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Effects of Polyvinyl Alcohol and Hydroxypropyl Methylcellulose Combination on Physical Stability and Irritability of Gluthathione Peel-Off Masks

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ABSTRACT

Glutathione is an antioxidant composed of cysteine containing -SH functional groups. Its concentration decreases with age, causing aging symptoms. Gluthathione has hydrophilic properties with a log P of -1.4, which requires a reverse micelle system by adding surfactants such as Tween 80 and Span 80 to adjusts the log P to 2-3 to aid the penetration of stratum corneum for dermal delivery. Anti-aging strategies involve cosmetics including peel-off masks, which require effective polymer for ease of application. This study combines polyvinyl alcohol (15,16,17%) and hidroxypropyl methylcellulose (3,4,5%) to create a suitable gel base for glutathione peel-off masks. This research aims to determine the influence of the PVA and HPMC combination as a base for glutathione peel-off masks in terms of real-time physical stability and irritation effects using the HET-CAM method. The glutathione preparation utilized the reverse micelle method, followed by the formulation of the peel-off mask with a base comprising an equivalent of 2% reverse micelle glutathione. The quality of the combination was assessed for stability, including organoleptic properties, pH, viscosity, spreading ability, and drying time. The HET-CAM irritation score was utilized for irritation testing. This study found that the concentration of gelling agents PVA and HPMC affects the physical stability (organoleptic, pH, drying time, spreading ability, and viscosity) of the glutathione peel-off mask. Additionally, the HET-CAM irritation test demonstrates that all three dosage forms (F1, F2, and F3) exhibit non-irritating characteristics, with scores within the non-irritating range of 0.0 to 0.

***Keywords*:** glutathione, peel-off mask, PVA, HPMC, reverse micelle, HET-CAM

Introduction

Glutathione is an antioxidant with thiol groups in its cysteine component.[1] Glutathione is generally considered a safe substance for use as a dietary supplement. It inhibits the enzyme tyrosinase, converts dopaquinone to pheomelanin, and reduces reactive oxygen species and free radicals that impact tyrosinase activation.[2] The decline in glutathione concentration with age leads to aging symptoms.[2] Glutathione has low oral bioavailability due to poor absorption resulting from the action of glutamyl transpeptidase in the digestive system. Potential side effects arise when administering glutathione intravenously into the bloodstream. One approach to circumvent this is through topical application. Glutathione demonstrates hydrophilic properties with a log P value of -1.4, while the optimal log P value for substance penetration through the stratum corneum ranges from 2 to 3.[4,5,6]

Skin penetration ability through intercellular and transcellular pathways can be improved by enhancing the partition coefficient value (log P) using a reverse micelle system in the form of microemulsions, which are thermodynamically stable and visually transparent, consisting of a mixture of water, oil, and surfactants.[5,7]

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When a glutathione solution is prepared in the form of a reverse micelle system with the addition of Tween 80 and Span 80, there is an enhancement in glutathione approaching a log P range of 2-3, allowing it to penetrate the stratum corneum[5] effectively. Consequently, the lipophilicity of glutathione needs to be increased for topical application.

This study investigated the effectiveness of reverse micelle glutathione in formulating peel-off masks for anti-aging treatment. Peel-off masks provide several advantages compared to other types of masks, including ease of application and removal.[8,9] Peel-off masks offer various benefits, such as the capacity to relax facial muscles, cleanse, invigorate, moisturize, and soften the facial skin.[9] These masks provide a moisturizing effect on the skin, establish a uniform thin film layer, contribute to skin tightening, and can dry within 15-30 minutes. Peel-off masks must be easy and comfortable to use.[10] thus necessitating a suitable gel-based polymer in formulating the glutathione peel-off mask.

The advantages of peel-off masks include easy application, non-greasy feel, and convenient removal due to the formation of an occlusive film that can be easily peeled off. Peel-off masks can also enhance the permeability of active substances because of their occlusive nature.[9] One gelling agent used in the production of peel-off masks is polyvinyl alcohol (PVA).[11] PVA is a water-soluble synthetic polymer with various functions, including viscosity enhancement.[12] The gel produced by polyvinyl alcohol readily dries and forms a robust and transparent film that adheres well to the skin.[11] Kaplan.[13] stated that a commonly employed polymer for peel-off mask formulations is polyvinyl alcohol (PVA), although the resulting film layer tends to be rigid.[10] Thus, a combination of PVA and hidroxypropyl methylcellulose (HPMC) was utilized to obtain a peel-off mask formulation with film quality that can be quickly released or lifted like an elastic membrane upon application without irritation.

