Indonesian Journal of Pharmacy

VOL 34 (1) 2023: 112–119 | RESEARCH ARTICLE

Optimization of Gelling Agent and Sunscreen Activity Test on 4-OH-chalcone Gel Using *In Vitro* Method

Abdul Karim Zulkarnain[1]*, Jumina[2], Galih Titiasari Kharismawati[3] and Fina Anjani Larasati[3]

1. Laboratory of Physical Pharmacy, Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada Yogyakarta, Skip Utara 55281 Yogyakarta.
2. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Skip Utara 55281 Yogyakarta.
3. Faculty of Pharmacy, Universitas Gadjah Mada Yogyakarta, Skip Utara 55281 Yogyakarta.

Article Info

Submitted: 24-03-2022
Revised: 23-07-2022
Accepted: 02-12-2022

*Corresponding author
Abdul Karim Zulkarnain

Email:
akarimzk08@gmail.com

ABSTRACT

4-OH-chalcone is suspected of absorbing and reducing ultraviolet radiation intensity exposed to the skin. It was formulated in gel dosage form using a combination of 0.50-1.00% w/w carbopol content, 19-20% w/w propylene glycol content, and 0.50-1.00% w/w CMC-Na content. This study aimed to optimize the three gel bases and evaluate the physical properties, physical stability, and activity of 4-OH-chalcone gel as a sunscreen using *in vitro* method. Simplex Lattice Design (SLD) method was used to optimize the gel base using Design Expert software version 10.0. The activity of 4-OH-chalcone gel as sunscreen was determined spectrophotometrically by determining the Sun Protecting Factor (SPF) value, percent transmission of erythema (% TE), and percent transmission of pigmentation (% TP). Data were analyzed statistically using SPSS Statistical software. The optimum formula of 4-OH-chalcone gel was 0.50% w/w carbopol content, 0.50% w/w carboxymethylcellulose sodium content, and 20.0% w/w propylene glycol content which had a pH of 5.59, viscosity of 37.4 dPa.s, spreadability of 28.8 cm^2, adhesive time of 1.28 seconds, SPF value of 28.65, %, TE value of 16.44%, %TP value of 0.24%, and relatively stable physical properties.

Keyword: gel, 4-OH-chalcone, SLD, SPF

INTRODUCTION

Indonesia is tropical, so it gets sun exposure throughout the year. Sun rays benefit living beings, especially humans (Tahir et al., 2002). Sun's ultraviolet (UV) rays exposure to human skin can cause damage to the skin, such as reddishness, bleeding, aging, and increased risk of skin cancer. (Lann et al., 2016). UV rays are divided into three types, namely UV-A (320-400 nm), UV-B (290-320 nm), and UV-C (200-290 nm) (Wilkinson and Moore, 1982). UV-A rays pose a lower risk of erythema than UV-B rays (Juzeniene and Moan, 2012). UV-A rays are less cytotoxic and mutagenic than UV-B rays. UV-B rays are more efficient in inducing erythema, while UV-A rays are more efficient in inducing tanning effects (Young et al., 1998). UV-C rays have the greatest energy and destructive power. UV-C radiation cannot reach the earth's surface because it is blocked by the ozone layer that covers the earth, but as pollution increases, the ozone layer becomes thinner, allowing UV-C radiation reaches the earth's surface (Jones, 2000). Sunscreen is a preparation that can reduce the harmful effect of UV rays on the skin through a reflection or absorption mechanism of UV radiation (Draelos and Thaman, 2006). Sunscreen is divided into two types, namely physical and chemical sunscreens. Physical sunscreen is a translucent product that can reflect and scatter UV radiation (Helms et al., 2008). Chemical sunscreens protect the skin from UV radiation by absorbing UV radiation at certain wavelengths (Shi et al., 2012). Chemical sunscreens generally have benzene nuclei substituted in *ortho* and *para* positions conjugated with carbonyl groups (Taufikkurohmah, 2005).

