

Effect of Quercetin and Avobenzone Concentration on Physical Characteristics and In Vitro Activity of Sunscreen

Budipratiwi Wisudyarningsih*, Kuni Zu'aimah Barikah, & Fernanda Indah Lita

Herbal Drug Innovation Research Group, Faculty of Pharmacy, University of Jember, Indonesia

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ABSTRACT: Quercetin is a flavonoid compound that has great potential activity, one of which is a chemical absorber of ultraviolet (UV) radiation, especially UV B radiation. The objective of the present study was to optimize quercetin as a UV B absorber combined with avobenzone as a UV A absorber in sunscreen cream formulation. Optimization was carried out by factorial design method using Design Expert 11.0 Software, with viscosity, pH, Sun Protection Factor (SPF) value, Percent of Erythema Transmission (% TE), and Percent of Pigmentation Transmission (% TP). The results showed that the quercetin factor was decreasing viscosity, pH, % TE, and % TP value but increasing the SPF value of the formulation. Avobenzone factor affected the increase of viscosity, pH, and SPF value but decreased the % TE and TP value. The interaction of quercetin and avobenzone significantly affected decreasing the viscosity and pH value but increased the SPF < % TE, and % TP value. Evaluation of optimum formula obtained by the overlay contour plot on the software resulting viscosity cream at 338,33 dPas, pH value at 5,40; SPF value at 33,31; % TE at $2,62 \times 10^{-3}$ % and % TP at $1,56 \times 10^{-5}$ %.

Keywords: sunscreen; quercetin; avobenzone; factorial design.

Introduction

Sunlight has ultraviolet (UV) radiation consisting of UVA, UVB, and UVC [1]. UV radiation is needed in the production of vitamin D [2]. On the other hand, UV radiation has negative effects such as pigmentation, erythema, premature aging, and skin cancer [3,4]. Exposure to high-intensity UV rays can also cause melasma which occurs in 40% of women in Southeast Asia [5].

Naturally, human skin has a natural protection system against UV rays such as the thickening of the stratum corneum and the formation of melanin. At high-intensity UV exposure, additional protection is needed in the form of sunscreen creams [6]. A good sunscreen cream must be able to protect the skin against both UVA and UVB. Quercetin is one of the anti-UVB sunscreen compounds, so it is necessary to add anti-UVA compounds, one of which is avobenzone, which can protect the skin superiorly from most of the UVA spectrum [7,8]. A previous study combined quercetin with sunscreen agents TiO₂, ZnO, and rutin at 10%, but the high concentration affected the acceptability of the cream [4].

In this study, the oil cream in water (o/w) was chosen for the formulation of sunscreen creams because it is non-sticky and easy to clean with water, making it more comfortable to use [9]. A new formula for a sunscreen facial cream that has better effectiveness and acceptability is needed due to the community's increasing needs. This research was conducted to obtain the optimum formula and know the effect of the combination of quercetin and avobenzone on the physicochemical characteristics, namely viscosity and pH as well as the effectiveness of sunscreen facial cream which were assessed using the value of Sun Protection Factor (SPF), percent erythema transmission (% TE), and percent pigmentation transmission (%TP).

Methods

Material

Quercetin (Sigma Aldrich, Indonesia), avobenzone (CV. Cipta Anugerah Bakti Mandiri, Indonesia), stearic acid (PT. Bratachem, Indonesia),

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*Corresponding Author: Budipratiwi Wisudyarningsih
Herbal Drug Innovation Research Group, Faculty of Pharmacy, University of Jember,
Sumbersari, Kabupaten Jember, Jawa Timur Indonesia, 68121 | Email: wisudyarningsih@unej.ac.id

Table 1. The design of a modified sunscreen facial cream formulation from a research formula [24]

