CHAPTER V

DISCUSSION

1. Formulation of sunscreen preparations

From the physical appearances of the prepared sunscreen emulsion shows that sunscreen emulsion prepared using modified emulsion bases consisting of sunscreen agents and water resistant ingredients are physically stable when stored in the hot air oven at 40 ^{0}C, 50 ^{0}C and 60 ^{0}C for 3 months and Freeze - thaw cycle (six cycles). Table 13 shows that the pH values of the prepared sunscreen creams were 6.6-7.4 because basic formula of oil in water emulsion contains triethanolamine which has pH of alkaline resulted in higher pH of some preparations. Table 18 shows content analysis of sunscreen agent in various sunscreen creams. The data showed percent labelled amount of octyl dimethyl PABA, octyl methoxycinnamate and oxybenzone. They were between 87.00-101.71, 91.75 - 105.75, 83.00 - 113.33, respectively. These content would result in penetration into the skin and SPF values. In this investigation percent labelled amount of micronized titanium dioxide were not analyzed because it was very stable and did not penetrate in to the skin, it could not extract from an emulsion base.

2. Determination of the *in vitro* SPF values of sunscreen emulsions using SPF - 290 analyzer

This study focused on using the SPF-290 analyzer to evaluate and compare SPF of sunscreen emulsions with an *in vivo* method if they showed any correlation between them. Table 19 shows SPF valued of all formula. Formula 1 and 10 contained only 5% micronized titanium dioxide and showed SPF value only 2.1± 0.2, 2.3 ± 0.1 respectively. Formula 19 contained 5% micronized titanium dioxide and 3% silicone oil 350 cps in oil in water emulsion and showed

the SPF value of 3.0 ± 0.3. Formula 28 contained 5% micronized titanium dioxide and 3% silicone oil 350 cps in water in oil emulsion and showed the SPF value of 3.8 ± 0.7 as shown in Table 20; but when compared between oil in water and water in oil emulsion base there were not significant difference. The formula contain only single sunscreen agent showed lower SPF value of 2.1-5.9 shown in Table 20. While formula contain two sunscreen agents, medium SPF value, between 6.1-11.6 were resulted. Table 22 showed high SPF value, all of these formula contained three sunscreen agents; sunblock agent ($MiTio_2$); UVB absorber (octyl dimethyl PABA and octyl methoxycinnamate); UVA absorber (oxybenzone). SPF value were between 14.8 - 22.3. The type of emulsion base did not made the significant difference between the formula as shown in Table 23 when calculated with ANOVA, but the comparative between the same sunscreen agent using t test seven pairs of them showed non significant different eleven pairs of them showed significant different. From these data most of them showed significant different so the type of emulsion could be effect on the SPF value. Table 24 shows the comparative SPF value between the ordinary emulsion base with and without silicone oil 350 cps ; it shows no significant different data when calculated with ANOVA, but the comparative between the same sunscreen agent but different in silicone content using t test studies four pairs of them showed non significant different eleven pairs of them showed significant different explain that the addition of silicone oil to the emulsions caused the significant different in SPF value of sunscreen products because silicone oil could improve polarity of the emulsion to give more lipophilic resulted in higher its extinction coefficient. So the emulsion containing silicone oil give higher SPF.

Additionally, the *in vivo* and *in vitro* correlation of the obtained SPF values was determined so as to foresee the future use of the *in vitro* results as

preliminary checkups for future *in vivo* experiments. Since the SPF-290 analyzer's user guideline allows for 20% variation of the obtained mean SPF values, SPF ranges were used instead of SPF means when the comparisons between *in vitro* and *in vivo* results. The SPF results obtained from the *in vitro* experiments pointed out that the SPF-290 analyzer predicted SPF values of sunscreen emulsions Formula 1-36.

3. *In vitro* skin penetration

Selected formula 26, 27 ,35 and 36 (High-SPF) were used to study *in vitro* skin penetration because of High-SPF sunscreen products are famous to use and have opportunity toxicity more than Low-SPF sunscreen products.

Table 25 and 26 shows that sunscreen agents have been fully investigated, there is little published data describing either their penetration into the skin or their permeation through the skin. All sunscreen agents are lipophilic substance. The receptor fluid used was physiological saline with albumin to made the same condition as in the skin fluid.

