80°C (■) by Franz-diffusion cell

As can be seen in Figure 3.4 and 3.5, penetration rates of OMC through fresh skin and skin kept at -20°C were comparable and penetration rates of OMC through fresh skin and skin kept at -80°C were also quite similar. This result agree with previous studies done by K.R. Brain *et al* .[49] which indicated the permeation of diethanolamine (DEA) was only a little grater through frozen than through fresh skin. Therefore, skin kept at -20°C was used for the rest of the experiments.

3.1.3 Study of transdermal penetration of various sunscreen through baby mice skin by Franz diffusion cell method.

Sunscreens tested in the study include:

- 4 commercial organic UV filters; octyl methoxycinnamate (Eusolex®2292), butyl methoxy dibenzoylmethane (Eusolex®9020), 4-methyl benzylidene camphor (Eusolex®6300) and Eusolex UV pearl OMC.

- 4 newly developed small organic UV filters; di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate, 2-ethylhexyl-2,4,5-trimethoxycinnamate, dihexyl-2,4,5-trimethoxybenzalmalonate and diethyl-2,4,5-trimethoxybenzalmalonate.

- 8 polymeric UV filters; poly-(3-hydroxy-propoxy)cinnamic acid, poly-(6-hydroxy-hexyloxy) cinnamic acid, poly-(11-hydroxy-undecyloxy) cinnamic acid, poly penta ethylene glycol cinnamate, poly-(*p*-propoxycinnamate)-co-(*p*-undecyloxycinnamate), 4-methoxycinamoylphthaloyl chitosan, 4-methoxycinamoylphthaloyl irradiated chitosan and poly [vinyl 2,4,5 trimethoxycinnamate (vinyl alcohol)]copolymer.

Table 3.1 Percent penetration* of the commercial sunscreens (in solution forms) through baby mice skin after 24 h.

UV filters	% penetration after 24 h (mean±S.D.)
OMC	70.60±10.08
BMDBM	24.26±1.82
4-methylbenzylidene camphor	14.70±1.78
Eusolex UV pearl OMC	0±0

Table 3.2 Percent penetration* of newly developed small organic UV filters (in solution forms) through baby mice skin after 24 h.

UV filters	% penetration after 24 h (mean±S.D.)
OMC	70.60±10.08
di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate	7.76±1.76
dihexyl-2,4,5-trimethoxybenzalmalonate	6.06±1.20
diethyl-2,4,5-trimethoxybenzalmalonate	0±0
2-ethylhexyl-2,4,5-trimethoxycinnamate	8.63±1.44

*determined from the receptor fluid.

Table 3.3 Percent penetration* of polymeric UV filters (in solution forms) through baby mice skin after 24 h.

UV filters	%penetration after 24 h (mean±S.D.)
OMC	70.60±10.08
poly-(3-hydroxy-propoxy)cinnamic acid	0±0
poly-(6-hydroxy-hexyloxy)cinnamic acid	0±0
poly-(11-hydroxy-undecyloxy)cinnamic acid	0±0
poly penta ethylene glycol cinnamate	0±0
poly-(*p*-propoxycinnamate)-co-(*p*-undecyloxycinnamate)	0±0
4-methoxycinamoylphthaloyl chitosan	0±0
4-methoxycinamoylphthaloyl irradiated chitosan	0±0
poly[vinyl-2,4,5-trimethoxycinnamate(vinyl alcohol)]copolymer.	0.14±0.12

Table 3.4 Percent penetration* of the interested UV filters (incorporated into the lotion) through baby mice skin after 24 h.

UV filters	%penetration after 24 h (mean±S.D.)
OMC	89.46±6.72
BMDBM	44.88±4.88
di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate	0±0
2-ethylhexyl-2,4,5-trimethoxycinnamate	9.84±2.66

It can be seen that OMC, BMDBM and 4-methyl benzylidene camphor (table 3.1) could all penetrate the baby mice skin. OMC shows the most transdermal penetration amongs the three. This result agree well with previous studies showed indicate transdermal penetration of OMC [13] BMDBM [23] and 4-methyl benzylidene camphor [18]. The encapsulated OMC (UV-pearl) product shows no transdermal absorption. No transdermal absorption of the UV pearl OMC can be easily explained through the encapsulation of OMC into a polymeric bead.

It can be seen that di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate, dihexyl-2,4,5-trimethoxybenzalmalonate and 2-ethylhexyl-2,4,5-trimethoxycinnamate (table 3.2) could very slightly permeated through the baby mice skin while diethyl-2,4,5-trimethoxybenzalmalonate shows no transdermal absorption at all.

The polymeric UV filters (Table 3.3) such as poly-(3-hydroxy-propoxy)cinnamic acid, poly-(6-hydroxy-hexyloxy) cinnamic acid, poly-(11-hydroxy-undecyloxy)cinnamic acid, poly penta-ethylene glycolcinnamate, poly-(*p*-propoxycinnamate)-co-(*p*-undecyloxycinnamate), 4-methoxycinamoylphthaloyl chitosan, 4-methoxycinamoylphthaloyl irradiated chitosan and poly[vinyl-2,4,5-trimethoxycinnamate(vinyl alcohol)]copolymer shows no transdermal absorption. This agrees will with the facts that all the test polymeric UV filters possesed M.W. of > 2000. Such high M.W. help retard the transdermal penetration of the compounds.