Material Name	Function	F(1)	F(a)	F(b)	F(ab)
Quercetin	Active ingredients	0.5%	3%	0.5%	3%
Avobenzone	Active ingredients	2%	2%	4%	4%
Stearic acid	Emulsifying agent	14%	14%	14%	14%
Cetyl alcohol	Stiffening agent	1.5%	1.5%	1.5%	1.5%
Sorbitol	Humectant	6%	6%	6%	6%
TEA	Emulsifying agent, alkalizing agent.	2%	2%	2%	2%
Tween 80	Emulsifying agent	10%	10%	10%	10%
BHT	Preservative	0.1%	0.1%	0.1%	0.1%
Propylene glycol	Additional solvent	6%	6%	6%	6%
Glycerin	Additional solvent, preservative	4%	4%	4%	4%
Distilled water	Solvent	53.9%	51.4%	51.9%	49.4%

cetyl alcohol (PT. Bratachem, Indonesia), butylated hydroxytoluene (PT. Bratachem, Indonesia), sorbitol (PT. Bratachem, Indonesia), triethanolamine (PT. Bratachem, Indonesia), tween 80 (PT. Bratachem, Indonesia), propylene glycol (PT. Bratachem, Indonesia), glycerin ((PT. Bratachem, Indonesia), distilled water (PT. Bratachem, Indonesia), methylene blue (PT. Bratachem, Indonesia), isopropanol (PT. Bratachem, Indonesia), 96% ethanol (PT. Bratachem, Indonesia).

Formulation of sunscreen facial cream

In the formula that has been designed (Table 1), there are two types of material groups, namely oil phase ingredients including avobenzone, stearic acid, cetyl alcohol, butylated hydroxytoluene, and aqueous phase including sorbitol, triethanolamine, tween 80, and distilled water. The oil phase and the water phase are heated separately in a water bath at 70°C. Then the heated water phase is added to the hot mortar, and after that, the oil phase is added with constant stirring counterclockwise. Then when the mortar temperature had dropped, added quercetin which had been dissolved with propylene glycol and glycerin, and stirred until homogeneous.

Organoleptic Testing

Organoleptic testing is carried out through visual observation of the characteristics of the creams including the color, texture, and smell.

Viscosity Testing

Viscosity testing (30g of cream) conducted using Viscometer (Rion VT-04, Japan), and the test value is

seen on the tool scale [10,11]. The spindle used is spindle number 2.

PH Testing

The pH value was measured by preparing 1g of the cream and dissolving it in 100 ml of distilled water. Then dip the calibrated pH meter (Elmetron CP 502, Poland) into the test cream [12].

In Vitro SPF Value Testing

The in vitro SPF value can be obtained using the UV-Vis spectrophotometry (Shimadzu, Japan) method using 96% ethanol as solvent. The cream was weighed as much as 0.5 g, then dissolved in 25 ml of 96% ethanol (20,000 ppm). The absorbance of cream was measured at a wavelength of 290-320 nm with intervals of 5 nm [13]. The calculation of the SPF value is done using the following equation:

$$\text{SPF}_{\text{spectrophotometric}} = \text{CF} \sum_{290}^{320}$$

- CF = correction factor
- EE = erythema effect spectrum
- I = solar intensity spectrum
- EExI = constant whose value has been determined
- A = absorbance of sunscreen product

Testing The Value of %TE and %TP

%TE and %TP values can be obtained using the UV-Vis spectrophotometry (Shimadzu, Japan) method. A small amount of cream (5 mg) was dissolved using 10 ml propanol and shake until homogeneous. The absorbance

Table 2. Organoleptic test results

Formula	Texture	Mass	Color	Smell
F(1)	Gentle	Cream	Yellow	No smell
F(a)	Gentle	Cream	Deep yellow	No smell
F(b)	Gentle	Cream	Pale yellow	No smell
F(ab)	Gentle	Cream	Slightly intense yellow	No smell

was measured at a wavelength of 292.5-372.5 nm with an interval of 5 nm. Calculation of %TE values at 292.5-337.5 nm and %TP at 322.5-372.5 nm [14]. The calculation of the %TE and %TP values is done using the following equation:

$$A = -\log T$$

A = absorbance value

T = percent transmission value at concentration 1g/L

$$\%TE = \frac{\Sigma(TFe)}{\Sigma Fe}$$

$\Sigma (T.Fe)$ = amount of erythema flux transmitted by sunscreen at 292.5-337.5 nm

ΣFe = the total amount of solar erythema flux

T = erythema transmission percent value

Fe = erythema flux constant

$$\%TP = \frac{\Sigma(TFp)}{\Sigma Fp}$$

$\Sigma (T.Fp)$ = amount of pigmentation flux transmitted by sunscreen at 322.5-372.5 nm

ΣFp = the total amount of sunlight pigmentation flux

T = percent value of pigmentation transmission

Fp = pigmentation flux constant

Optimum Formula Determination and Verification

Based on the determination and setting of the optimum formula criteria in the Design Expert 11.1.2.0 software, the optimum formula is selected with a desirability value close to 1 [15], then verification of the optimum formula was carried out with three test replications to compare the suitability of the results of the design expert's predicted response with the observative results. Statistically analyzed using the one-sample t-test method with a 95% confidence level. The data is not significantly different if the p-value

is > 0.05 and significantly different if the p-value is < 0.05 [16].

