Correlation study of *in vitro* and *in vivo* test for SPF (Sun Protection Factor)

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Summary

In this study, we evaluate the correlation between *in vitro* and *in vivo* determination of SPF of sunscreen products containing various ingredients depending on emulsification system.

For *in vitro* approach, we determined SPF by the method of Diffey and Robson using an Transpore™ tape(3M Health care,USA) and SPF 290-analyzer(Optometrics Co.USA). SPF values and standard deviations are calculated and displayed after completion of the run. *In vivo* SPF values are determined according to KFDA (the Korea Food and Drug Administration) method in panels of Fitzpatrick's skin type II or III.

We investigated the difference in SPF data of sunscreen ingredient according to emulsification system. The *in vivo* SPF data is high in water-in oil(W/O) emulsion than in oil-in water(O/W) emulsion samples. The difference may be due to the particular behavior in each vehicles and its presence on skin surface may produce a different sunscreen film. We obtained the corrlation coefficient between in vitro and in vivo SPF data for O/W (R-squre=0.72)and W/O emulsion(R-squre=0.77). From these results, we suggest the improvement of methodology using Transpore™ tape as substrate to increase the predictability of *in vitro* method.

Keywords : Sun protection factor, SPF test .

Introduction-

Sunlight is a part of our everyday life but can also be harmful to human skin. Sunscreen have been used in cosmetic products for many years to prevent deleterious effects of sunlight on skin. The photoprotective efficacy of sunscreen product is generally expressed as its sun protection factor which is a ratio of the time required for a given irradiation to produce minimal perceptible erythema(MED :minimal erythemal dose) in sunscreen-protected skin to the time required for

unprotected skin. The *in vivo* measurement of SPF is complicated and time-consuming procedure not really suitable for broad screening during product development. Many publications deals with the problem of the *in vitro* determination of SPFs [1]. The point of *in vitro* method is that *in vitro* SPF must correlate with the result of *in vivo* data , the costs must remain low and the materials needed must be readily available, Many substrate have been tested for sunscreen application, including synthetic skin, mouse or human epidermis. Although *in vitro* SPF testing is not well harmonized word widely, this method has provided pre-scan tool prior to *in vivo* testing. The reliablility of *in vitro* method is determined by the degree of correlation with *in vivo* method and is a important information for routinely using.

We adopted Diffey and Robson method[2] using Transpore™ tape(3M Health care,USA) as substrate and evlautate the correlation between *in vitro* and *in vivo* determination of SPF of several sunscreen products with different formulations.

Materials and Methods

Samples

To investigate the effect of formuation to the degree of correlation and SPF, we designed water in oil emulsion and oil in water emulsion for the same UV filters.

Quatitative composition of oil in water emulsions(O/W) and water in oil emusion(W/O) and UV filters is described in Table1, Table 2 and Table 3.

Table 1.Formula of oil in water emulsion sample

INCI name	% (g)
Cetearyl alcohol	1.20
Glycerol stearate	1.00
PEG-100 stearate	1.00
Polysorbate 60	1.20
Sorbitan sesquioleate	0.30
Dicaprylyl carbonate	8.00
Di-C12-13 alkyl malate	8.00
Methyl paraben	q.s.
Propyl Paraben	q.s
Water	Adjusted to 100
Disodium EDTA	0.02
Glycerin	10.0
Imidazolidinyl urea	q.s.
Sunscreen ingredient	

Table 2.Formula of oil in water emulsion sample water in oil

INCI name	%(g)
Ozokerite	1.00
Beeswax	1.00
Cetyl PEG/PPG-10/1 Dimethicone	2.50
Sorbitan sesquioleate	0.50
Methyl paraben	q.s.
Propyl Paraben	q.s.
Dicaprylyl carbonate	15.00
Cyclomethicone	3.00
Cylclomethicone and Dimethicone crosspolymer	4.00
Water	Adjusted to 100
Disodium EDTA	0.02
Glycerine	10.00
Sodium chloride	1.20
Imidazolidinyl urea	q.s.
Sunscreen ingredient	

The 6 following UV filters in table 3. were incorporated in O/W, W/O formulations.