All the above experiments were done using sunscreen solutions. To ensure that the penetration of sunscreen chemicals tested above can be used to predict the penetration of UV filters in the form of personal care products, similar experiment were done using skin lotion incorporated with the test UV filters. Five mL of this lotion was mixed with 500 mg of UV filters. This gave the lotion with sunscreen concentration of 0.1 mg/μL. Twenty μL of this lotion was then applied to 2.27 cm^2 mouse skin, giving the final coverage of the sunscreen chemical on mouse skin of 0.88 mg/cm^2.

It can be seen that OMC, BMDBM could penetrate the baby mice skin well (table 3.4). 2-Ethylhexyl-2,4,5-trimethoxycinnamate incorporated into the lotion could very slightly permeated through the baby mice skin while di(2-ethylhexyl-2,4,5-trimethoxybenzalmalonate shows no transdermal absorption at all. This result agree well with the results done using sunscreen solutions.

This result indicates that skin penetration ability of sunscreen chemical is primarily governed by chemical structure of the sunscreen. Formulation although can affect the penetration rate, but at the minor role.

3.2 Determination of percutaneous absorption by suction blister

3.2.1 Analysis of sunscreen in suction blister fluid by UV/Vis spectrophotometry

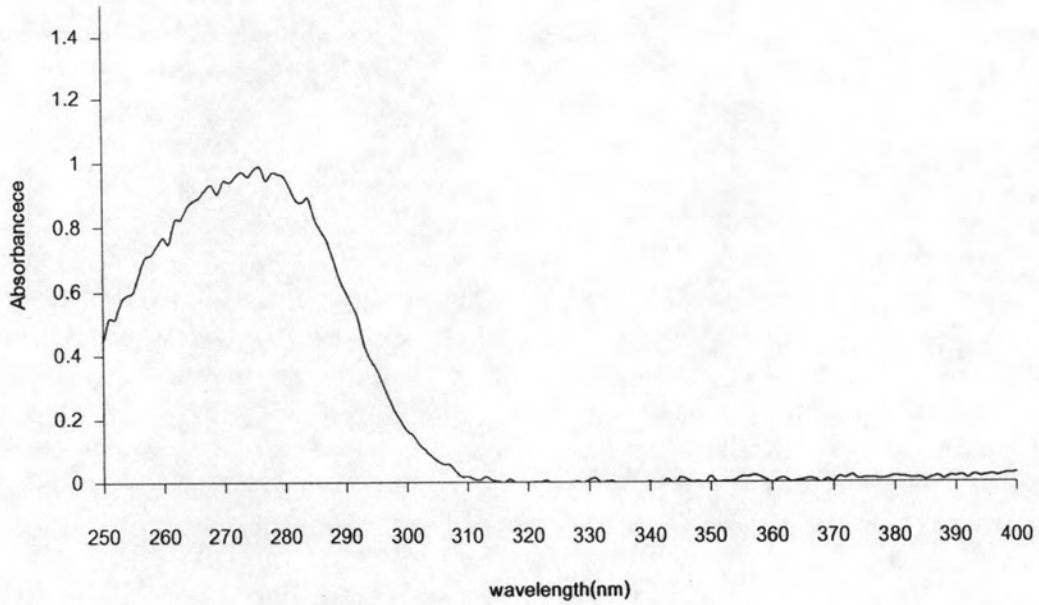


Figure 3.6 UV absorption spectrum of the suction blister fluid.

From Figure 3.6, it can be seen that UV absorption spectrum of SBF contains no absorption bands beyond 300 nm, as a result, presence of UVA and UVB filters in the SBF can be detected by UV absorption spectrometry.

When sunscreens were added into the SBF at 10 ppm concentration, absorption bands of the sunscreens can still be detected clearly. (Fig. 3.7) This makes it possible to quantitate amount of sunscreen in SBF using UV absorption spectrometry.