Topical application must fulfill various requirements, including safety aspects. The safety of topical application can be evaluated through

irritability, which can be assessed through several methods, including the hen's egg test on the chorioallantoic membrane (HET-CAM) method.[14]

This study examined the enhancement of the lipophilicity of glutathione using a reverse micelle system with the addition of surfactants Span 80 and Tween 80. The enhancement in the penetration and effectiveness of glutathione is anticipated to lead to better outcomes. Additionally, combining PVA and HPMC gelling agents in a 20% ratio is expected to yield stable glutathione peel-off masks that are effective for the skin upon application.

Materials and Method

Ingredients

The materials utilized in this study include glutathione (PT. Acerchem International INC, Shanghai, China), HPMC type 2208 (PT AIE BE ANTWERP ZW.W W3P CLD), PVA type 100-27 (G) (BASF, Indonesia), phenoxyethanol, PR (BASF, Indonesia), glycerine type 3026 (BASF, Indonesia), Span 80 (PT. Evonik Industries), Tween 80 (PT. Evonik Industries), KH2PO4 (Sumber Ilmiah Persada, Surabaya), NaOH (Sumber Ilmiah Persada, Surabaya), and fertile chicken eggs (Makassar chicken farm).

Tools

The equipment used in this research comprised IKA® T25 Digital Ultra-turax, Thermo Scientific Cimarec^{+}, pH meter SI Analytics Lab 865, OHAUS® analytical balance, CHRIST freeze dryer, magnetic stirrer, Brookfield cone-and-plate viscometer, Atech thermoshaker, as well as Sonic gram balance.

Method

Glutathione Preparation

Prior to the addition of surfactants, the aqueous phase preparation was carried out. Two grams of glutathione were dissolved in 20 ml phosphate buffer solution with a pH of 6 ± 0.05. Subsequently, surfactants were introduced to achieve a hydrophilic-lipophilic balance (HLB) value of 7. It was attained by mixing 2.52 ml of Span 80 with 7.47 ml of Tween 80. The mixture was freeze-dried at 26°C for 30 hours.[5] However, the freeze-drying of glutathione yielded non-dry results due to the oily nature of Tween 80 and Span 80. The freeze-dried product was subjected to partition coefficient determination by dissolution in the n-octanol phase. This evaluation was conducted using a thermoshaker at 37°C ± 0.05°C for 30 minutes, with three replications.

Peel-off mask Preparations

Developed respectively PVA and HPMC at a temperature of 70-80° C. Then glycerin and phenoxy ethanol are added, after which glutathione reverses micelles (Table 1).

Stability Testing

Physical stability assessment was conducted in real time under room temperature conditions (25 ± 1°C) with a humidity level of 60±5% RH for 30 days. During the first and final weeks, evaluations were carried out for organoleptic parameters, pH, homogeneity, spreadability, drying time, viscosity, and foldability of the glutathione peel-off masks.

Organoleptic

This procedure involved visual observation of the clarity, phase separation, color, and odor of the dosage form. The expected outcomes included a precise dosage form without phase separation, odorless characteristics, and no color changes.[15]The testing was performed before and after the 30-day stability period.

pH Measurement

The pH measurements was conducted at room temperature using the SI Analytics Lab 865 pH meter pre-calibrated with standard pH 7 and acidic pH 4 buffer solution. The dosage form was diluted at a ratio of 1:10 with distilled water, and the electrode was immersed into the dosage form until a constant pH reading was achieved. The pH of the dosage form should be within the range of skin pH, namely 4.5-6.5. Observations were made on newly prepared and those previously stored dosage forms.[15] The testing was performed before and after the 30-day stability period.

Viscosity Test

A Brookfield cone-and-plate viscometer was used for this test. The stationary plate formed the base of the sample cup, which could be moved, allowing the sample to be added and replaced without affecting calibration. The system was accurate within ±1.0% of the full-scale range, with a reproducibility of ±0.2%. It operated within a temperature range of 0°C – 100°C.[15] The testing was performed before and after the 30-day stability period.

Spreadability

This test involved placing 0.5 grams of the dosage form at the center of a scaled circular glass and then placing another unscaled circular glass on top, applying a load (using a Sonic gram balance). The diameter was measured at intervals of one minute. The load (grams) was increased in 5-gram increments until a constant spread diameter was achieved. Measurements were performed with three replications. A graph was plotted between the load and the average spread diameter before and after storage. A good gel mask exhibits a spread diameter between 5cm and 7cm, and the data can be analyzed for the gel's diameter increase.[16] The testing was performed before and after the 30-day stability period.

