Research Article

Optimization of Carbopol, CMN-Na, Gelatin, and in vitro Activity Test of 4-Hydroxy Chalcone Gel as Sunscreen

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Abstract: The 4-hydroxychalcone compound is a flavonoid derivative that has benzene rings with unsaturated chains and a chromophore group, which can absorb UV rays and reduce the intensity of radiation exposure to the skin. To achieve desired activity and also considering the ease of use, the 4-hydroxychalcone must be formulated in topical dosage forms such as gel. This study aims to examine the effect of gelling agents on the physical properties of gel preparations, optimum formula stability, and activity of 4-hydroxychalcone gel as a sunscreen in vitro. Base optimization was carried out using Design Expert software version 10 with the simplex lattice design method and variations in gelling agent concentrations, namely carbopol, sodium carboxymethylcellulose (CMC-Na), and gelatin. The activity of 4-hydroxychalcone sunscreen gel was determined spectrophotometrically by determining the Sun Protecting Factor (SPF) value, the percentage of erythema transmission (%TE), and the percentage of pigmentation transmission (%TP). Data were analyzed statistically with SPSS software. The results showed that the optimum formula composition was obtained at a concentration of 0.67% carbopol, 2% CMC-Na, and 1.83% gelatin. The optimum formula of 0.5% 4-hydroxychalcone gel was stable during storage at room temperature and did not experience syneresis during 72 hours of storage at ± 10°C. The 0.5% 4-hydroxychalcone gel gave an SPF value of 27.37 % TE was 1.76% and % TP was 10.21%, therefore it is categorized as a sunblock.

Keywords: 4-hydroxychalcone gel, stability, sunscreen

1. INTRODUCTION

Ultraviolet light has several good benefits, like as the formation of cholecalciferol (vitamin D3), which plays a role in bone formation and can be used to treat psoriasis and vitiligo if exposed to UV radiation at sufficient and regular times [1].

Skin that is exposed to excessive sunlight can have detrimental effects such as erythema, pigmentation, and premature aging [2,3,4]. UV light contributes to skin cancer due to DNA damage mechanisms and inhibition of protection in the skin [5]. The skin must be protected from the harmful effects of UV radiation with skin protection ingredients called sunscreens [6]. Sunscreen is used to reflect or absorb UV rays, to reduce the intensity of UV radiation that enters the skin [7,5]. Sunscreen preparations generally contain active photoprotector ingredients [8].

The structure of the chalcone compound has a chromophore group that has the potential as a sunscreen, antioxidant. Sunscreen activity can be seen from the absorption of the maximum

wavelength of the UV-Vis spectrophotometer [9,10,11]. The hydroxychalcone and hydroxyflavone series of compounds have antioxidant activity [12]. The sunscreen formulation for gel preparations has the advantage of good spreading power on the skin surface, provides a cooling effect through slow evaporation on the skin, and is easily washed off with water. Topical preparations in gel form have the advantage of being able to release the drug more quickly than with cream and lotion dosage forms [13]. Gel dosage forms are easily spread evenly when applied to the skin, cause a cooling sensation, do not leave marks on the skin, and are easy to use [14]. CMC-Na is widely chosen as a gelling agent because of its ability to provide high viscosity at low concentrations [15]. Carboxymethylcellulose sodium in the concentration range of 1.25-2.5% can be used as a gelling agent [16].

Compounds that have chromophore groups can absorb UV rays, both UV-A and UV-B, hence they can reduce the intensity of their exposure to the skin [17]. The form chalcone compound is a crystalline powder that is soluble in chloroform, ether, and benzene, slightly soluble in ethanol, and insoluble in water. The melting point of chalcone is in the range of 55-57ºC and the boiling point of chalcone is 208ºC [18]. The 4-hydroxychalcone compound is a chalcone derivative compound with a basic framework of 1,3-diphenylprop-2-en-1-one in which two aromatic rings are joined by three carbon chains which have a carbonyl part with α,β-unsaturated [19].