Table 3. Sunscreen ingredient

INCI name	%(g) of experimented
Octyl methoxycinnamate (OMC)	3%,7%,10%
Isoamyl-p-methoxycinnamate (IMC)	3%,5%,7%
Bis-Ethylhexyloxylphenol Methoxyphenyl Trazine (EPMT)	1%,2%,3%
Titanium oxide/C12-15 alkylbenzoate/polyhydroxystearic acid/alumina/aluminum stearate, (Solaveil CT-100)	1%,2%,3% (as TiO_2)
Titanium dioxide/silica/alumina (TiO2 MT500SA)	1%,2%,3% (as TiO_2)
Titanium/water/simethicone (Eusolex T-aqua)	1%,2%,3% (as TiO_2)

In vitro Method

In vitro SPF measurements were obtained using an Optometirics SPF-290S Sunscreen Analyzer(Optometrics USA, Inc.) equipped with an integrating sphere accessory. Covering both the UVB and UVA spectral regions, the system automatically scans over 290 to 400 nm wavelength range, accumulating and storing data at 5 nm intervals. A monochromatic protection factor (MPF) ,the reciprocal of the sample transmittance, is calculated into SPF.

$$SPF_{Sem} = \frac{\sum_{290}^{400} E_\lambda B_\lambda}{\sum_{290}^{400} \frac{E_\lambda B_\lambda}{MPF_\lambda}}$$

Eλ = Spectral irradiance of terrestrial sunlight under controlled conditions, and
Bλ = Erythemal effectiveness. (1)

In vivo Method

In vivo SPF values are determined according to KFDA (the Korea Food and Drug Administration) method in panels of Fitzpatrick's skin type II or III.

As UV-radiation source, we used Oriel Solar simulator(Oriel Co. U.S.A). The SPF value is defined as the UV energy required to produce a minimum erythemal dose(MED), or redness, on protected skin divided by the UV energy required to produce an MED on unprotected skin:

$$\mathrm{SPF} = \frac{\text{MED of protected skin}}{\text{MED of unprotected skin}} \quad (2)$$

Condition of the test

Application rate of preparation	2.0 mg/cm^2
Calculation of SPF	Arithmetic mean
Evaluation after exposure	18 hr after irradiation
Subject (n)	10
Test area	24 cm^2

]

Results

The measured SPF values ar shown in table 4. and illustrated in Fig.1,Fig2,Fig3,Fig4,Fig5 and Fig6.

	O/W		W/O	
	In vitro	*In vivo*	*In vitro*	*In vivo*
Octyl methoxycinnamate(OMC) 3%	7.35±1.05	4.95±0.68	8.25±1.22	5.28±0.38
Octyl methoxycinnamate(OMC) 7%	11.51±0.56	9.55±0.64	11.69±1.02	9.73±0.68
Octyl methoxycinnamate (OMC) 10%	13.11±1.30	9.78±0.56	14.10±1.02	12.97±5.63
Isoamyl-p-methoxycinnamate 3%	8.80±1.03	5.42±1.21	8.66±1.28	5.13±0.79
Isoamyl-p-methoxycinnamate 7%	10.65±0.97	9.34±1.46	10.27±0.59	12.90±4.16
Isoamyl-p-methoxycinnamate 10%	12.77±0.98	11.60±0.80	12.23±0.69	15.00±2.40