Drying Time

A quantity of 0.2 g of gel was evenly applied onto an object's glass surface to form a one mm thin layer thickness. The gel was left to dry and then peeled off, with the time taken recorded.[17] The testing was performed before and after te 30-day stability period.

Irritation Testing

The hen's egg test on the chorioallantoic membrane (HET-CAM) method was employed to assess irritability in this study. This method is convenient and exempt from ethical approval when the embryo age of the animal is less than half of the total incubation period.[19] Fertilized Leghorn chicken eggs were placed in an incubator at a temperature of 37°C. Subsequently, the eggs were rotated for ten days, ensuring the air chamber was positioned at the top. On the 10th day, the eggs were examined to distinguish between the fertilized and unfertilized. Fertilized eggs were selected, and the air chamber was marked. The marked eggshell was then opened using sterile scissors after softening the shell with a sterile 0.9% NaCl solution. Subsequently, the outer egg membrane was moistened with warm, sterile 0.9% NaCl solution and incubated for 5-20 minutes to facilitate the removal of the outer egg membrane. A 300mg sample was placed onto the CAM and allowed to stand for 20 seconds. Sodium lauryl sulfate and water were used as positive and negative irritant controls, respectively. The CAM was subsequently cleansed using a sterile 0.9% NaCl solution. The observation commenced at the 301st second after cleaning the CAM from the sample. The occurrence time of hemorrhage, lysis, and coagulation on the CAM was observed and recorded. Hemorrhage is characterized by bleeding in the vascularized CAM. Lysis is indicated by the disappearance of small blood vessels, potentially due to bleeding, dystonia of these delicate vessels, or actual disintegration. Coagulation encompasses both intravascular coagulation (thrombosis) and extravascular coagulation of proteins on the CAM, which typically increases CAM opacity Irritation scores were then calculated using the following formula.[20]:

IS = ((301-t(h) x 5) / 300) (301-t(l)) x 7 / 300 ((301-t(c) x 9) / 300

Explanation:
t(h) = the time when initial vascular bleeding is visually detected
t(l) = the time when initial vascular lysis is visually detected
t(c) = the time when initial vascular coagulation is visually detected

The irritation resulting from this experiment was classified based on the following range of values:
0.0 - 0.9 = non-irritating
1.0 - 4.9 = mild irritation

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5.0 - 8.9 = moderate irritation
9.0 - 21.0 = severe irritation

Hagino.[21] stated that liquid samples were used in quantities of 200 µL and applied onto the CAM utilizing a silicone rubber ring to confine the contact area. On the other hand, solid or powdered samples were sieved using a No. 200 sieve (nominal size: 75 mm). In this study, 0.2 g of each sample was applied to the silicone rubber ring. Samples that did not easily pass through the sieve were ground as frequently as necessary. Additionally, filter paper with a diameter of 18 mm was employed for semi-solid samples, which were then placed on the CAM surface. The test samples were applied into the CAM for 20 seconds, followed by rinsing the CAM samples with sterile NaCl and observing allergic reactions. The sample application onto the CAM can be seen in Figure 2.

Results and Discussion

Real-time testing was conducted under room temperature conditions (25 ± 1°C) with a humidity level of 60±5% RH to assess physical stability (Table 2). The results of the organoleptic evaluation before and after 30 days of storage using real-time testing indicated the absence of significant changes in the glutathione peel-off mask dosage form. Before and after storage, the color remained opaque white, the odor exhibited a distinctive characteristic, and the physical state did not indicate any phase separation.

Table 1: Formulation of Glutathione Reverse Micelle Peel-off Mask System

Ingredients	Functions	Formula (%)		
		F1	F2	F3
Glutathione reverse micelle equivalent 2%	active substance	2% equivalent	2% equivalent	2% Equivalent
Polyvinyl Alcohol (PVA)	Gelling agent	16	15	17
Hydroxy Propyl Methyl Cellulose	Gelling agent	4	5	3
Glycerin	humectants	10	10	10
Phenoxyethanol	Preservative	1	1	1

Table 2: Organoleptic test results on Real-Time stability on Days 0 and 30 of the RM Glutathione Peel-off Mask

Formula	Organoleptic characteristics			
	Day -0		Day -30	
Formula 1	Cloudy white, distinctive aroma, viscous		Yellowish-white, distinctive aroma, viscous	
Formula 2	Cloudy white, distinctive aroma, viscous		Yellowish-white, distinctive aroma, viscous	
Formula 3	Cloudy white, distinctive aroma, viscous		Yellowish-white, distinctive aroma, viscous	

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Table 3: pH, viscocity, and spreadability test results on Real-Time stability on Days 0 and 30 of the RM Glutathione Peel-off Mask