The 4-OH-chalcone is one of the chalcone compounds that is a flavonoid derivative (Anonymous, 2015). The 4-OH-chalcone has chalcone quality in general. Chalcone has two aryl rings connected to the α and β unsaturated ketone group in their structure and is one of the flavonoid compounds (Solomon and Lee, 2012). Flavonoids can potentially be developed as sunscreens because flavonoids have a chromophore group that can absorb both UV-A and UV-B, reducing light exposure to the skin (Shovyana and Zulkarnain, 2013).

The 4-OH-chalcone can be formulated as sunscreen gel. Topical gels have advantages such as releasing the drug faster than creams and lotions (Kumar and Kumar, 2011). The gel can spread easily when applied to the skin, give a cold sensation, does not imprint on the skin, and is easy to use (Anggraeni et al., 2012). The gel can be made by a gelling agent such as carbopol, sodium carboxymethylcellulose, and propylene glycol. Carbopol can be used as a gelling agent with a content of 0.50-2.00% (Rowe et al., 2009). Sodium carboxymethylcellulose (CMC-Na) can be used as a gelling agent with a content of 0.50%-2.00% (Nursal et al., 2010). Propylene glycol can be used as a humectant content of 19-20% (Wang and Li, 2005). The SLD method can optimize the gel formula, which can predict the response based on experimental data (Bolton and Bon, 2009). Sunscreen activity test on the gel using *in vitro* method can be performed by measuring the Sun Protection Factor (SPF) value, percent transmission of erythema (% TE), and percent transmission of pigmentation (% TP) of the gel spectrophotometrically (Wilkinson and Moore, 1982). SPF value of sunscreen can be determined spectrophotometrically by measuring its absorbance every 5 nm at wavelengths of 290-320 nm. Sunscreen's %TE value can be determined spectrophotometrically by measuring its percentage of transmittance every 5 nm at a wavelength of 292.5-337.5 nm. Sunscreen's %TP value can be determined spectrophotometrically by measuring its percentage of transmittance every 5 nm at a wavelength of 292.5-372.5 nm. The gel formula was optimized by optimizing carbopol content of 0.5%-0.1%, sodium carboxymethylcellulose content of 0.50%-2.00%, and propylene glycol content of 19-20%. Responses used to carry out optimization in this study were pH, viscosity, and spreadability. Three responses were used to determine the gel optimum formula by SLD method using *Design Expert®* version 10.0 software.

MATERIALS AND METHODS

The materials consisted of 4-OH-chalcone (Faculty of Mathematics and Sciences), carbopol (pharmaceutical grade), sodium carboxymethylcellulose (pharmaceutical grade), propylene glycol (pharmaceutical grade), TEA (pharmaceutical grade), benzoic acid (pharmaceutical grade), *aqua rosae* (pharmaceutical grade), aquadest (pharmaceutical grade), ethanol 96% (pro analytic), and chloroform (pro analytic).

The instruments consisted of glassware (Pyrex®), analytical balance (Mettler Toledo®), refrigerator, electric stove, plastic pot, stirrer (Stuart®), spreadability test equipment, adhesiveness test equipment, viscosimeter Brookfield (DV-I Prime), stopwatch (Alba®), oven, pH-meter (HANNA H1 5211), sonicator (JP Selecta®), cuvette, and UV-Vis spectrophotometer (Hitachi®).

Sunscreen activity test on 4-OH-chalcone

SPF value, % TE, and % TP of 2.5 x 10^{-4}% w/v 4-OH-chalcone in ethanol p.a. 96% were measured spectrophotometrically.

Preparation of gel base

Carbopol was dispersed in a mixture of triethanolamine and aquadest for 24 hours (mixture 1), and Sodium carboxymethylcellulose was dispersed in hot aquadest (mixture 2). Meanwhile, benzoic acid was dissolved in hot aquadest (mixture 3). Mixture 1, mixture 2, mixture 3, appropriate aqua rosae, and 20 grams of propylene glycol were mixed in a Beaker glass, and then aquadest was added until the mixture mass reached 100 grams (mixture 4). Mixture 4 was stirred using a stirrer until it became a homogeneous gel mass (Table I).