Bis-Ethylhexyloxylphenol Methoxyphenyl Trazine 1%	3.37±0.79	2.94±0.70	5.07±1.56	3.96±0.94
Bis-Ethylhexyloxylphenol Methoxyphenyl Trazine 2%	6.67±7.36	4.68±0.83	9.24±3.13	6.50±1.59
Bis-Ethylhexyloxylphenol Methoxyphenyl Trazine 3%	11.37±4.02	7.60±0.00	12.00±8.08	8.00±2.16
Solaveil CT-100 3.33% (1% as TiO_2), OMC 5%	11.00±1.30	9.10±1.67	13.43±1.53	10.00±0.00
Solaveil CT-100 6.66% (2% as TiO_2), OMC 5%	15.00±0.98	12.40±1.50	15.34±3.24	15.90±1.34
Solaveil CT-100 9.99% (3% as TiO_2), OMC 5%	16.10±0.08	15.00±1.56	18.24±1.12	21.20±2.12
TiO2 MT500SA 1.18% (1% as TiO_2), OMC 5%	11.50±0.35	6.40±1.01	11.61±3.21	7.40±0.52
TiO2 MT500SA 2.35% (2% as TiO_2), OMC 5%	13.30±1.11	7.30±1.60	15.47±4.13	12.35±0.98
TiO2 MT500SA 3.53% (3% as TiO_2), OMC 5%	14.80±1.27	7.90±1.50	16.15±4.33	12.98±1.95
Eusolex T-aqua 3.33% (1% as TiO_2), OMC 5%	13.70±0.27	8.60±2.12	12.90±3.75	11.55±1.31
Eusolex T-aqua 3.33% (1% as TiO_2), OMC 5%	16.10±1.29	10.40±1.97	13.40±3.84	13.47±1.63
Eusolex T-aqua 3.33% (1% as TiO_2), OMC 5%	17.40±1.78	11.30±0.93	17.50±3.84	19.15±1.55

