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In-vitro Assessment of Effectiveness and Photostability Avobenzone in Cream Formulations by Combination Ethyl Ascorbic acid and alpha Tocopherol Acetate

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ABSTRACT

Avobenzone is UVA sunscreen active substances, which are unstable when exposed to UV radiation, especially UVA. Theaim of this study is to determine the effectiveness and photo stability avobenzone when combined with ethyl ascorbic acid and alpha tocopherol acetate with various concentrations of ethyl ascorbic acid and alpha tocopherol by in vitro using spectrophotometric method. The photostability study of the eight variations of formula showed that the formula3, 4, 5, 6, 7 and 8 had significant differences with Formula 1 ($p<0,05$) and the best photostability represented by the formula 4 (avobenzone 2%, ethyl ascorbic acid2%) with avobenzone concentrations decrease by 11,82% for 15 hour using UVA lamp irradiation 4,7 mW/cm^2. In effectiveness of SPF (Sun Protection Factor) value determination showed moderate protection category (SPF ±6). The percent transmission of erythema and pigmentation test showed that cream had effectiveness as sunscreen by showing the category as sun block on the area pigmentation and not to erythema areas at concentration 1000 µg/ml.

INTRODUCTION

The sunlight that reaches the Earth's surface is composed of ultraviolet (200-400 nm), visible (400-800 nm) and infrared (> 800 nm) radiation. Ultraviolet light emitted is divided into three regions, namely the UVC (200-290 nm), UVB (290-320 nm) and UVA I region (340-400 nm) and UVA II region (320-340 nm) (Gonzalez *et al.,* 2007; Bonda and Marinelli, 1999; Chaudhuri *et al.,* 2006). The skin need protect from ultraviolet radiation hazard. Sunscreen is one effort to minimize of ultraviolet radiation penetration into the skin. Indonesia is a tropical country where the sun exposure is high to require the preparation of cosmetics that acts as a sunscreen (WHO, 2013). The Environmental Working Group (EWG) that founded in 1993 by Ken Cook and Richard Wiles reviewed over 1800 sunscreens and more than 257 brands and found morethan75% of sunscreens contain toxic chemicals that can increase the risk of cancer and other health problems. Chemical sunscreens identified hazards include PABA (Para amino benzoic acid), menthylanthranilate, oxybenzone, 4-metilbenzilidin camphor, 3-benzilidin camphor, Octylmethoxycinnamate, homosalate, octisalate, and octocrylene. FDA announced to use sunscreen safety and agreed that avobenzone can be used as chemical sunscreens(EWG's, 2013). The good sunscreen should have photo-stability. Photostability is defined as the ability of a molecule to remain intact with irradiation filter or sunscreen(Gonzalez *et al.,* 2007; Hojerova *et al.,* 2011), because they are deliberately selected as the molecules that absorb UV radiation (Nash and Tanner, 2014).

Avobenzone is significantly degraded by UV radiation and sunlight thus the effectiveness is reduced in skin protection (Rai *et al.,* 2012; Beasley and Meyer, 2010). One hour of sunlight exposure reduces avobenzone absorbance by 36% and mechanism stabilization of avobenzonecan be prepared by free radical scavenging (Korac and Khambholja, 2011; Gonzalez *et al.,* 2007). SPF value and UVA filters photostability can be improved by antioxidant with potential scavenging of reactive singlet oxygen (ROS) (L'Alloret *et al.,* 2012; Rai *et al.,* 2012; Latha *et al.,* 2013; Afonso *et al.,* 2014). Vitamins C and E are antioxidants that can counteract reactive singlet oxygen (ROS) (Latha *et al.,* 2013; Miura *et al.,* 2008; Hojerova *et al.,* 2011).

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The effectiveness of sun-screen preparations can be determined in-vitro by calculating the value of SPF (Sun Protection Factor), percent transmission of erythema (%Te) and percent transmission of pigmentation (%Tp) (Pelizzo *et al.,* 2012; Gonzalez *et al.,* 2007; Mishra and Chattopadhyay, 2012; Hupel *et al.,* 2011) .