Physical properties test

Physical properties tests on 13 run formulas conducted to obtain the optimum formula consisted of organoleptic observation, pH measurement, viscosity test, and spreadability test.

Determination of gel base optimum formula

The optimum formula was determined by processing the physical properties data of 13 run formulas using Design Expert® software version 10.0 (Table I). The observed physical properties in the optimization step were pH, viscosity, and spreadability. Response target and degree of importance were adjusted to obtain the optimal gelling agent composition. The physical properties of the gel base optimum formula were compared with 4-OH-chalcone gel. 4-OH chalcone gel is a gel base optimum formula that contains 0.5% b/b 4-OH chalcone compound.

Table I. Composition of 13 run formulas

Materials (% w/w)	***Run***												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
***carbopol**	0.74	0.74	0.94	0.50	0.50	0.74	1.00	0.63	0.82	1.00	0.74	0.50	0.94
***CMC-Na**	0.50	0.74	0.76	0.80	0.50	0.50	1.00	1.00	0.99	0.50	0.74	0.96	0.76
Propylen glykol*	19.75	19.52	19.29	19.70	20	19.76	19.00	19.37	19.19	19.50	19.52	19.54	19.29
TEA	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Benzoic acid	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Aqua rosae	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Aquadest ad	100	100	100	100	100	100	100	100	100	100	100	100	100

Physical properties data of both gels were compared using independent samples t-test to determine whether the addition of 4-OH-chalcone gives effect(s) on the physical properties of the gel base optimum formula.

Verification of gel base optimum formula

The optimum formula suggested by Design Expert® software version 10.0 was made with the same method as the previous 13 run formulas. The gel base optimum formula's physical properties on week 0 were tested, and then the actual gel base optimum formula's physical properties were compared with the gel base optimum formula's physical properties predicted by Design Expert® software version 10.0 (Table II). The observed physical properties included pH, viscosity, spreadability, and adhesion power. One sample t-test with a confidence level of 95% was performed on the gel base optimum formula's physical properties data to determine whether the actual gel base optimum formula's physical properties differed significantly from the physical properties predicted by Design Expert® software version 10.0.

Physical properties evaluation of gel base optimum formula and 4-OH-chalcone gel

The physical properties evaluation of the gel base optimum formula and 4-OH-chalcone gel consisted of organoleptic observation, pH measurement, viscosity test, spreadability test, adhesion power test, and gel syneresis test.

Measurement of pH

The pH was measured using a pH-meter HANNA H1 5211, calibrated using buffers with pH of 4.0, 7.01, and 10.01. Based on SNI, sunscreen preparations must have pH values from 4.5 to 8.0 (Yumas, 2016). This test was conducted by dipping the pH meter electrode into preparation and then observing the pH detected by the pH meter.

Viscosity test

A viscosity test was performed using a viscosimeter Brookfield DV-I Prime with spindle number seven. The spindle was dipped in the gel-filled container so that the spindle was immersed perfectly, and then the viscosimeter was turned on. The spindle rotation speed was set to 100 rpm and rotated for 15 s (Fitrianingsih, 2018). According to SNI, sunscreen preparation should have a viscosity range of 2.000-50.000 cps, equivalent to 20-500 dPa.s (Standar Nasional Indonesia 164954, 1998).

Spreadability test

The gel with a mass of 0.5 g was placed on a scaled round glass, and then the gel was covered with another round glass that had been weighed and known for its weight. A load of 50 grams was added every minute until the total weight reached 250g The average spread diameter of gel at longitudinal and transverse positions was recorded every minute after the addition of round glass and load (Fitrianingsih, 2018). Good topical preparations have spread diameters of 3-5 cm or equivalent to a spread area of 7.1-19.6 cm^2.

Adhesiveness test

Gel with a mass of 0.1 g was placed on an object glass with an area of 2.5 cm x 3.5 cm. The glass object, whose weight was known, was placed on top of the gel. The top glass object was pressed with a load of 1 kg for 5 min. The circuit was attached to test equipment with a load of 80 grams. The load was dropped, and the time taken until two object glasses separated after the load was noted (Fitrianingsih, 2018).