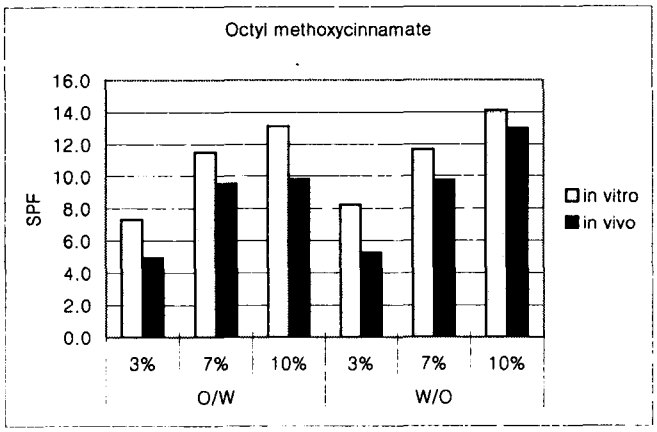

Fig.1 Comparative SPF of OMC

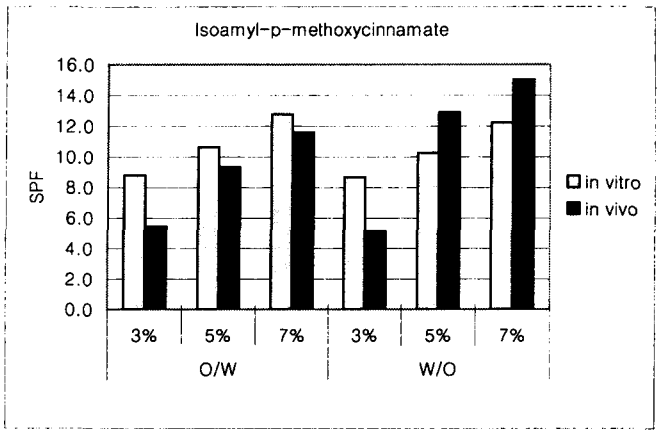

Fig.2 Comparative SPF of IMC

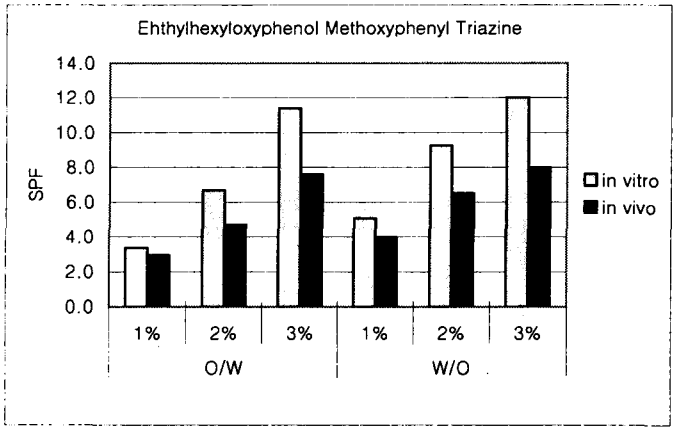

Fig.3 Comparative SPF of EPMT

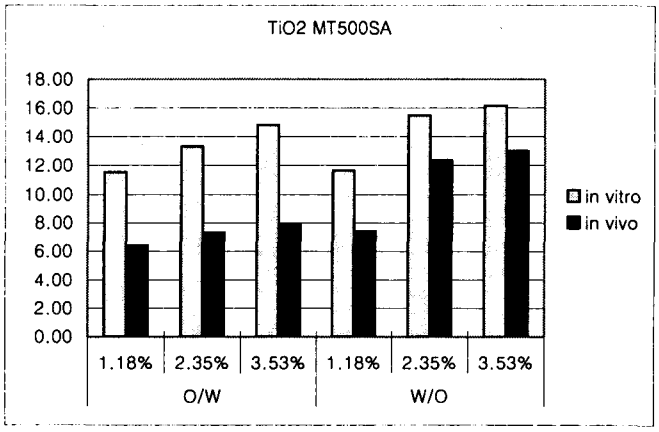

Fig.4 Comparative SPF of TiO2 MT500SA

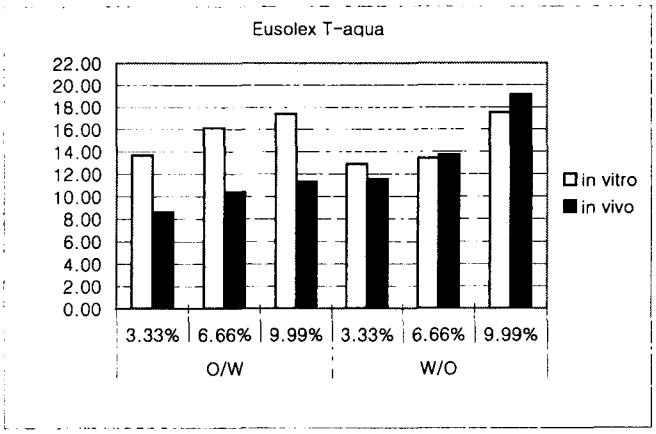

Fig.5 Comparative SPF of Eusolex T-aqua

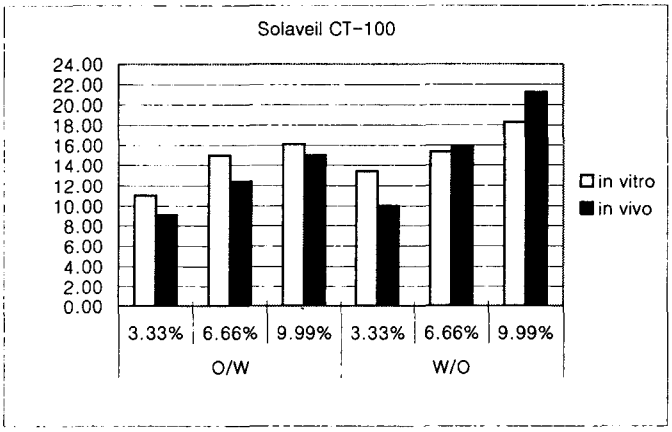

Fig.6 Comparative SPF of Solaveil CT-100

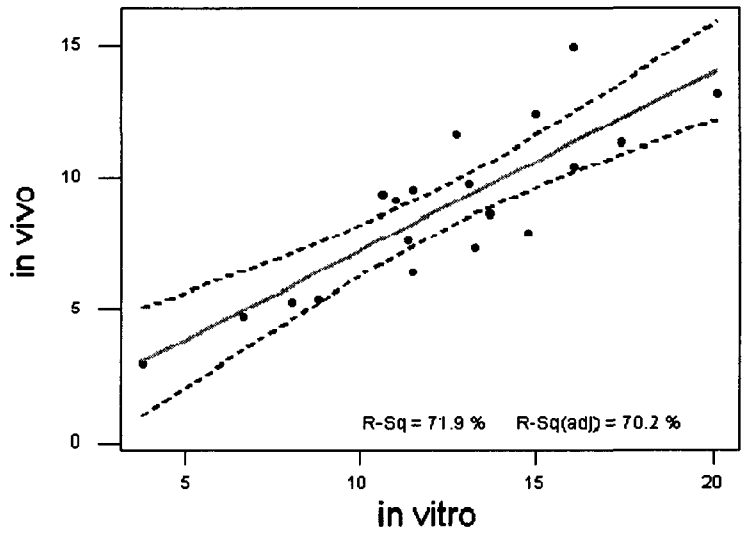

Fig.7. Plots of SPF measured by *in vitro* and *in vivo* method of O/W emulsion samples.