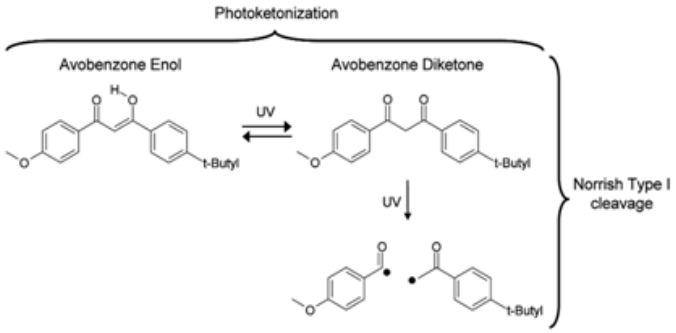

Fig. 1: Photoketonization reaction (reversible) and Norrish Type I reaction (irreversible) of Avobenzone.

The aim of this study was to determine avobenzone photostability that combined with ethyl ascorbic acid and alpha-tocopherol acetate to assess avobenzone levels before and after UVA lamp exposure, and determine effectiveness include the SPF value, percent transmission of erythema and pigmentation.

MATERIALS AND METHOD

Chemicals

Avobenzone, ethyl ascorbic acid, alpha tocopherol acetate were purchased from Sigma (St Louis, USA), ethanol 96% (Merck), cream bases were purchased from local industry in Bandung, Indonesia.

Apparatus

The apparatus used included analytical balance (Sartorius), double beam Shimadzu UV/Visible spectro-photometer, UVA Lamp 4,7mW/cm^2, UV radiometer, SPSS for window version 18.0, hot plate, homogenizer (IKA), rhion viscometer, pH strip (acid indicator), thermometer, centrifugation, ultrasonic, and other glassware commonly used in the laboratory.

Stock Solution

20 mg avobenzone was weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol 96% to give 200 µg/ml solution.

Maximum wavelength determination of avobenzone (λ max)

The maximum wavelength is determined at a concentration of 6 µg/ml of avobenzone diluted with ethanol 96%. The measurements were taken in the range of 200 to 400 nm

Preparation of Calibration Curve

Appropriate dilutions of the stock solution were done separately to get 2, 3, 4, 6, 8 and 10 µg/ml. The absorbances were measured at 356 nm (λ max of Avobenzone), and the calibration curves were plotted.

Cream Formulations

All of formulas was used for the preparation of oil-in-water creams containing F1 (basic as negatif control), F2 (avobenzone 2% as positive control), F3 (avobenzone 2% and ethyl ascorbic acid 0,5%), F4 (avobenzone 2% and ethyl ascorbic acid 1%), F5 (avobenzone 2% and ethyl ascorbic acid 2%), F6 (avobenzone 2%, ethyl ascorbic acid 0,5%, and alpatocopherolacetatate 1%), F7 (avobenzone 2%, ethyl ascorbic acid 1%, and alpha tocopherol acetate 1%), F8 (avobenzone 2%, ethyl ascorbic acid 2.0%, and alpha tocopherol acetate 1%).

Evaluation of Sunscreen Cream

Organoleptic observations

Organoleptic testing was done by looking at changes in color, odor (rancidity), and the occurrence of phase separation or rupture of cream.

Homogeneity test

Homogeneity testing was observed by checking the particles size between two object glasses to determine the formation of coarse particles.

pH Measurements

The pH measurements were carried out with pH strip acid indicator.

Viscosity Measurements

The viscosity cream measurements were carried out on rhion viscometer using spindle number 2 and the measurement were carried out in triplicate and calculated the average (Kumar, *et al.,* 2011).

Centrifuge test

Centrifuge testing has been done by centrifuging a cream preparation at a speed of 3800 rpm for 5 hour, and phase separation was observed (Lachman *et al.,* 1994; Elya *et al.,* 2013).