Syneresis test

The Syneresis test was performed by storing the gel for 72 h at ± 10°C. The volume of separation in the gel at 24, 48, and 72 h was noted, and then the percentage of syneresis was calculated.

Gel physical stability test

The gel physical stability test conducted in this study consisted of organoleptic observation, pH measurement, viscosity test, spreadability test, and adhesion power test on gel base optimum formula and 4-OH-chalcone gel after being stored at room temperature during weeks 1, 2, 3, and 4.

Accelerated stability test

The accelerated stability test was performed using the freeze-thaw cycling method and centrifugation treatment. The accelerated stability test using the freeze-thaw cycling method was performed by storing gel for three cycles. A storage cycle consisted of 24 hours of storage at 8°C and 24 h of storage at 45°C. The accelerated stability test using centrifugation treatment was performed by centrifuging gel for 30 min at 3000 rpm. The F value was used as a parameter to determine the stability of the gel. The F value has a range from zero to one. The closer F value to one indicates better stability of the gel.

Sunscreen *in vitro* activity test

The SPF value was determined by putting the 0.5-gram gel base formula optimum and 4-OH-chalcone gel into the 10 mL volumetric flask separately. Each gel was dissolved with less than 10 mL mixture of ethanol 96% and chloroform with a ratio of 1:1 (Pratama and Zulkarnain, 2015). The sample was sonicated for 10 min, and the solvent was added until it reached the mark. The sample was filtered with filter paper, and the solution was diluted a hundred times with solvent. The absorbance of solutions was measured every 5 nm at a wavelength of 290-320 nm, and then the SPF value of each gel was calculated. The %TE and %TP values were determined by measuring the percent transmittance of the same solution as used in the determination of SPF value every 5 nm at wavelengths of 292.5-337.5 nm to determine the %TE value and 292.5-372.5 nm to determine the %TP value. The %TE and %TP values of each gel were calculated.

RESULTS AND DISCUSSION

Sunscreen Activity of 4-OH-chalcone

The 4-OH-chalcone with content of 2.5 x 10-4% w/v in the solvent gave an SPF value of 100.02, %TE value of -0.119%, and %TP value of -0.222%.

Physical Properties of 13 Run Formulas, Gel Base Optimum Formula, and 4-OH-chalcone Gel

The organoleptic observations result of 13 run (Table I) formulas showed that 13 run formulas had identical characteristics: slightly cloudy, smelled like *aqua rosae*, and were homogeneous. The result of pH measurement on 13 run formulas showed that all run formulas met the pH requirements of sunscreen preparations according to SNI because they had pH in the range of 4.5-8.0. Run 7 produced the lowest pH, while run 12 produced the highest pH.

Viscosity is a scale that states the flowing hindrance of a substance. The higher the viscosity, the greater the force needed for a substance to flow at a certain rate (Attwood and Florence, 2008). The result of the viscosity test showed that run 7 produced the highest viscosity, while run 5 produced the lowest viscosity. The spreadability test result showed that run 5 produced the highest spreadability, while run 7 produced the lowest spreadability. Topical preparations that spread easily on the skin are preferred because they are easier and more convenient.

Determination of gel base optimum formula

The parameters used to determine the optimum formula in this study were pH, viscosity, and spreadability. The observed parameters had a significant model and no a significant lack of fit test result, so those parameters could be used as determining parameters to determine the optimum formula. The goal for the pH parameter was in range. The goal for the viscosity parameter was to target. The goal for the spreadability parameter was maximized. Design Expert® software version 10.0 provides six solutions formula close to the desired optimization target. The chosen solution is a formula with the highest desirability value. The chosen optimum formula has a desirability value of 0.996. The chosen optimum formula has carbopol content of 0.50%, sodium carboxymethylcellulose content of 0.50%, and propylene glycol content of 20.0% (Figure 1). The optimum formula was predicted to have a pH of 5.56, a viscosity of 35.81 dPa.s, and a spreadability of 30.75 cm^2. Design Expert® software version 10.0 provides the SLD equation as follows:

pH response	=	4.62 A + 5.49 B + 5.56 C
Viscosity response	=	135.03 A + 95.24 B + 35.81 C
Spreadability response	=	-25.66 A+19.47 B+30.75 C–29.48 AB– 44.39 AC-19.97+101.95 ABC