Result and Discussion

Organoleptic

The results of all formulations have the same texture and odor characteristics (Table 2). The difference in each formula lies in the color (Figure 1), but the four formulas do not cause a yellow color on the skin when applied according to the desired dosage specifications. The higher concentration of quercetin causes a higher intensity of yellow color. The higher concentration of avobenzone causes a lower intensity of yellow color.

Viscosity

Based on (Table 3), the results of the viscosity test showed that the highest viscosity value was obtained at F(b) 492.44 and the lowest viscosity value was obtained at F(a) 300.90. The test results are by the requirements for the viscosity value of topical creams, namely 200-5000 dPas [17], as well as by the expected cream viscosity specifications of 200-700 dPas.

Based on the calculation of factor effects, coded factor equations, and analysis of variance, it was found that quercetin and quercetin-avobenzone interaction had a significant effect on reducing viscosity, while avobenzone had a significant effect on increasing viscosity. These results can also be observed in the contour plot (Figure 2), the red color indicates an increase and the blue color indicates a decrease in response.

Differences viscosity of the creams can occur because the formation of emulsions from cream creams using emulsifying in the form of TEA-stearate is influenced by the pH value [18].

pH Value

Based on (Table 3) the results of the pH test of the cream showed that the highest pH value was obtained at F(b) 6.39 and the lowest pH value was obtained at F(ab)

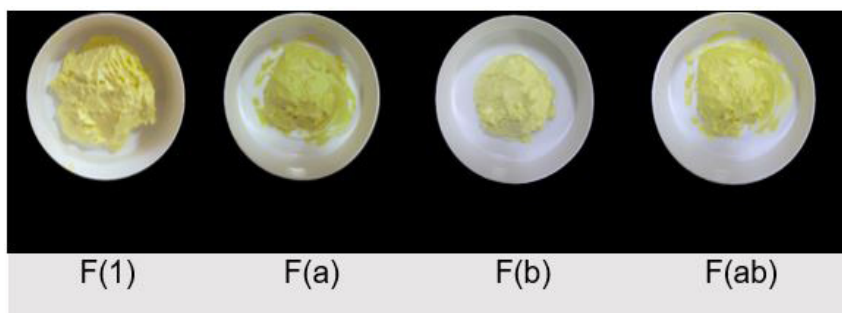


Figure 1. The result of sunscreen facial cream formulation

5.18. The results of this test are by the requirements for the pH range of topical creams in the range of 4.5-6.5 [12], but the results of the pH F(b) test did not comply with the requirements for the pH value of quercetin stability in the range of 1-6 [19].

Based on the results of factor effect calculations, coded factor equations, and analysis of variance, it can be concluded that quercetin and quercetin-avobenzone interaction have a significant effect on reducing pH, while the avobenzone has a significant effect on increasing pH. These results can also be observed in the contour plot (Figure 2).

Differences in pH value can occur because quercetin is an acidic compound that can lower pH [20], while avobenzone can cause cream more alkaline [21]. The interaction between quercetin-avobenzone can lower pH, but the decrease in pH is not as great as the decrease in pH caused by quercetin. Quercetin can lower the pH of a cream due to its acidity. In aqueous solution at pH 7.4, quercetin will react with peroxy radicals, where catechol only has a secondary role. The pKa of quercetin in water/methanol was 8.45, indicating that most of it would dissociate at pH 7.4 [27].

SPF Value

Based on (Table 3), all formulations yielded an SPF value of the ultra-protection category [22]. The highest

SPF value is owned by F(ab) 34.63606 and the lowest SPF value is owned by F(1) 31.4599. The protective power of sunscreen creams will increase as the SPF value increases [23].