The SPF value of the preparation can be evaluated at a wavelength of 290-320 nm and then Mansur's mathematical equation is applied [20]. The Food Drug Administration (FDA) categorizes SPF into 3 groups based on their ability to protect the skin: SPF values (2<12) are minimal sunburn protection, SPF values (12-30) are medium sunburn protection, and SPF values > 30 are high sunburn protection [21].

Hence, the study aims to evaluate the effect of gelling agents on the physical properties of gel preparations, optimum formula stability, and activity of 4-hydroxychalcone gel as a sunscreen in vitro.

2.   MATERIALS AND METHODS

2.1. Materials

4-hydroxychalcone compound obtained from Faculty of Mathematics and Natural Sciences UGM. While, carbopol, CMC-Na, gelatin, TEA, citric acid, propylene glycol, DMDM hydantoin was obtained from Brataco Indonesia. Chloroform, and ethanol analytical grade are obtained from Merck Indonesia. Analytical balance (Mettler Toledo®), stirrer (Stuart®), viscometer (Brookfield DV-I Prime), stopwatch (Alba®), pH meter (HANNA H1 5211), adhesion tester (Tekfar Lab), spreadability tester (Tekfar Lab), UV-Vis spectrophotometer (Hitachi®), sonicator (JP Selecta®), ultra turrax T25 (JANKE & KUNKEL IKA®-Labortechnik).

2.2. Gel Base Optimization

Gel base optimization was carried out using the Simplex Lattice Design method using Design Expert® 10 software. The gel formula with a variation of 3 components produced 10 formulas and 3 validation formulas which can be seen in Table 1.

Table 1. Gel base formula resulting from Design Expert® software

Material (%b/b)	Run												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Carbopol	0.50	0.50	0.50	0.83	0.83	1.00	0.67	0.67	0.67	0.5	0.67	0.50	1.0
CMC-Na	2.50	2.50	2.00	2.00	2.17	2.00	2.33	2.17	2.17	2.17	2.17	2.33	2.00
gelatin	1.50	1.50	2.00	1.67	1.50	1.50	1.50	1.67	1.67	1.83	1.67	1.67	1.50
Propylene glycol	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
DM Hydantoin	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
TEA	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Citric acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aquades ad	100	100	100	100	100	100	100	100	100	100	100	100	100

Notes: Run formulas 8, 9, and 11 are used to validate the SLD equation

2.3. Gel Formation

Carbopol and CMCNa were dispersed with distilled water (aquades) in different containers and allowed to stand for 24 hours. The gelatin was developed in hot water. The carbopol was stirred using a stirrer at 750 rpm until homogeneous and then triethanolamine was added, stirring was carried out until a clear gel base was formed. CMC Na was stirred using a stirrer until homogeneous, then mixed with carbopol and stirred until homogeneous (mixture 1). Gelatin solution was added to the first mixture, and stirred until homogeneous until a gel mass was formed. DMDM Hydantoin was mixed in propylene glycol. This solution was added to the gel mass that had been formed and stirred using ultra turrax until homogeneous.

2.4. Physical Properties and Gel Stability Test

2.4.1. Viscosity Test

Viscosity testing used a Brookfield DV-I Prime viscometer with spindle number seven. The spindle rotation speed was 100 rpm and the rotation time was 15 seconds [20].

2.4.2. Spreadability Test

The spreadability test was carried out to ensure even distribution of the gel when applied to the skin which was carried out immediately after the gel was made [22]. A total of 0.5 grams of gel was placed in the middle of a round glass scale, covered with another round glass that had been weighed beforehand, and allowed to stand for 1 minute. The diameter of the distribution of the preparations was measured from various sides. A load of 50 grams was added to the glass, allowed to stand for 1 minute, and the diameter of the distribution of the preparation was recorded as before. For each time interval of one minute, 50 grams was added to the load until the final load was 250 grams [20,23].