Annotation: A = carbopol content (% b/b); B = CMC-Na content (% b/b); C = propylene glycol (% b/b)

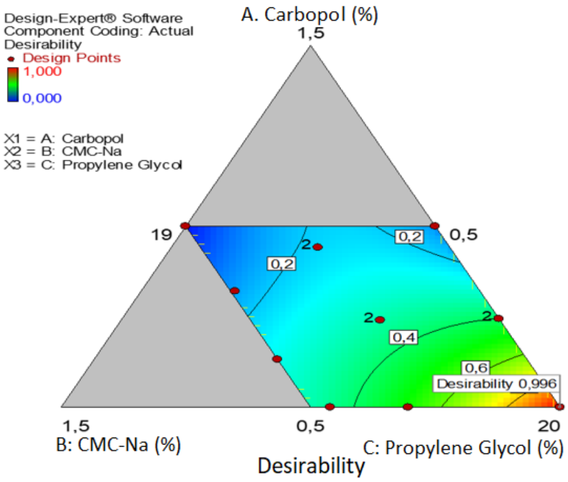

Figure 1. Contour plot desirability of suggested optimum formula using a combination of carbopol content of 0.5-2.5% w/w, sodium carboxymethylcellulose content of 0.5-1.0% w/w, and propylene glycol content of 19.0-20.0% w/w

The component that gives the greatest positive influence on pH response is carbopol content. Carbopol has a greater pKa value than sodium carboxymethylcellulose, so carbopol becomes less influential in decreasing the pH of the gel than sodium carboxymethylcellulose. Moreover, in this study, carbopol was used with a lower amount than sodium carboxymethylcellulose. Propylene glycol can give acidic pH in the produced gel (Rowe et al., 2009). The addition of carbopol became less influential in decreasing the pH of the gel compared to propylene glycol because carbopol was used with a smaller amount than CMC-Na in this study. The components that gave the most significant negative influence on pH response were interactions between carbopol and CMC-Na. The dispersion of carbopol and CMC-Na can provide an acidic pH, so adding the amount of carbopol and CMC-Na will cause a considerable decrease in the pH of the produced gel (Rowe et al., 2009). The three gelling agents optimized in this study could give acidic pH to the produced gel. However, in this study, it was assumed that the interaction between carbopol and CMC-Na significantly reduced the pH of the produced gel.

The component that gave the most significant positive influence on viscosity response was the interaction between carbopol and CMC-Na. Carbopol gel will reach its highest viscosity at a relatively neutral pH, and CMC-Na has a relatively neutral pH; therefore, the addition of carbopol accompanied by the addition of CMC-Na will cause a significant increase in gel viscosity (Rowe et al., 2009). The component that had the most significant negative influence on the viscosity response was interactions between carbopol and propylene glycol. Carbopol gel will reach its lowest viscosity at a relatively neutral pH, and sodium carboxymethylcellulose gel will reach its highest viscosity at a relatively neutral to base pH (Rowe et al., 2009). Carbopol, sodium carboxymethylcellulose, and propylene glycol have acidic pH, so the increase in the number of the three gelling agents will cause a considerable decrease in viscosity through the influence of pH (Riddick et al., 1985, Rowe et al., 2009).

The component that had the most significant positive influence on the spreadability response was the interaction between carbopol, CMC-Na, and propylene glycol. Carbopol will form a gel structure that makes it difficult to flow at a relatively neutral pH, and sodium carboxymethylcellulose will form a gel structure that makes it difficult to flow at a relatively neutral to base pH. Carbopol and sodium carboxymethylcellulose have relatively acidic pKa values, so adding these two materials will form an easy-to-flow gel structure (Rowe et al., 2009). The easy-to-flow gel structure will certainly have good spreadability. The component that gave the most significant positive influence on spreadability response was the interaction between carbopol, sodium carboxymethylcellulose, and propylene glycol.