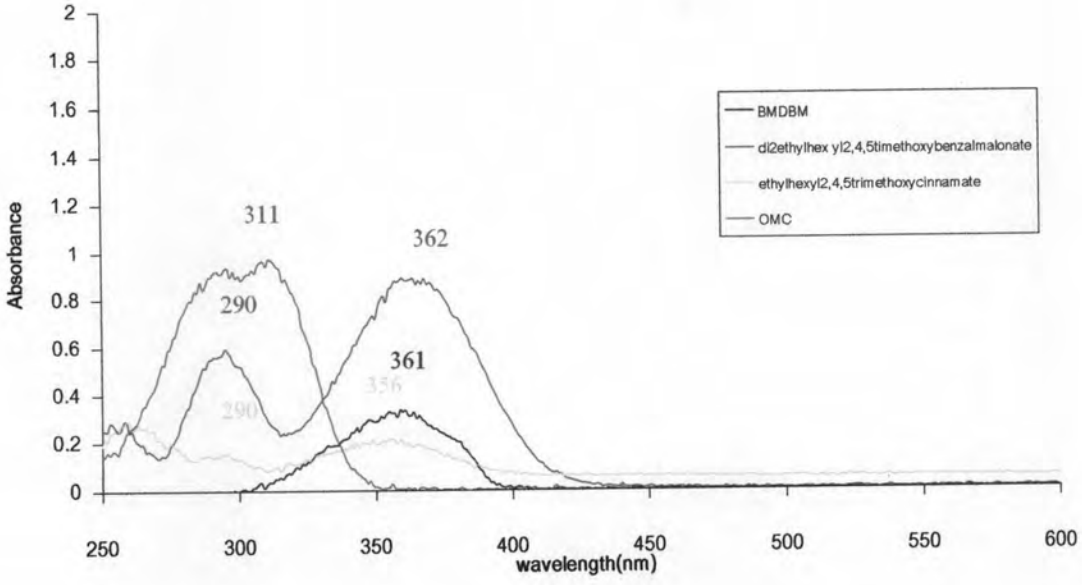
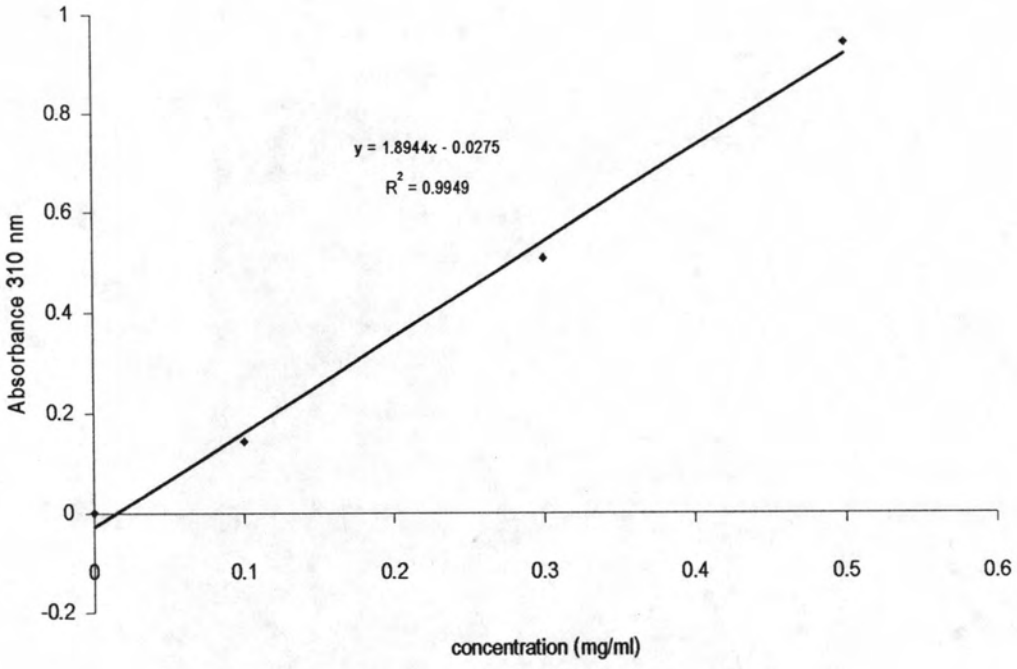
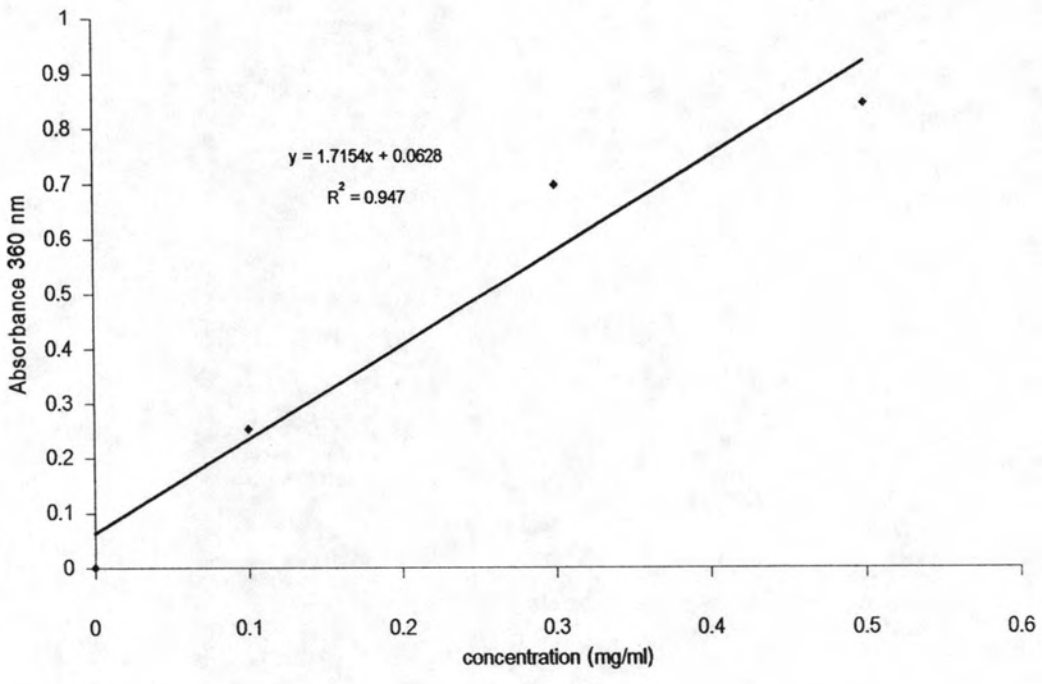