2.4.3. Adhesion Test

A total of 0.10 grams of gel was placed and spread on an object glass with an area of 2.5 cm x 3 cm then covered with another glass object on top of the preparation. The object glass was pressed against a load of 1 kg for 5 minutes and then placed on the test apparatus. A load weighing 30 grams was released and the time was recorded when the weight fell until the two glass objects were separated [23].

2.4.4. pH Test

The testing pH of all run formulas used a calibrated pH meter. The testing of the pH of gel preparations used a pH meter HANNA H1 5211. The pH test was carried out by dipping the pH meter electrode which had been calibrated with a pH buffer of 4.01; pH 7.01 and pH 10.01 into the gel preparation, then the pH of the preparation was recorded [24].

2.4.5. Syneresis Test

Several preparations with the same weight were placed in a porcelain cup that had been weighed beforehand. The preparations were stored for 72 hours at ±10°C and a decrease in gel weight was observed at 24, 48, and 72 hours. Gel syneresis was calculated using the following formula [25].

$$\text{Syneresis (\%)} = \frac{\text{berat awal (g) – berat akhir (g)}}{\text{berat awal (g)}} \times 100\% \quad \ldots\ldots\ldots (1)$$

2.5. Determination of Sunscreen Activity

2.5.1. Sample Preparation

The gel base and 0.5% 4-hydroxychalcone gel were weighed as much as 0.25 grams; 0.50 grams; 0.75 grams; 1.0 grams; and 1.25 grams.   Each sample was dissolved in 8 mL of a mixed solution of 96% chloroform and ethanol at a ratio of 1:1. The sample was sonicated for 10 minutes until the undissolved polymer turned white. The sample was put into a 10 mL measuring flask and the solvent was added up to the tera mark. The sample solution was filtered using filter paper that had previously been saturated. A total of 1 mL of the filtered solution was taken and put in a 10 mL measuring flask and the solvent was added up to the tera mark. Replication was carried out three times.

2.5.2. Determination of SPF Value

The absorbency of the sample solution was read every 5 nm at a wavelength range of 290-320 nm. The SPF value was calculated using the Mansur equation:

$$SPF = CF x \sum_{290}^{320} EE\,(\lambda) x I(\lambda) x Abs\,(\lambda)\ldots.(2)$$

Notes: SPF = *Sun Protecting Factor*

Abs = absorbency, $EE(\lambda)\ x\ I(\lambda)$= constant

CF = correction factor = 10

The value of EE x I had been determined according to the constants in the table [26].

2.6. Determination of Erythema Transmission Percentage and Pigmentation Percentage

The transmittance percentage of the sample solution was read at a wavelength of 292.5-372.5 nm to obtain the %TP value and at a wavelength of 292.5-337.5 nm to obtain the %TE value [27]. The

categories of sunscreen activity based on the resulting %TE and %TP values were listed as follows: sunblock (%TE <1 and %TP 3-40), extra protection (%TE 1-6 and %TP 42-86) standard suntan (%TE 6-12 and %TP 45-86), Fast tanning (%TE 10-18 and %TP 45-86).

$$\%Te = \frac{\sum(\%T \times Fe)}{\sum Fe} \ldots\ldots\ldots (3) \qquad \%Tp = \frac{\sum(\%T \times Fp)}{\sum Fp} \ldots\ldots\ldots (4)$$

Notes: Sunscreen activity was based on %TE and %TP values

%TE= erythema transmission; Fe = erythema effectiveness factor; %Tp = pigmentation transmission; Fp = pigmentation effectiveness factor; %T = transmission percent

3. RESULTS AND DISCUSSIONS

The formula was developed from previous studies, 3 gelling agent namely Carbopol CMC-Na, and gelatin to enhance 4-Hydroxy Chalcone solubility and provide sufficient stable formulation. The optimum formula was determined using Design Expert software with the Simplex Lattice Design method. The resulting formulas were as many as 13 formulas. The response to the physical properties of the thirteen formulas used to obtain the optimum formula can be seen in Table 2.