Gel Base Optimum Formula Verification

The optimum formula was verified by comparing the optimum formula's actual physical properties and the optimum formula's predicted physical properties using one sample t-test with a confidence level of 95%. Physical properties data were processed using SPSS Statistical software. The statistical analysis results indicated that the experiment results' pH, viscosity, and spreadability differed significantly from the predicted values. This could be due to a variable that fails to be controlled in making the gel: the dimensions of the container used to mix the ingredients to make the gel (Table II).

Table II. Optimum formula's physical properties verification

Response	Prediction	Experiment	Sig. (2-tailed)	Interpretation
pH	5.56	5.58	0.000	Significantly different
Viskosity (dPa.s)	35.81	37.6	0.066	Not Significantly Different
Spreadability (cm^2)	30.75	28.3	0.045	Significantly different

Table III. Physical properties comparison of gel base optimum formula and 4-OH chalcone gel

Formula	pH	Viscosity (dPa.s)	Spreadability (cm^2)	Adhesiveness (second)
Gel base optimum	5.58 ± 0.001	37.6 ± 0.33	28.3 ± 0	1.39 ± 1.14
4-OH-chalcone gel	5.59 ± 0.008	37.4 ± 0.69	28.8 ± 0.5	1.28 ± 0.71

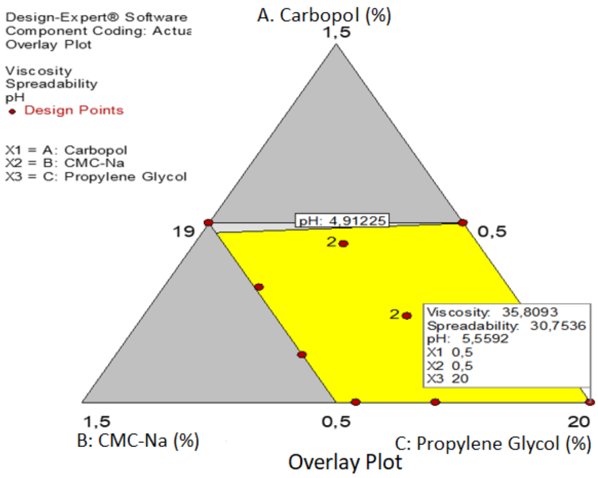

Figure 2. *Superimposed contour plot* of physical properties responses from suggested optimum formula using a combination of carbopol content of 0.5% w/w, CMC-Na 0.5% w/w, and propylene glycol 20.0% w/w

Physical Properties Comparison of Gel Base Optimum Formula and 4-OH chalcone Gel

The physical properties of the gel base optimum formula and the 4-OH-chalcone gel were compared using two sample-independent t-tests (Table III). The compared physical properties in this study consisted of pH, viscosity, spreadability, and adhesiveness. The statistical analysis result showed a significant difference between the pH of the two gels. This could be due to the addition of 4-OH chalcone, which had a relatively neutral pH, so adding 4-OH chalcone in the gel base optimum formula could change its pH value to 5.59. The statistical analysis result showed no significant difference between the two gels' viscosity, spreadability, and adhesiveness. Gel base optimum formula and 4-OH-chalcone gel had pH value, viscosity, spreadability, and adhesiveness that met the required criteria.

Physical Stability Test and Syneresis Test

Organoleptic observation showed no color, odor, or homogeneity changes on the gel base optimum formula and 4-OH-chalcone gel. This indicated that the two gels were stable for four weeks of storage.

The statistical analysis result showed significant pH changes in the gel base optimum formula and 4-OH-chalcone gel in the second week of storage. This indicated that the pH of the two gels was unstable for four weeks of storage at room temperature. This could be due to the formation of a less stable gel structure which was unstable in storage conditions, causing changes in the gel structure under storage conditions. Changes in a gel's structure can affect the gel's pH. The statistical analysis result showed no significant changes in the viscosity and adhesiveness of the two gels after being stored for four weeks at room temperature.