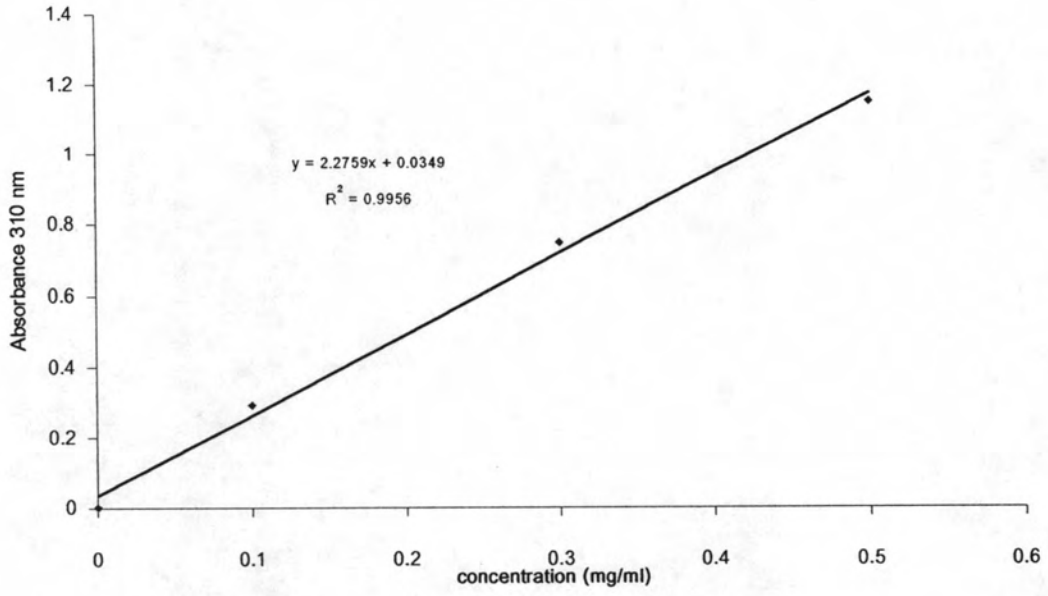
Figure 3.7 UV spectrum of 10 ppm sunscreens in suction blister fluid.

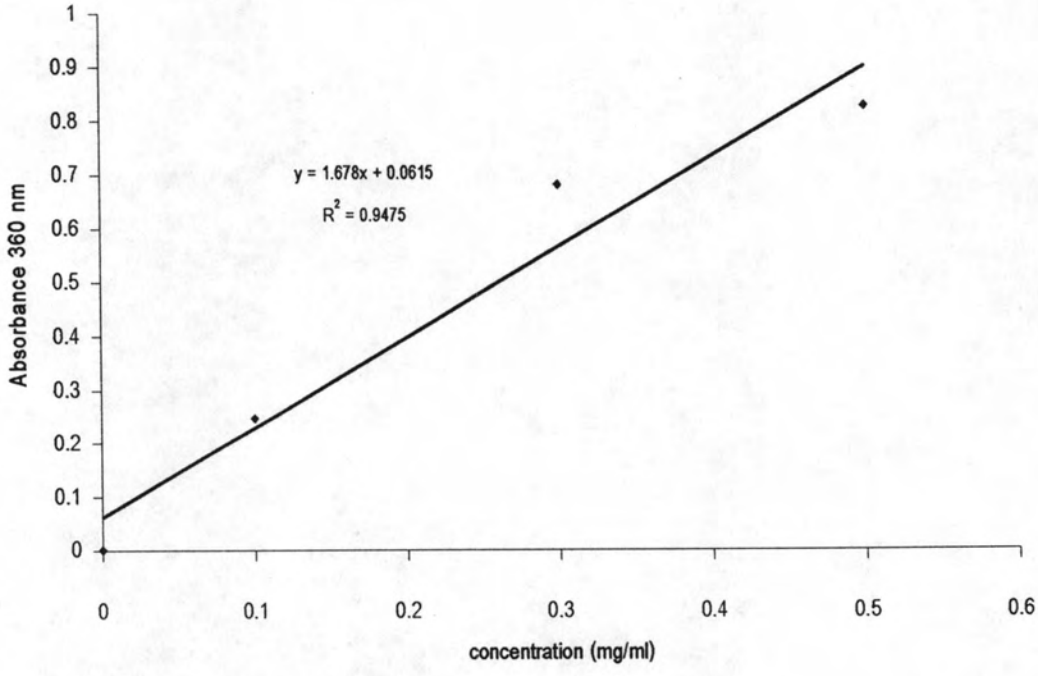
Since the suction blister fluids from different volunteers migth be different, calibration curve of OMC and BMDBM were contructed for each individual. Specific amount of the sunscreen was added into SBF and the resulting solution was subjected to UV-absorption measurement at its λmax. Figure 3.8 shows the calibration plots of OMC and BMDBM constructed using SBF from five volunteers. There graphs were used for the determination of sunscreens in SBF withdrawn from the corresponding volunteers after the blister roof skin had been applied with sunscreen spiked lotion. (3.2.2)