Table 2. The response of the 13th physical properties of the run formula

Run	pH	Viscosity (dPas)	Spreadability (cm²)	Adhesion (second)
1	5.1	122,4 ± 17,82	16,58 ± 0,72	7,29 ± 0,54
2	5.1	125,8 ± 11,83	15,61 ± 0,87	7,36 ± 0,24
3	5.3	109,2 ± 2,35	16,71 ± 0,32	10,47 ± 0,62
4	5.1	159,9 ± 11,07	12,67 ± 0,27	4,68 ± 0,50
5	5.1	201,2 ± 5,34	11,24 ± 0,41	6,36 ± 1,15
6	5.4	225,7 ± 5,02	10,54 ± 0,35	7,82 ± 0,51
7	5.3	170,7 ± 12,98	12,66 ± 0,32	6,28 ± 0,16
8	5.0	139,9 ± 8,00	14,33 ± 0,57	7,23 ± 1,26
9	5.0	143,7 ± 8,53	14,85 ± 1,01	7,12 ± 0,49
10	5.3	110,0 ± 4,09	18,24 ± 0,73	8,28 ± 0,67
11	5.2	135,0 ± 6,81	15,66 ± 1,63	5,55 ± 0,74
12	5.4	142,3 ± 4,36	14,94 ± 0,98	7,23 ± 0,63
13	5.4	211,0 ± 7,56	10,93 ± 0,30	7,89 ± 0,72

Table 3. Independent variables and dependent variables on the SLD to determine the optimum formula

Trial Variables	Limitation		
Free Variables	**Low Composition**	**High Composition**	**Target**
Carbopol	0,5 %	1,0 %	*Minimize*
CMC-Na	2,0 %	2,5 %	*Minimize*
Gelatin	1,5%	2,0 %	*Maximize*
Dependent variable	**Lower limit**	**Upper limit**	**Target**
Viscosity	140 dPas	170 dPas	*in range*
Spreadability	12 cm²	16 cm²	*Maximize*
Adhesion	5 seconds	8 seconds	*in range*

The formula chosen was the formula that had the highest desirability value of 0.779. Superimposed from the contour plot all responded in terms of viscosity, spreadability, and adhesion of the base gel, as shown in the yellow area in Figure 1.
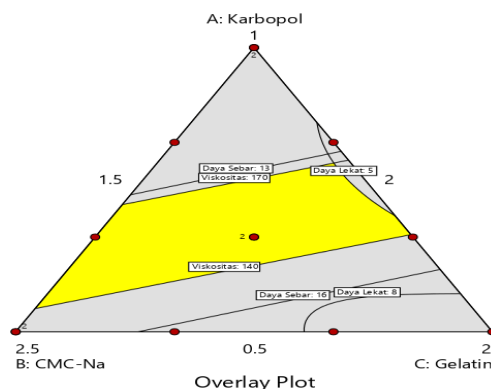

Figure 1. Overlay plot of the response to the optimum formula for viscosity, spreadability, and adhesion tests

Table 4. Optimum formula verification using one sample t-test

Response	Prediction	Test	*Sig. (2-tailed)*	Notes
Viscosity (dPa.s)	140	139 ± 5,41	0.736	Not significantly different
Spreadability (cm²)	15.116	15.22 ± 0,96	0.834	Not significantly different
Adhesion (second)	5.48	5.50 ± 0,93	0.530	Not significantly different

The optimum formula obtained from the experimental results analyzed by the simplex latice design (SLD) program was a formula with a carbopol concentration of 0.67%, CMC Na of 2%, and gelatin of 1.83%. This optimum formula was used for the preparation of 0.5% 4-hydroxychalcone gel.