Table IV. Sunscreen activity test result

Material/formula	SPF value	%TE value	%TP value
The 4-OH-chalcone	100.02±0.00	-0.12±0,02	-0.22±0.00
Gel base optimum formula	0.09±0.02	98.05±0.62	96.59±0.33
The 4-OH-chalcone gel	28.85±0.01	16.44±0.07	0.24±0.01

This indicated that the viscosity and adhesion of the two gels were stable for four weeks of storage at room temperature.

The syneresis test result showed that the gel base optimum formula and 4-OH-chalcone gel gave 0% of syneresis percentage. This indicated that the two gels were stable during 72 hours of storage at 10°C because there were no observed syneresis events.

Accelerated Stability Test

The accelerated stability test results using the freeze-thaw cycling method and centrifugation treatment showed that the gel base optimum formula and 4-OH-chalcone gel gave an F value of 1. This indicated that the two gels were stable under extreme conditions, such as changes in storage temperature and force treatment.

Sunscreen Activity Evaluation of Gel Base Optimum Formula and 4-OH-chalcone Gel

The SPF value of the gel base optimum formula with a concentration of 0.5 w/v in solvent and 4-OH-chalcone gel with a concentration of 0.5% w/v in solvent were 0.09 and 28.65, respectively. Gel base optimum formula could generate minimal sunburn protection, while the 4-OH chalcone gel could generate medium sunburn protection.

The %TE and %TP values of the gel base optimum formula with a concentration of 0.5 % w/v in solvent were 98.05% and 96.59%, respectively. The %TE and %TP values of 4-OH-chalcone gel with a concentration of 0.5% w/v in solvent were 16.44% and 0.24%, respectively (Table IV). The gel base optimum formula with a concentration of 0.5% w/v in solvent did not protect against UV light. The 4-OH-chalcone gel with a concentration of 0.5% w/v in solvent was categorized as a sunscreen with extra protection and categorized as a fast tanning *sunscreen* by %TE value, as a *sunblock* by %TP value.

CONCLUSION

The optimum formula obtained was a formula with carbopol content of 0.50% w/w, sodium carboxymethylcellulose content of 0.50% w/w, and propylene glycol content of 20.0% w/w. The 4-OH-chalcone gel had physical properties that met the required criteria and were relatively capable of maintaining physical stability. The 4-OH-chalcone gel with a concentration of 0.50% w/v in ethanol 96%:chloroform (1:1) gave an SPF value of 28.65 (medium sunburn protection), %TE value of 16.447% (fast tanning), and %TP value of 0.24% (sunblock).

ACKNOWLEDGEMENT

This research was supported by the Faculty of Pharmacy, Gadjah Mada University, through the “Hibah Berkualitas Prima” scheme.

REFERENCES

Attwood, D., and Florence, A.T. (2008). Physical Pharmacy, Pharmaceutical Press, London, Chicago

Anggraeni, Y., Hendradi, E., & Purwanti, T. (2012). Preparation and Release Characteristics of Diclofenac Sodium in Niosomal System with Gel Base Carbomer 940, PharmaScientia, 1(1), 1-10.

Anonymous. (2015). 4-Hydroxychalcone, https://pubchem.ncbi.nlm.nih.gov/compound/4-Hydroxy-chalcone, 22 June 22, 2020.

Bolton, S., & Bon, C. (2009). *Pharmaceutical Statistic: Practical and Clinical Applications*, 5th Ed., CRC Press, Florida.

Draelos, Z.D., & Thaman, L.A. (2006). *Cosmetic Formulation of Skin Care Products*, 75, 135-137, 157-161, Taylor and Francis Group, New York.

Fitrianingsih, V. (2018). Gelling Agent Optimization of Karbopol, CMC Natrium, and Gelatine as well as activity test of Gell 3-Nitrokalkon as a sunscreen in vitro, Skripsi, Fakultas Farmasi Universitas Gadjah Mada, Yogyakarta.

Helms, R.A., Quan, D.J., Herfindal, E.T., & Gourley, D.R. (2008). *Textbook of Therapeutics: Drug and Disease Management*, 8th Ed., 221-222, Lippincott Williams & Wilkins, USA.