Formula	pH characteristics		Viscocity (cps)		Spreadability (slope cm/g)	
	Day -0	Day -30	Day -0	Day -30	Day -0	Day -30
Formula 1	4.71 ± 0.02	5.60 ± 0.11	24260 ± 793	36706 ± 501	0.2291 ± 0.015	0.2691 ± 0.072
Formula 2	4.53 ± 0.01	5.16 ± 0.12	9275 ± 257	27953 ± 582	0.2990 ± 0.016	0.2789 ± 0.019
Formula 3	4.52 ± 0.02	5.41 ± 0.01	1118 ± 793	20286 ± 688	0.2329 ± 0.025	0.2579 ± 0.010

Table 4: Dry-time test results on Real-Time stability on Days 0 and 30 of the RM Glutathione Peel-off Mask

Formula	Dry-time (minute)	
	Day -0	Day -30
Formula 1	22.59 ± 0.42	26.08 ± 0.04
Formula 2	21.01 ± 0.55	26.43 ± 0.04
Formula 3	22.56 ± 0.41	25.41 ± 0.14

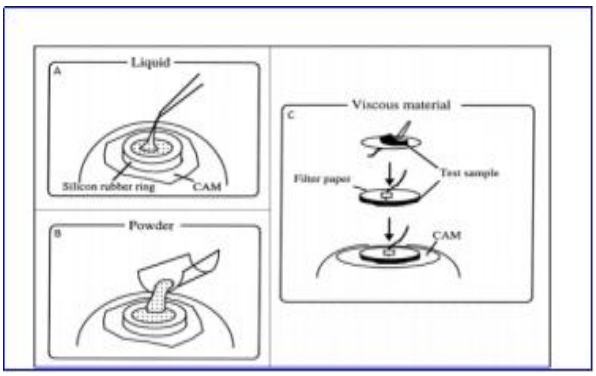

Figure 1: pplication of Samples to CAM, including liquid samples (A), powders (B), and semi-solid samples (C) [23]

After the organoleptic observation (Table 3**)**, the pH conditions of the three dosage forms, namely Formula 1 (PVA 16: HPMC 4), Formula 2 (PVA 15: HPMC 5), and Formula 3 (PVA 17: HPMC 3), showed significant differences. The pH test was conducted to assess the acidity level of the dosage forms to ensure that they do not induce irritation on the skin. The pH should align with the criteria of skin pH, namely within the range of 4.5-6.5. For topical dosage forms intended for use on the skin, a pH below 4.5 may lead to skin irritation, while a pH greater than 6.5 may cause skin dryness.[12] As for the viscosity, it is advisable for the dosage forms to fall within the range of 7100-83144 cps.[22]

On the assessment of viscosity stability, measurements were carried out on day 0 and day 30 using the Brookfield cone-and-plate viscometer at a rotational speed of 4 rpm. The viscosity measurements were replicated three times for each dosage form. Based on the table below, it is evident that the viscosity of the dosage form Formula 1 (PVA 16: HPMC 4) exhibited a significant difference between day 0 (9275±90 cps) and day 30 (27953 ± 358 cps). Similarly, Formula 2 (PVA 15: HPMC 5) showed a significant difference between day 0 (24260±583 cps) and day 30 (36706±501 cps). Additionally, Formula 3 (PVA 17: HPMC 3) displayed a significant difference between day 0 (11118±793 cps) and day 30 (20286±689 cps). All three dosage forms exhibited significant differences with p-values less than 0.05. From the testing, the highest viscosity was observed in Formula 2 (PVA 15: HPMC 5), indicating that increased usage of HPMC leads to higher viscosity in the dosage form. The varying concentrations of film-forming agents within each dosage form influence viscosity. According to Yuliani.[20] higher concentrations of film-forming agents can increase the viscosity of dosage form gel, as depicted in Table 3. A higher concentration of HPMC results in more hydroxyl groups binding with water, consequently leading to increased viscosity.[23]

Combining PVA and HPMC as film-forming agents yields a dosage form with substantial viscosity. Increased HPMC concentration enhances the polymer fiber count, thereby retaining more liquid bound by the gel-forming agent, resulting in elevated viscosity of the dosage form.[9] [11] During storage, viscosity might increase due to the thixotropic nature of the gel, where viscosity rises when left undisturbed.[11]