y = 1.8944x - 0.0275
R² = 0.9949
Absorbance 310 nm
concentration (mg/ml)
1
0.8
0.6
0.4
0.2
0
-0.2
0
0.1
0.2
0.3
0.4
0.5
0.6

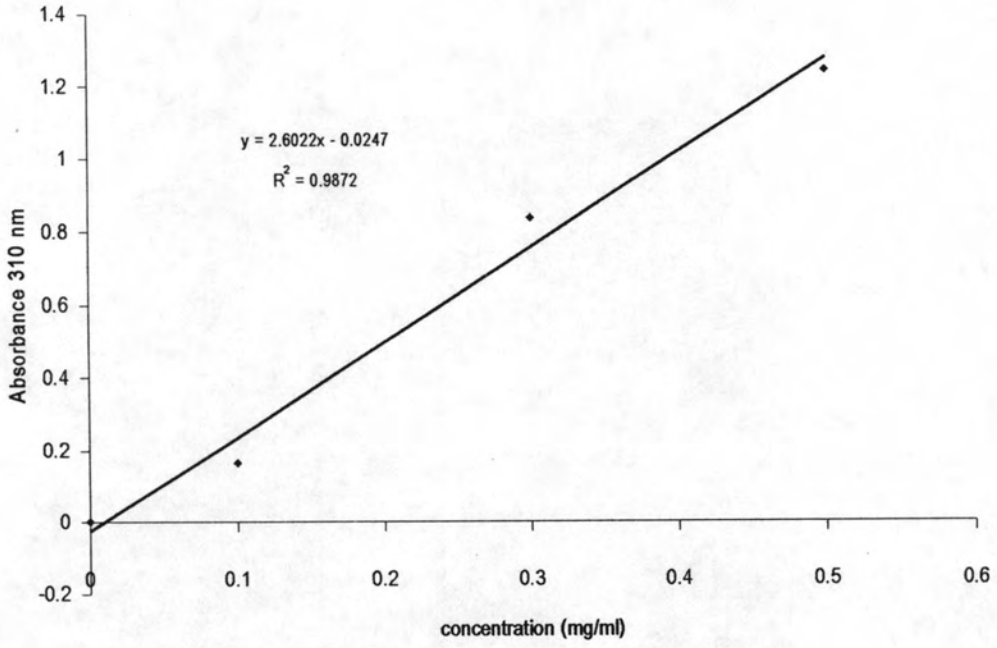

y = 1.7154x + 0.0628
R² = 0.947
Absorbance 360 nm
concentration (mg/ml)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6

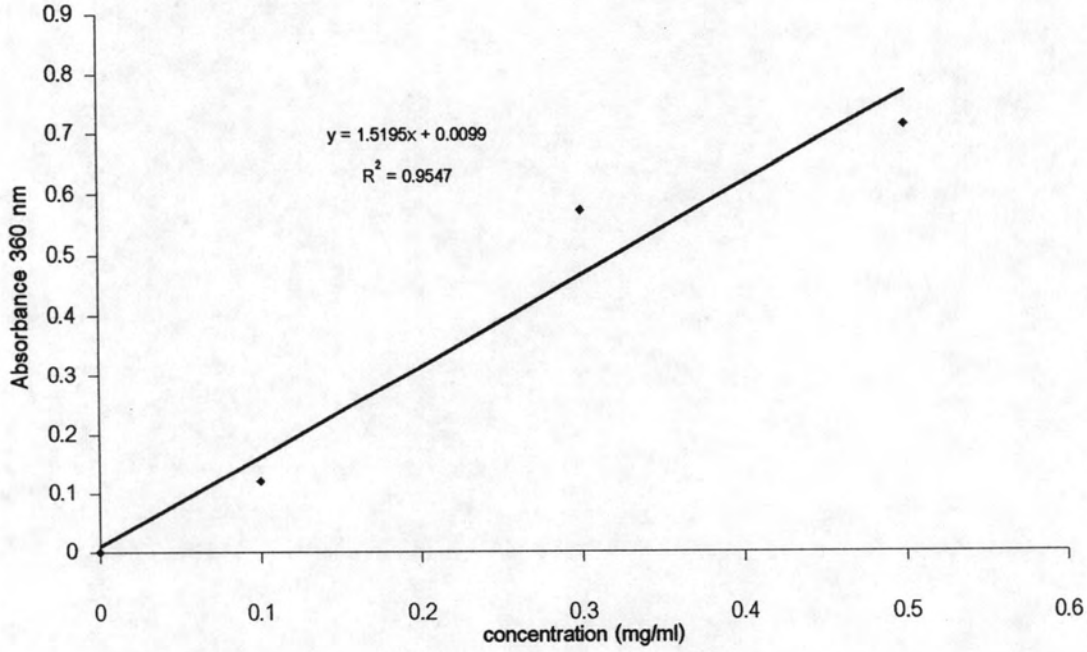
A


y = 2.2759x + 0.0349
R² = 0.9956
Absorbance 310 nm
concentration (mg/ml)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.1
0.3
0.5