Thermal Stability

Cream preparation stored for 1 month at room temperature (28±2 °C), and checked every week include organoleptic, homogeneity, viscosity and pH (Lachman, *et al.,* 1994).

Photostability Testing of Sunscreen Cream

Sample Irradiation

Cream weighed of 0.1 g and placed on object, irradiated by UVA 4,7mW/cm^2 for 15 hour and radiation dose gave 846 KJ/m2, 1.692 KJ/m2 and 2.538 KJ/m2. This correspond to UVA dose that reaches the earth's surface during 2-5 at sunny day. For a comparison of each formula are treated without irradiation. Every

time each formula is taken and stored in a place protected from sunlight (Venditti *et al.*, 2008).

Initial Absorbance Measurements

Cream (0.1 g) was weighed, dissolved on 96% ethanol and the absorbances was measured by UV spectrophotometerat 356 nm. Avobenzone levels were calculated using the regression line equation of calibration curve.

Absorbance Measurements after UVA irradiation

A cream (0.1 g) were UVA irradiation dissolved in 96% ethanol and absorbance was measured with UV spectrophotometer at 356 nm (λ max of Avobenzone), and avobenzone levels were calculated using the regression line equation of calibration curve.

Evaluation

The Data of photostability testing of avobenzone cream was performed between the level of avobenzone and length of exposure time to UV light and the significance of the differences between mean values (where $p < 0.05$) was analyzed by the block method of variance (ANAVA) from SPSS version 18.0.

Effectiveness Assessment of Sunscreen Cream

SPF (*Sun Protection Factor*)

The cream (0.1 g) was weighed, further diluted in ethanol 96% to obtain 1000 µg/ml solution. The solution was diluted to obtain 100, 200, and 500 µg / ml. The absorption data were obtained in the range of 290-320 nm, every 5 nm. The SPF could be calculated followed by the application of Mandur equation (Gonzalez *et al.*, 2007; Pelizzo *et al.*, 2012; Sayre *et al.*, 1980):

$$\text{SPF spectrophotometric} = CFx \sum_{290}^{320} \text{EF}(\lambda) \text{ x I}(\lambda) \text{ x Abs}(\lambda)$$

Where :CF = correction factor = 10; EE (λ) = erythemal effect spectrum; I (λ) = solar intensity spectrum; Abs = absorbance of sunscreen product. The values of EE (λ) x I (λ) are constants. It is determined by Sayre *et al (*1980).

Table 1: Normalized product function used in the calculation of SPF.

Wavelenght (λ nm)	EE (λ) x I (λ) normalized
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180
Total	1

SPF assessments refer to the FDA regulations that categorize the effectiveness of sunscreen based on SPF values (Diffey and Robson, 1989). SPF assessments refer to the FDA regulations that categorize the effectiveness of sunscreen based on SPF values (Diffey and Robson, 1989). In evaluating SPF, sample was treated at thickness of 2 mg/cm^2, the SPF was calculated as the ratio of MED (Minimal Erythema Dose) of sunscreen-protected skin compare to the MED of unprotected skin (Haywood *et al.*, 2003; Stephens *et al.*, 2011).

Table 2: Prediction of SPF value.

SPF	Protection Category
2-4	Minimum
4-6	Moderate
6-8	Extra
8-15	Maximum
≥ 15	Ultra

Percent Transmission of Erythema and Pigmentation

The cream (0.1 g) was weighed, dissolved in ethanol 96% to obtain 1000 µg/ml solution. The solution has been diluted into100, 200, and 500 µg / ml. The absorption data were obtained in the range of erythema and pigmentation wavelength 292,5-372,5 nm, every 5 nm(Agustin *et al.*, 2013). % Te and % Tp were calculated followed by equation:

$$\% Te = \frac{\sum Ee}{\sum Fe}$$

$$\% Tp = \frac{\sum Ep}{\sum Fp}$$

Where: %Te = percent transmission of erythema value; %Tp = percent transmission of pigmentation; Ee = Σ(%T x Fe); Ep = Σ(%T x Fp).