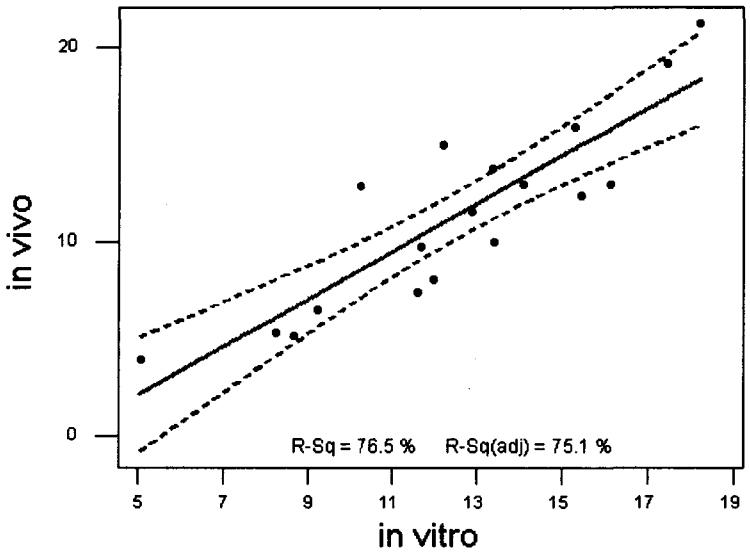

Fig.7. Plots of SPF measured by *in vitro* and *in vivo* method of W/O emulsion samples.

Discussion and conclusions

The results indicates that each sunscreen ingredients shows a increase dose-dependently (Table.1). We investigated the difference in SPF data of sunscreen ingredient according to emulsification system. The *in vivo* SPF data is high in water-in oil emulsion than in oil-in water emulsion samples. The difference may be due to the particular behavior in each vehicles and its presence on skin surface may produce a different sunscreen film. For all studied UV filters, there

were tendency that *in vitro* data is slightly higher than *in vivo* data in low SPF, but this is broken for relatively high SPF in water-in oil emulsion.

Fig.6, Fig.7 compare the *in vitro* SPF result with *in vivo* SPF result and least squares fit with 95% confidence intervals has been drawn for the data in dotted line. The results indicate that there was not so good correlation between *in vitro* and *in vivo* SPF for each O/W and W/O emulsion.

In vitro method based on spectrophotometric determination of changes in the spectral absorption properties of UV filter is different from the basis of *in vivo* method and it have to be calculated in considering of biological effect(erythema) and natural sun irradiations. So, it is very difficult to get highly accurate and the same data from *in vitro* method compared with *in vivo* data. To claim the real sunscreen effect to consumer, *in vivo* test is necessary and *in vitro* method can be used for formulator to pre-scan UV filters, or to assess the photostablilty [3],[4].

From the published literatures, there are indications that specific type of formulations may not correlate and attempts to improve the predictability using other substrates[5],[6]. Since the *in vitro* method is much faster and less expensive than using human volunteers, we need to study for the cause of difference between *in vitro* and *in vivo* methods. Perhaps the substrate does not react to UV irradiation as human skin *in vivo*. Or, the measurement system may not report the protective efficacy as human skin.

In conclusion, there were difference between emulsification system for the same sunscreen ingredients and it may provide preliminary information for formulations. We obtained the corrlation coefficient between in vitro and in vivo SPF data for O/W (R-squre=0.72)and W/O emulsion(R-squre=0.77). From these results, we suggest the improvement of methodology using Transpore™ tape as substrate to increase the predictability of *in vitro* method.

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