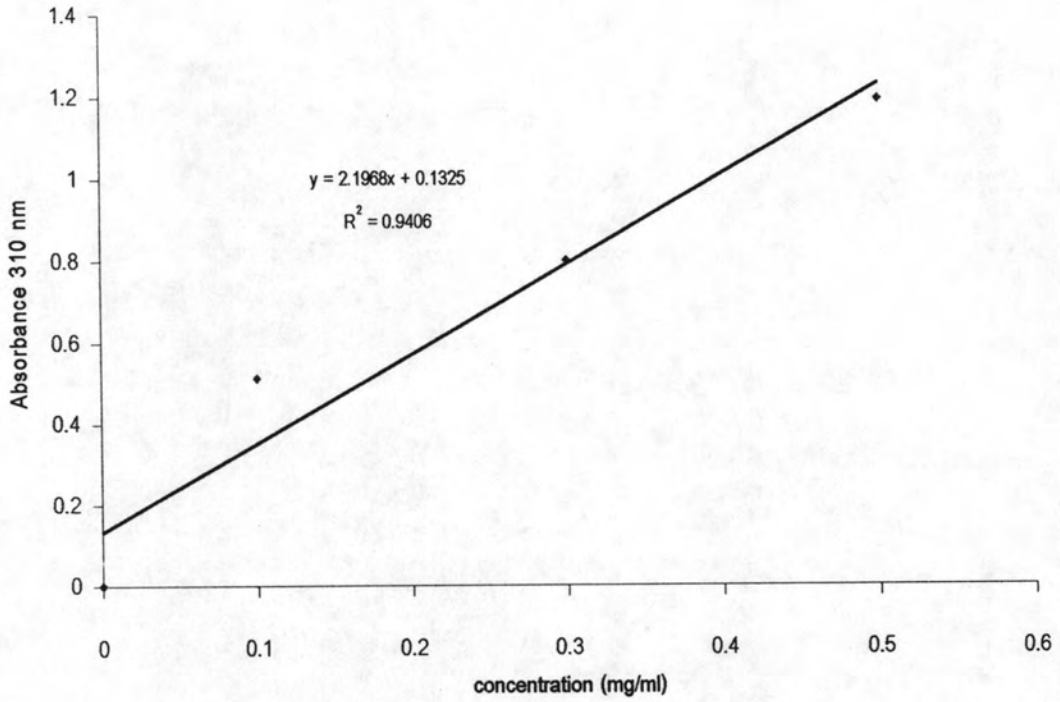

y = 1.678x + 0.0615
R² = 0.9475
Absorbance 360 nm
concentration (mg/ml)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

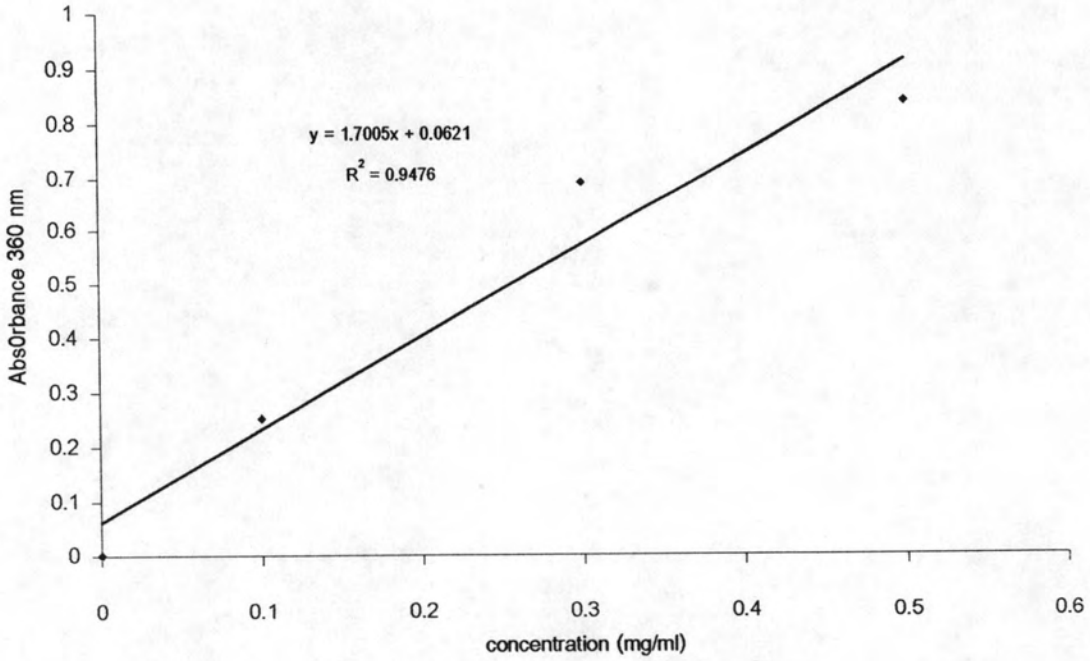
B


y = 2.6022x - 0.0247
R2 = 0.9872
Absorbance 310 nm
concentration (mg/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
0.1
0.2
0.3
0.4
0.5
0.6