Table 3: Transmission of erythema.

Wavelength Range (nm)	Erythema Fluks (Fe)
290-295	0.1105
295-300	0.6720
300-310	1.0000
310-315	0.2008
315-320	0.1125

Table 4: Transmission of Pigmentation.

Wavelength Range (nm)	Pigmentation Fluks (Fp)
320-325	0.1079
325-330	0.1020
330-335	0.0936
335-340	0.0798
340-345	0.0669
345-350	0.0570
350-355	0.0488
355-360	0.0456
360-365	0.0356
365-370	0.0310

Table 5: Rating Sunscreen Category.

%Te	%Tp	Sunscreen Category
<1	3-40	Sun block
1-6	42-86	Ultra protection
6-12	45-86	Suntan
10-18	45-86	Fast Taning

Table 6: The results of measurement of cream absorbances before and after UVA irradiation

Formula	Avobenzone level before irradiation (%)	Avobenzone level after irradiation (%)			Avobenzone level decreased (%)
		5 jam	10 jam	15 jam	
F1	115.87 ± 0.119	107.21 ± 0.05	95.94 ± 0.063	86.50 ± 0.065	29.37
F2	117.02 ± 0.141	107.15 ± 0.087	97.11 ± 0.241	88.27 ± 0.260	28.75
F3	111.72 ± 0.174	104.82 ± 0.304	98.92 ± 0.074	91.03 ± 0.145	20.69
F4	112.02 ± 0.048	109.64 ± 0.395	105.34 ± 0.378	100.18 ± 0.059	11.84
F5	116.25 ± 0.144	106.03 ± 0.032	96.72 ± 0.233	88.82 ± 0.257	27.43
F6	119.40 ± 0.246	110.75 ± 0.292	101.12 ± 0.253	91.79 ± 0.118	27.61
F7	117.04 ± 0.104	110.04 ± 0.08	104.91 ± 0.143	99.30 ± 0.024	17.74
F8	115.10 ± 0.091	111.22 ± 0.145	104.93 ± 0.140	97.32 ± 0.165	17.78

Table 7: The Results of Effectiveness - SPF (Sun Protection Factor) Testing.

Formula	SPF value			
	100 µg/ml	200 µg/ml	500 µg/ml	1000 µg/ml
Formula 0 (Negative control)	0,039	0,110	0,415	0,987
Formula 1 (Positive control)	0,524	1,136	2,911	5,889
Formula 2	0,5s63	1,144	2,918	6,024
Formula 3	0,584	1,146	2,931	6,024
Formula 4	0,594	1,209	3,043	6,190
Formula 5	0,570	1,173	2,972	6,074
Formula 6	0,611	1,227	3,091	6,250
Formula 7	0,621	1,239	3,128	6,278
Formula 8	0,633	1,323	3,136	6,407

Table 8: The Results of Effectiveness – Percent Transmission of Erythema and Pigmentation at 100 µg/ml.

Formula	%Te	%Tp	Effectiveness Category
Formula 0 (Negative control)	98,834	101,462	Ineffectiveness
Formula 1 (Positive control)	88,844	67,549	Ultra Protection of UVA
Formula 2	87,904	67,017	Ultra Protection of UVA
Formula 3	87,736	67,041	Ultra Protection of UVA
Formula 4	87,132	65,844	Ultra Protection of UVA
Formula 5	87,629	66,787	Ultra Protection of UVA
Formula 6	87,116	68,309	Ultra Protection of UVA
Formula 7	86,756	65,937	Ultra Protection of UVA
Formula 8	86,631	65,844	Ultra Protection of UVA

Table 9: The Results of Effectiveness – Percent Transmission of Erythema and Pigmentation at 200 µg/ml