Based on the results of factor effect calculations, coded factor equations, and analysis of variance, it was found that each factor and its interaction increased the response of the in vitro SPF value, but there was no significant difference, because the concentration differences between formulas is not wide enough. The quercetin became the dominant factor in increasing the in vitro SPF value. These results can also be observed in the contour plot (Figure 2). Quercetin and avobenzone are chemical absorber sunscreen agents that can absorb high-intensity UV light energy [24].

%TE and %TP Values

Based on (Table 3) the results of the %TE and %TP tests on the four formulations produced %TE and %TP in the total block category with a value of <1 [22]. The protective power of sunscreen creams will be better when the %TE and %TP are getting smaller [25].

Based on the results of factor effect calculations, coded factor equations, and analysis of variance, it can be concluded that quercetin and avobenzone have a significant effect on reducing %TE and %TP, while their interaction has a significant effect on increasing %TE and

Table 3. Test result

Formula	Spread power	Viscosity (dPas)*	pH*	SPF In Vitro*	%TE*	%TP*
F(1)	6.03 ± 0.15	320.00 ± 5.00	5.54 ± 0.06	32.55256 ± 1.09266	9.18 × 10 ⁻² ± 2.82 × 10 ⁻²	5.08 × 10 ⁻³ ± 2.92 × 10 ⁻³
F(a)	6.10 ± 0.20	306.67 ± 5.77	5.52 ± 0.05	33.37701 ± 0.21721	1.27 × 10 ⁻² ± 6.72 × 10 ⁻³	5.20 × 10 ⁻⁴ ± 4.05 × 10 ⁻⁴
F(b)	4.67 ± 0.21	486.67 ± 5.77	6.20 ± 0.19	33.05571 ± 0.89460	2.02 × 10 ⁻² ± 4.99 × 10 ⁻³	1.11 × 10 ⁻⁴ ± 5.30 × 10 ⁻⁵
F(ab)	4.90 ± 0.10	335.00 ± 5.00	5.34 ± 0.16	33.94483 ± 0.69123	2.76 × 10 ⁻³ ± 9.79 × 10 ⁻⁴	1.20 × 10 ⁻⁵ ± 6.00 × 10 ⁻⁶

Data are presented as mean ± standard deviation (n=3)
*Test response included in a factorial design