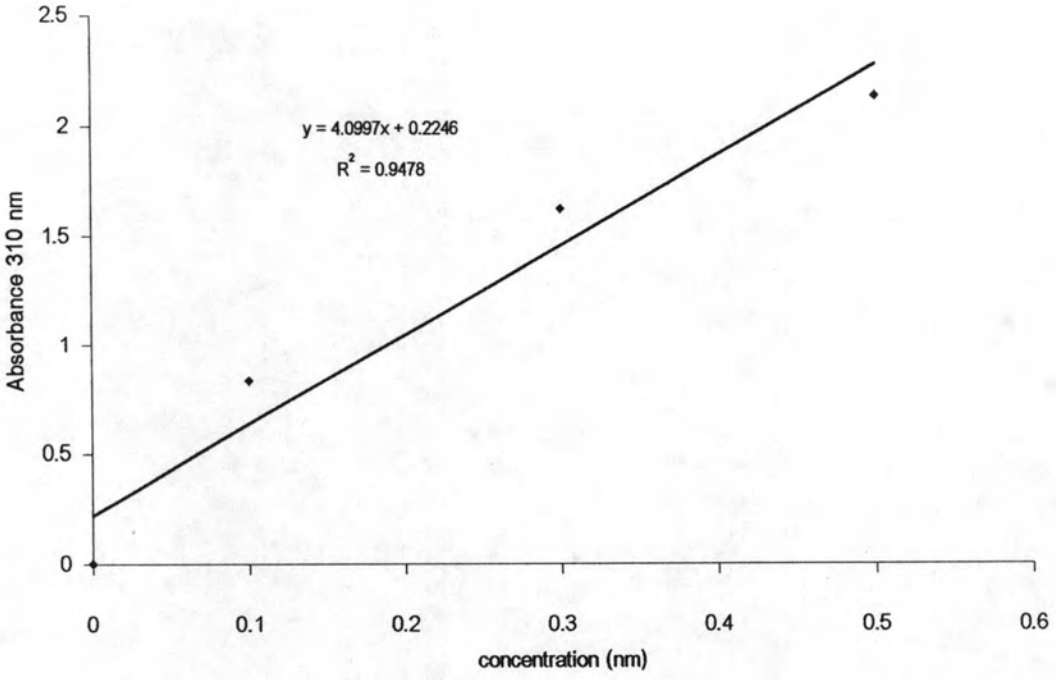

y = 1.5195x + 0.0099
R2 = 0.9547
Absorbance 360 nm
concentration (mg/ml)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6