The phenomenon of percutaneous absorption can be visualized as a composite of a series of steps in sequence, that is, adsorption of a penetrant molecule onto the surface layers of stratum corneum. Table 25 shows that sunscreen agents remained in stratum corneum more than $87.94 \pm 0.52\%$ while it penetrated through epidermis only 0.32 - 2.06% and less penetration to dermis only 0.02-0.15%. Table 26 shows the penetration of sunscreen agents through the skin into receptor fluid. There was no any sunscreen agent in the receptor fluid. The receptor fluid was the representative of peripheral circulation.

All of sunscreen agents are lipophilic. All of the sunscreen agent are accumulate at the stratum corneum only small proportion of sunscreen agents was partition into epidermis and dermis. They showed no sunscreen agents in receptor fluid or systemic circulation.

4. Determination of the *in vivo* SPF values of sunscreen emulsions based on US-FDA method

In the US-FDA method, the standard homosalate which has the SPF value of 4 is used to calibrate the procedure in each group of volunteers with a particular skin type. The typical method is normally performed on volunteers who have type I and type II skins. We adopted this method to evaluate the *in vivo* SPF values of sunscreen creams in 20 Thai volunteers of whole type III and type IV skins. The average *in vivo* SPF value of standard homosalate in Thai volunteers was found to be 4.10 which showed no statistically significant difference at a 90% confidence interval from the claimed value as tested in volunteers with type I and type II skins. Therefore, it is confirm that the *in vivo* SPF evaluation in Thai volunteers adhered to the standard of US-FDA. Table 28 showed the lower SPF value of formula 26, 27, 35 and 36 because the skin type of Thai volunteers differs greatly from that of volunteers in western countries including the United States of America where the US-FDA procedure was originated. Thai volunteers usually have type III and type IV skins whereas volunteers in the western world have type I and type II skins. According to the US-FDA method, only volunteers with type I and type II skins are to be included in the study. It should be realized that people with type III and type IV skins have a much high content of melanin pigment in the skin than those with type I and type II skins. People with type I and type II skins are also more vulnerable to the harmful effect of UV radiation and indicate lower minimal erythema dosage level than other groups.

It should also be awared that determination of minimal erythema in people with darker skin color is much more difficult than that done in people with lighter skin color since the later group of people show a rapid and clear territory of erythema without any negative impact from the skin pigment.

Theoretically, the SPF evaluation is independent on the skin types of volunteers according to the following equation.

SPF = MED protected skin / MED unprotected skin

Consequently, volunteers who have more melanin in the skin are prone to have higher UV dose that produces minimal erythema than those who have lighter skin color when their skins are not protected by the use of sunscreen. When the skins of volunteers who have darker skin color are protected from the UV radiation by applying sunscreens with specific SPF value, the minimal erythema dose will increase proportionally and hence the SPF value remains the same. However, an important consideration must be taken during the appraisal of individual minimal erythema dose for unprotected skin, especially in volunteers with plenty of melanin. As mentioned earlier, justification of MED, though performed by a dermatological expert, can be misinterpreted by the ability of melanin to conceal the point where minimal erythema emerges. Therefore, the next point where more than minimal erythema appears will be selected which corresponds to a 25% increase in UV dose according to the US-FDA criteria in determining dosage interval. As a consequence, 1 MED for a particular Thai volunteer might be misinterpreted and the value of 1.25 MED is obtained instead. Since the MED for unprotected skin will later be used to calculated the estimated MED for protected skin, the error in determining this dose will lead to an even greater overdose of MED for protected skin particularly for products with high SPF values such as those above 15.

5. Comparison between SPF values obtained from US - FDA procedure and SPF - 290s analyzer

The comparison between the *in vitro* SPF results and the *in vivo* SPF results was more interesting. The results obtained from this study revealed that the *in vivo* SPF values from US-FDA method of standard homosalate, was well within the range of *in vitro* SPF result. When the correlation of the *in vivo* data obtained from US-FDA method and the *in vitro* data obtained from SPF-290 analyzer was determined in Formula 26, 27, 35 and 36 the correlation coefficient (r) was 0.5658, this indicate very low correlation. One should be aware of substituting the *in vitro* SPF data for the *in vivo* data since there still are certain limitations of the SPF-290 analyzer which could render the interpersonal determination of SPF subject to large variation. There are several factors which affect the SPF determination. The examples of these factors include the difference in transpore membrane characteristics between batches, the volume of sunscreen product applied on the membrane, how evenly the sunscreen emulsion or lotion being spread on the membrane, and the time required for emulsion or lotion to dry before analysis. Certainly, the use of this instrument requires trained personnel and if the above limitations have been overcome, the more reliable data will be obtained.