3.1. Verification of the Response of the Physical Properties of the Base Gel and 0,5% 4-hydroxychalcone Gel

The physical properties of the optimum base gel formula were compared with the physical properties of 0.5% 4-hydroxychalcone gel and statistical analysis tests were carried out to determine differences in physical properties between the two gels. The results of the analysis showed that the response of viscosity, adhesion, and spreadability on the base gel was not significantly different from the response on 0.5% 4-hydroxychalcone gel. These results indicated that the addition of 0.5% 4-hydroxychalcone did not affect the physical properties of the gel.

3.2. Optimum Formula Stability

The optimum gel base formula and 0.5% 4-hydroxychalcone gel that had been prepared were then tested for physical stability which included pH, viscosity, spreadability, and adhesion of the preparations at week 0, 1, 2, 3, and 4 which were stored at room temperature. The 0.5% 4-hydroxychalcone gel had a lower pH compared to the base gel. During storage, the pH of the two optimum formulas tended to decrease but was still within the pH range of normal skin, which is between 5 to 6.5, so it can be concluded that the change in pH is still within the pH range that is safe for the skin.

Table 5. Response of Optimum Formula Gel Base and 0.5% 4-hydroxychalcone Gel during storage

Formula	pH	Viscosity (dPa.s)	Spreadability (cm²)	Adhesion (second)
Gel base	5.52 ± 0,031	149.45 ± 1,55	14.3 ± 0,93	6.59 ± 2,35
4-Hidroksikalkon Gel	5.15 ± 0,048	151.24 ± 2,69	13.9 ± 0,85	6.38 ± 1,71

Based on the data obtained, Tukey's posthoc one-way ANOVA statistical analysis was then performed to determine the optimum stability of the physical properties of the formula during storage. Base viscosity was stable for 4 weeks of storage at room temperature, the viscosity obtained was still good during storage.

The spreadability of the two preparations slightly decreased every week. The decrease in the spreadability of the preparations observed for 4 weeks was not significant, that was greater than 0.05. Statistically, the spreading power of the base can be said to be stable during storage at room temperature. Both preparations during storage experience an increase in adhesion. The adhesion of the base and 0.5% 4-hydroxychalcone gel was stable for 4 weeks of storage at room temperature.

3.3. Syneresis Test

Syneresis happens when water comes out of the gel. The gel will shrink and tend to squeeze the water out of the gel so that the gel looks smaller and denser. A high syneresis rate indicates that the gel was physically unstable on storage for 24, 48, and 72 hours [28]. The optimum formulation of gel base and 0.5% 4-hydroxychalcone gel did not experience syneresis during 72 hours of storage at ± 10°C.

3.4. Determination of Activity Assay of 0,5% 4-hydroxychalcone Gel as a Sunscreen

The 0.5% 4-hydroxychalcone gel test obtained an SPF value of 27.37 and was in the sunblock category. The SPF value of the gel base was constant and did not provide a protective effect, while the SPF value of 0.5% 4-hydroxychalcone gel increased with increasing concentration of the sample solution. The % erythema/pigmentation transmission value of a sunscreen makes better sunscreen activity because the UV radiation that is transmitted to the skin is getting smaller [27]. Large %TE and %TP values of the gel base mean that the UV radiation that is transmitted is large, so it can be concluded that the gel base does not provide a protective effect on the skin. The increased concentration of 4-hydroxychalcone gel caused a decrease in %TE 1.76% and %TP 10.21%, meaning that less UV radiation is transmitted so it provides a greater protective effect.

4. CONCLUSION

The optimum formula was obtained with a composition of 0.67% carbopol, 2% CMC Na, and 1.83% gelatin. The optimum formula of 0.5% 4-hydroxychalcone gel was stable during storage at room temperature and did not experience syneresis during 72 hours of storage at ± 10°C. 0.5% 4-hydroxychalcone gel gave an SPF value of 27.37 and % TE of 1.76% and % TP of 10.21%, therefore it can be categorized as a sunblock.

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