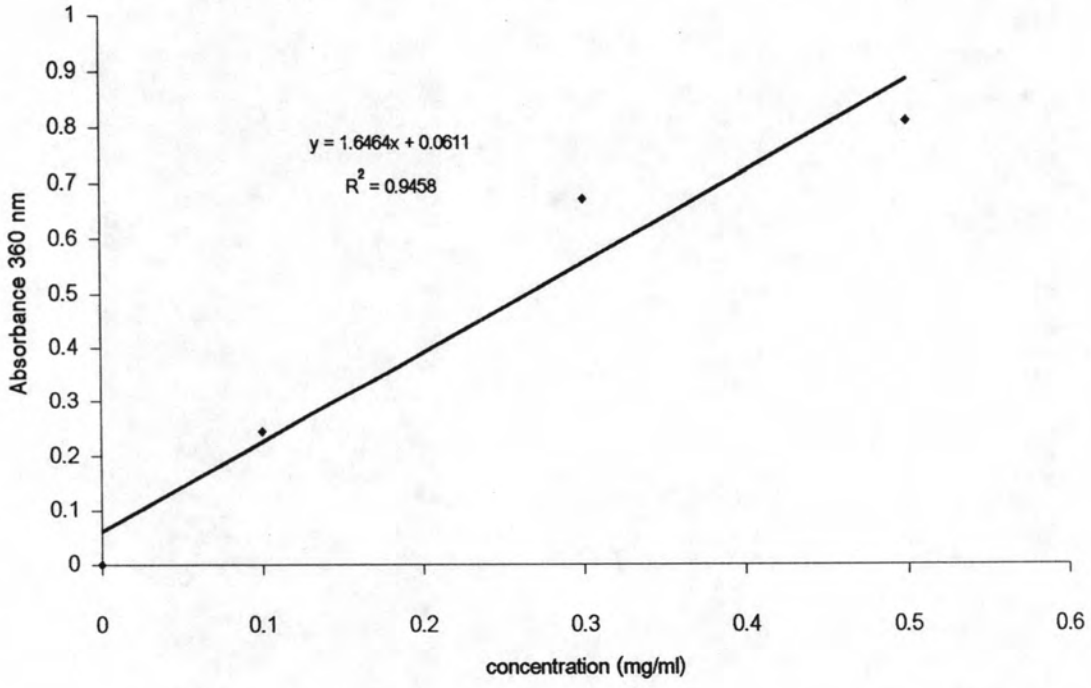
C


y = 2.1968x + 0.1325
R² = 0.9406
Absorbance 310 nm
concentration (mg/ml)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.1
0.3
0.5


y = 1.7005x + 0.0621
R² = 0.9476
AbsOrbance 360 nm
concentration (mg/ml)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

D





E

Figure 3.8 Calibration curves of OMC and BMDBM in suction blister fluid (SBF) for each volunteer; A) first volunteer, B) second volunteer, C) third volunteer, D) fourth volunteer, E) fifth volunteer

3.2.2 Study of transepidermal penetration of various sunscreen through volunteer human skins by suction blister method.

In this experiment, four UV filters; OMC, BMDBM, 2-ethylhexyl-2,4,5-trimethoxycinnamate and di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate, were blended into the lotion to give the final concentration of 0.1 mg/μL. When 20 μL of the UV filter spiked 5 mL lotion were applied to 0.88 cm^2 skin at the suction blister roof, the final coverage of the sunscreen on the skin was 2.27 mg/cm^2. The suction blister fluid was then collected after appropriate times.The appropriate time was determined by performing a test on OMC penetration using this suction blister technique. After the skin at the suction blister was applied with 2.27 mg/cm^2 OMC, SBF was collected at 15, 30, 90 min and 3 hr. Maximum concentration of OMC in SBF could be found at 3 hr. (see appendix Fig.C.1) Therefore, for the following experiments, SBF was collected after the skin at the suction blister roof had been applied with sample for 3 hr.

Quantitative analysis of sunscreen in SBF was done using calibration curve (3.2.1). The results (Table 3.5) indicates that OMC and BMDBM could very well penetrate the human epidermis into the SBF. This result agree with previous studies done by Potard *et al.* [14] and Heyden *et al.* [12] which indicated the transdermal absorption of these two sunscreens.

When comparing the results of the penetration of sunscreens obtained from Franz diffusion cell experiment which use the whole baby mouse skin (epidermis+dermis) and those obtained from the suction blister experiment which represent the penetration through human epidermis, it is obvious that the two experiments agree to each other well. Both experiments revealed that OMC and BMDBM can penetrate the skin well while 2-ethylhexyl-2,4,5-trimethoxycinnamate and di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate can neither penetrate baby mice skin nor human epidermis. Statistical analyses using t test and Pearson's test indicate good correlation between the Franz diffusion cell experiment and the suction blister experiment (table 3.6 and 3.7)

The thickness of baby mice skins were measured by micrometer. Full thickness skin is approximately 1.76± 0.172 mm or 1760±163.7 μm (mean± S.D.)(see appendix page 72). Human epidermis is 1.5-3.0 mm. thick [50]. Corrrelation between human epidermis and the used baby

mice skin is probably a result of a coincialently similar thickness between the two skins. This result, therefore, encorages the used of baby mice skin for transdermal penetration test.

Table 3.5 Percent penetration* of UV filters through human epidermis after the skin at the suction blister roof had been applied with sunscreen lotion for 3 hr.

UV filters	%penetration (mean±S.D.)
OMC	38.38±6.62
BMDBM	34.42±11.6
2-ethylhexyl-2,4,5-trimethoxycinnamate	0±0
di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate	0±0

*determined from the withdrawn SBF.

Table 3.6 Comparing between percent penetration by diffusion cell and percent penetration by suction blister. (Lotion form)

UV filter	Suction blister method (mean±S.D.)	Diffusion cell method (mean±S.D.)	Correlation coefficient (r)	$\alpha = 0.05$
OMC	38.38±6.62	40.45±3.77	0.8677	significant
BMDBM	34.42±11.6	30.73±5.40	0.9917	significant
2-ethylhexyl-2,4,5-trimethoxycinnamate	0±0	2.39±0.14	0	-
di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate	0±0	0±0	0	-

Note:data was shown in appendix page 70

Table 3.7 Comparing between percent penetration by diffusion cell and percent penetration by suction blister. (Solvent form)

UV filter	Suction blister method (mean±S.D.)	Diffusion cell method (mean±S.D.)	Correlation coefficient (r)	$\alpha = 0.05$
OMC	38.38±6.62	31.60±5.43	0.9168	significant
BMDBM	34.42±11.6	23.47±2.29	0.8860	significant
2-ethylhexyl-2,4,5-trimethoxycinnamate	0±0	1.51±0.50	0	-
di(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate	0±0	2.63±1.53	0	-

Note:data was shown in appendix page 70