The results of the spreadability testing before and after 30 days of storage are presented in Table 5.10. For Formula 1 (PVA 16: HPMC 4), the slope of spreadability is 0.2990 ± 0.016 cm/g on day 0 and 0.2789 ± 0.019 cm/g on day 30. For Formula 2 (PVA 15: HPMC 5), the spreadability slope is 0.2291 ± 0.015 cm/g on day 0 and 0.2691 ± 0.072 cm/g on day 30. For Formula 3 (PVA 17: HPMC 3), the spreadability slope is 0.2329 ± 0.025 cm/g on day 0 and 0.2759 ± 0.010 cm/g on day 30. The p-values for all three dosage forms are ($p < 0.05$), indicating no significant differences. All dosage forms were subjected to a load of 35 grams before and after stability testing, yielding the following spreadability values: F1 PVA 16: HPMC 4 displayed 5.3 cm (before) and 5.9 cm (after), F2 PVA 15: HPMC 5 exhibited 5.2 cm (before) and 5.4 cm (after), and F3 demonstrated 5.5 cm (before) and 6.1 cm (after). Formula 2 exhibited the widest spreadability. A large spreadability diameter implies a large surface area that the dosage form can cover. Based on the spreadability test results, it can be concluded that an increase in HPMC leads to reduced spreadability. This decrease is due to the increase in molecular unit size resulting from solvent absorption, causing the trapped fluid to increase resistance to flow and spread.[24] Dry time testing (Table 4) day 0 21.01-22.59 minutes and day 30 25.41-26.43 minutes, the driest time quickly after stability F3 (PVA 17: HPMC 3) the higher the PVA the more dries quickly.[12]

Irritability testing was conducted to ensure the safety of the peel-off masks as a topical dosage form using the HET-CAM method, which uses eggs as a medium for sample application and measures irritability based on the occurrence of hemorrhage, lysis, and coagulation on the chorioallantoic membrane (Yuliani *et al.,* 2016). In this testing, all three dosage forms were assessed and compared with K+ (1% sodium lauryl sulfate) and K- (0.9% sterile NaCl). The irritability testing results presented in Table 5 and Table 6 indicate that the irritation score for K+ is 7.60 ± 0.20. This score falls within the range of 5.0-8.9, suggesting moderate irritation for K+. On the other hand, K- and F1, F2, and F3 exhibit similar results, falling within the range of 0.0-0.9, suggesting that K- and F1, F2, and F3 are non-irritating. These results imply that the peel-off masks using Tween 80 and Span 80 as surfactants are safe and non-irritating. Tween 80 and Span 80 are non-ionic surfactants in the form of emulsifiers known to be safe for use and are derivatives of sorbitan esters.[5]

The results of the HET-CAM test can be linked to the pH of the dosage forms, which fall within the range of typical skin pH values. Based on these findings, it can be inferred that the peel-off mask has the potential to be developed into various topical dosage forms for skincare applications.[25]

The HET-CAM testing requires meticulous attention to the timing of visual analysis. It is crucial to ascertain whether hemorrhage (marked by the oozing of blood from blood vessels on the CAM egg), lysis (characterized by the disappearance of blood vessels on the CAM egg), or coagulation before CAM occurs.[14]

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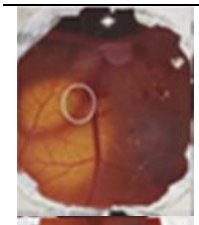
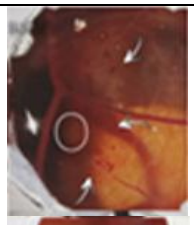
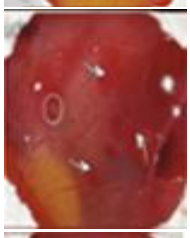
Table 5: Irritation test using the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM)

Sample	Treatment	
	Before	After
Positive control		
Negative control		
Formula 1		
Formula 2		
Formula 3		

→ = bleeding process; ○ = lysis process; control positive = Sodium lauryl sulfate (1%); negative control = Sterile NaCl (0.9%)

Table 6: Results of the HET-CAM Irritability Test for Glutathione Reverse Micelle

Sample	Average of score irritation ± SD
Positive control	7.60 ± 0.20
Negative control	0.00 ± 0.00
Formula 1	0.00 ± 0.00
Formula 2	0.00 ± 000
Formula 3	0.00 ± 0.00

Positive control = Sodium lauryl sulfate (1%);negative control = Sterile NaCl (0.9%); Formula = Peel-off mask glutathione reverse micelle formula

Conclusion

The combination of HPMC and PVA yields statistically significant differences in the physical stability of the glutathione peel-off gel mask as well as influencing organoleptic properties, pH, spreadability, drying time, and viscosity. The irritation test results demonstrate that all three dosage forms and the negative control are safe and non-irritating based on the HET-CAM method.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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