Jones, A.E. (2000). A Spectroscopic Study of Sunscreens, *Tesis*, Durham University, United Kingdom.

Juzeniene, A., & Moan, J. (2012). Beneficial Effects of UV Radiation other than Via Vitamin D Production, *Dermatoendocrinology*, 4 (2), 109-117.

Kumar, V.R., & Kumar, S. (2011). Formulation and Evaluation of Mimosa pudica Gel, *International Journal of Pharmacy and Pharmaceutical Sciences*, 3 (1), 55-57.

Lann, K.L., Surget G., Couteau C., Coiffard L., Cerantola S., Gaillard F., Larnicol M., Zubia M., Guerard F., Poupart N., & Pouvreau V.S. (2016). Sunscreen, Antioxidant, and Bactericide Capacities of Phlorotannins from the Brown Macroalgae Halidrys siliquosa, *Journal of Applied Phycology*, 28 (6), 3547–3559.

Nursal, F.K., Indriani, O., & Dewantini, L.A. (2010). Use of Na-CMC as a Gelling Agent in Formula Toothpaste Ethanol Extract 70% Guava Leaves (Psidium Guajava L), Pharmacy UHAMKA, 1 (1), 45- 51.Rowe, R.C., Shesky, P.J., & Quinn, M.E., 2009, *Handbook of Pharmaceutical Excipient*, 6th Ed, Pharmaceutical Press. Inc, London.

Riddick, J.A., Bunger, W.B., Sakano, T.K. (1985) *Techniques of Chemistry* 4th ed.,

Shi, L., Shan, J., Ju, Y., Aikens, P., & Prud'homme, R.K. (2012). Nanoparticles as Delivery Vehicles for Sunscreen Agents, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 396, 122-129.

Shovyana, H.H., & Zulkarnain, A.K. (2013). Physical Stability and Activity of Cream W/O Ethanolic Fruit Extract of Mahkota Dewa (Phaleria macrocarpa (scheff.) Boerl,) as a Sunscreen, *Traditional Medicine Journal*, 18 (2), 109-117.

Solomon, V.R., & Lee H. (2012). Anti-Breast Cancer Activity of Heteroaryl Chalcone Derivatives, *Biomedicine & Pharmacotherapy*, 66 (3), 213-220.

Indonesian National Standard 164954.1998. (1998). Skin whitening cream. Jakarta: National Standardization Agency.

Tahir, I., Jumina, & Yuliastuti, I. (2002). Analysis of In Vitro and In Vivo UV Protection Activities of Several Cinnamic Ester Compounds Condensation Reaction Products of Substituted Benzaldehyde and Alkyl Acetates, Paper at National Seminar on Chemistry XI Department of Chemistry, FMIPA UGM.

Taufikkurohmah, T. (2005). Synthesis of p-Methoxy-Cynnamil-p-Metoxycinamate from Ethyl P-Methoxycinamat was Isolated from Dried Rhizoma Kaempferia galanga L. as Sunscreen Compound, *Indonesian Journal of Chemistry*, 5 (3), 193-197.

Wang, Q., & Li, L. (2005). Effects of Molecular Weight on Thermoreversible Gelation and Gel Elasticity of Methylcellulose in Aqueous Solution, *Carbohydrate Polymers*, 62 (3), 232–238.

Wilkinson, J.B., & Moore, R. J. (1982). *Harry's Cosmeticology*, 7th Ed. New York: Chemical Publishing Company.

Young, A.R., Chadwick, C.A., Harrison, G.I., Nikaido, O., Ramsden, J., & Potten, C.S. (1998). The Similarity of Action Spectra for Thymine Dimers in Human Epidermis and Erythema Suggests that DNA is the Chromophore for Erythema, *Journal of Investigative Dermatology*, 111 (6), 982–988.

Yumas, M. (2016). Formulation of Facial Cream Preparations with Active Ingredients Methanol Extract Non-Fermented Cocoa Seeds (Theobroma Cacao L) Combination of Bee Honey, *Journal of the Plantation Industry,* 11 (2), 75-87.