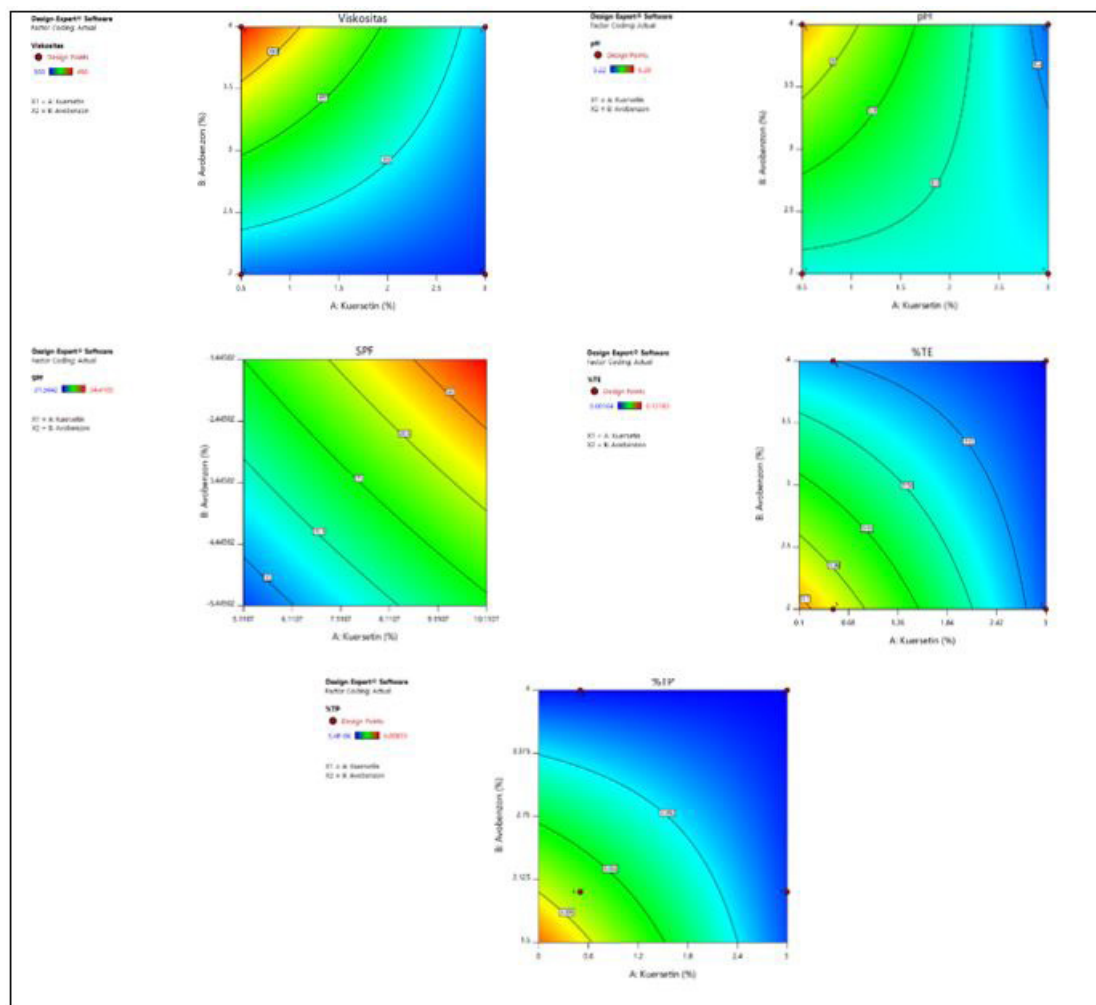


Figure 2. Contour plot of response viscosity, pH, SPF, %TE, and %TP

%TP. Quercetin became the dominant factor in reducing %TE, while avobenzone was the dominant factor in reducing %TP. These results can also be observed in the contour plot (Figure 2).

These results are because quercetin is a chemical absorber sunscreen agent that can absorb UVB rays that cause erythema [26], so the addition of quercetin can reduce the amount of UV rays that will be passed on to the deeper layers of skin and cause erythema indicated by decreasing %TE. Avobenzone is a chemical absorber sunscreen agent that can protect skin superiorly from most of the UVA spectrum that causes pigmentation [7,8] so that avobenzone can reduce the amount of UV rays that will be passed on to the deeper layers of the skin and cause pigmentation indicated by decreasing %TP.

Optimum Formula Determination and Verification Results

The selected optimum formula is cream formulation

using 3% quercetin and 4% avobenzone (F(ab)) with desirability value of 0.991. The value of each response is pH 5.337; viscosity 335,000 dPas; SPF 33,945; %TE 0.003; and %TP 0.000.

The results of verifying the optimum formula with a response to the value of viscosity, pH, SPF, %TE, and %TP yielded a value of $p > 0.05$. Data analysis results with a 95% confidence level are not significantly different if the value is $p > 0.05$ and the data are significantly different if the value is $p < 0.05$ [16], so it can be concluded that the observative results data are close to the predicted results or it can be stated that the optimization design model using a factorial design can predict the response correctly because there are no significant differences. Based on the research results, it can be seen that the formula used is appropriate to get the desired response or cream specification.

Conclusion

The addition of quercetin and quercetin-avobenzone interaction can reduce the pH and viscosity of cream, while the addition of avobenzone can increase the pH and viscosity of cream. This can happen because quercetin is acidic and avobenzone is basic. Apart from that, the pH value of creams using TEA stearate emulsifier will affect the formation of the emulsion. The more acid can reduce the viscosity of the resulting cream, meanwhile increasingly alkaline pH value of the cream can increase the viscosity of the cream. The addition of quercetin, and avobenzone, and their interactions can increase the SPF value, because quercetin and avobenzone are sun protective agents. The addition of quercetin and avobenzone can decrease the %TE and %TP values, while the interaction between the two can increase the %TE and %TP values. Quercetin is the dominant factor to reduce the %TE value because quercetin is a chemical sunscreen agent or a chemical absorber which has the ability to absorb UV B rays the cause of erythema is higher than the absorption of UV A rays, while avobenzone is the dominant factor to reduce the %TP value because avobenzone is a chemical sunscreen agent or a chemical absorber which has the ability to protect the skin superior to most pigmentation-causing UV A spectrum. The optimum formula that shows the best physicochemical characteristics and in vitro effectiveness of the best sunscreen on a sunscreen facial cream is 3% quercetin and 4% avobenzone. The selected optimum formula is in accordance with the formula F(ab).

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