Formula	%Te	%Tp	Effectiveness Category
Formula 0 (Negative control)	97,219	99,554	Ineffectiveness
Formula 1 (Positive control)	77,208	45,795	Ultra Protection of UVA
Formula 2	77,222	45,491	Ultra Protection of UVA
Formula 3	77,184	45,632	Ultra Protection of UVA
Formula 4	75,932	43,700	Ultra Protection of UVA
Formula 5	76,949	45,214	Ultra Protection of UVA
Formula 6	75,851	44,202	Ultra Protection of UVA
Formula 7	75,794	42,576	Ultra Protection of UVA
Formula 8	74,234	43,650	Ultra Protection of UVA

Table 10: The Results of Effectiveness – Percent Transmission of Erythema and Pigmentation at 500 µg/ml.

Formula	%Te	%Tp	Effectiveness Category
Formula 0 (Negative control)	90,561	93,217	Ineffectiveness
Formula 1 (Positive control)	51,956	15,414	UVA Sunblock
Formula 2	52,092	15,284	UVA Sunblock
Formula 3	51,996	15,211	UVA Sunblock
Formula 4	50,652	13,771	UVA Sunblock
Formula 5	51,337	15,003	UVA Sunblock
Formula 6	49,977	14,336	UVA Sunblock
Formula 7	49,511	13,661	UVA Sunblock
Formula 8	49,516	14,357	UVA Sunblock

Table 11: The Results of Effectiveness – Percent Transmission of Erythema and Pigmentation at 1000 µg/ml.

Formula	%Te	%Tp	Effectiveness Category
Formula 0 (Negative control)	79,365	82,322	Ultra Protection of UVA
Formula 1 (Positive control)	26,889	2,940	UVA Sunblock
Formula 2	26,200	2,883	UVA Sunblock
Formula 3	26,072	2,840	UVA Sunblock
Formula 4	25,179	2,473	UVA Sunblock
Formula 5	25,750	2,903	UVA Sunblock
Formula 6	24,762	2,582	UVA Sunblock
Formula 7	24,570	2,721	UVA Sunblock
Formula 8	23,915	2,407	UVA Sunblock

Keterangan : %Te = Percent transmission of erythema value., %Tp = Percent transmission of pigmentation value

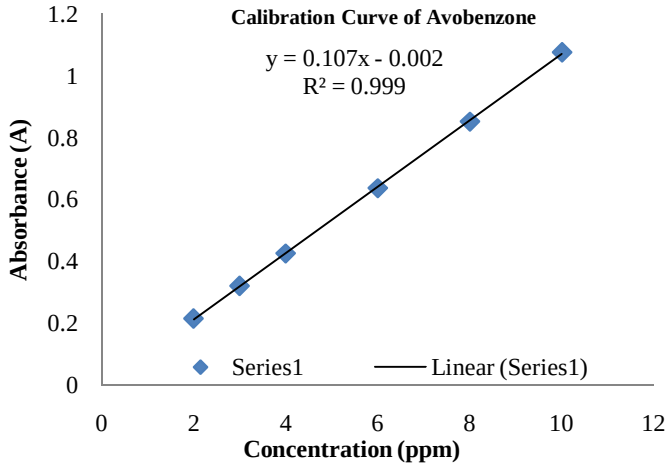

Fig. 2: Calibration Curve of Avobenzone (*λ* 356 nm).

CONCLUSIONS

The results of analysis of variance showed that the addition of ethyl ascorbic acid and alpha tocopherol acetate in cream formula gave the significant difference photostability with the formula 1 (avobenzone 2%) with 95% confidence level ($p \leq 0{,}05$).The formula 4 (avobenzone 2% and 2% ethyl ascorbic acid) was the best formula to stabilize avobenzone in preparation. The addition of ethyl ascorbic acid and alpha tocopherol acetate might improved the SPF value, but have been not able to improve the effectiveness of protection against erythema/UVB. While, the value of percent transmission of erythema (Te) and percent transmission of pigmentation (Tp) decreased.

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