EFFECT OF b-Cyclodextrin ON SPF VALUE AND INHIBITION OF KOJIC ACIDSTYROSINASE ACTIVITY IN VANISHING CREAM BASE FORMULATION (ON SUNSCREEN PRODUCT CONTAINED OXYBENZONE)

by Widji Soeratri

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INTRODUCTION

Newly, we found many cosmetics combined of sunscreen substance and lightening agent in their ingridients.

Kojic acid is a skin lightening agent which has very small molecular size, so easily absorbed up through the basal membrane. Because of small molecule, kojic acid hardly absorbed through the lipid membrane of its target sites, the melanocytes (Manosroi et al, 2005) and can penetrate into the systemic (Nakayama et al., 2005). It is likely absorbed through voids between cells on the skin. Kojic acid is produced mainly by microbial fermentation using aspergillus and penicillium spp. In in vivo test, cream containing kojic acid compounds have been reported as effective in preventing pigmentation changes in human skin due to exposure to UVA and UVB. This inhibition has been shown to be due to chelation of Cu, a prosthetic group in tyrosinase (Barel et all, 2001). Cream contained 1 % of kojic acid showed steady but slow whitening effect. 14C-labeled kojic acid cream was observed to be quickly absorbed from the skin to the liver, intestines, and kidneys in mice.

When the absorption was thus quick, the depigmentation agent did not stay at the epidermis where it had its target organ, melanocytes, for a long enough time to inhibit melanogenesis. Therefore, kojic acid was mixed with β-cyclodextrin to slow the absorption into the dermis (Elsner and Maibach, 2005). Losses due to kojic acid to the systemic Is not able to work effectively inhibits melanin formation took place in the basal membrane of skin that results are not optimal lightening effect. Addition of β-cyclodextrin reported can decrease penetration of kojic acid (Nakayama et al., 2005).

Cyclodextrins are cyclic oligosaccha-rides with a hydrophilic outer surface and a somewhat hydrophobic central cavity. Cyclodextrins are able to form inclusion complexes with many drugs by taking up the drug molecule, or a lipophilic moiety of the molecule, into the cavity (Loftsson and O'Fee, 2003).
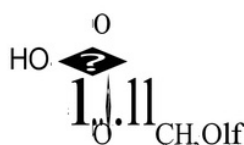
MW: 142.1
MP: 153-154oC
pKa: 7.90-8.
Log P (octanol-water): -0,64

Fig 1. Properties of Kojic Acid
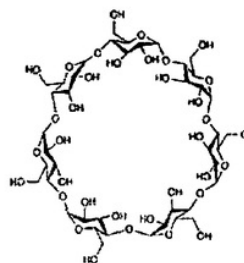5-hidroksi-2-hidroksimetil-4-pyron

Fig 2. β-cyclodextrin (Sweetman, 2007)

Sunscreen agents, such as: oxybenzone and octyl dimethyl PABA, are commonly present in whitening products to compensate for the photosensitivity effect caused by the whitening agent. This combination is more efficient but may have a profound effect on the efficacy of the sunscreen agent.

Therefore, this study was aimed to investigate the SPF value of sunscreen product contained oxybenzone and octyl dimetyl PABA (3:7) with the addition of kojic acid 1% b/b as whitening agent and their complex with (3-cyclodextrin in 1:1 molar equivalent. In this research, we also studied the effect of b-Cyclodextrin (BCD) on penetration of Kojic Acid, as lightening substance In Vanishing Cream Base Formulation (on Sunscreen Product Contained Oxybenzone), by determination of tyrosinase activity inhibition.

MATERIAL AND METHODE

Material

Oxybenzone, Octyldimethyl PABA and Kojic Acid were from Surya Oermato, (3-cyclodextrin p.a (Sigma Aldrich), Stearic Acid, cetyl alcohol, span 80, tween 80), Methyl paraben Propyl paraben, sorbitol 70%, Isopropanol p.a., (Brataco Chemicals). All of the active ingredients used here were in a pharmaceutical grade except for the chemical reagent isopropanol and β- cyclodextrin which were in pro analytical grade. While material for inhibition of tyrosinase activity test is the Mushroom tyrosinase, I-Tyrosine, NaH2PO4. 2H2O (p.a) and Na2HPO4 (p.a).

The SPF assay were carried out by using a Double Beam Spectrophotometer UV- Vis Perkin Elmer Lambda EZ 201, Ultrasonic Branson 3510, Hettich zentrifugen EBA 20, Mettler Toledo AL 204 analytical balance. Digital pH meter Schott CG 842 and spreading capacity measurer are also used for the organoleptic analysis.

Metode

The sunscreen product contained oxybenzone and octyl dimetyl PABA (3:7) with the addition of kojic acid 1% w/w as whitening agent and their complex with β-cyclodextrin in 1:1 molar equivalent. Vanishing cream as base was made with inversion technique.

The characteristics of the finished product evaluated were emulsion type, pH, spreadingability, and organoleptic such as colors, odors.

Table 1. Formula composition

Component	Percentage		
	F1	F2	F3
Kojic acid	1	1	1
β cyclodextrin	4	-	4
oxybenzone	-	3	3
Octyl dimethyl PABA	-	7	7
Vanishing cream base	Ad 100	Ad 100	Ad 100

Determination of SPF value

The SPF value was determined by Petro correlation for in vitro method. 2 mg/cm2 or 2 μL/ cm2 sunscreen agent for in vivo test was equivalent with 10 ppm sunscreen agent dissolved in isopropanol. An UV spectrum of this solution was then measured at 290-400 nm by using Double Beam UV-Vis Spectrophotometer Perkin Elmer Lambda EZ 201 at an interval of 2 nm which has absorbance for 0.05 or more. According to Petro, the absorbance was then converted into the absorbance for 10 ppm solution concentration for each wavelength. Then it was proceed in this following equation:

$$AUC_{\lambda_{p-a}}^{\lambda_p} = \frac{A_{p-a} + A_p}{2} \lambda_p - \lambda_{p-a}$$

Whereas:

AUC = Area under curve

Ap = Absorption on p wavelength

Ap-a = Absorption on p-α wavelength

The total AUC were obtained by totaling each AUC between 2 wavelengths in series from 290 nm till 400 nm which has an absorbance value above 0.0SO and the SPF value of a formula were obtained by inserting the total AUC into the equation below:

$$Lo_9 SPF = \frac{Total\ area}{An-11} \times Z$$

Whereas:

An = longest wavelength above 290 nm that has an absorbance higher than 0.050

Al = shortest wavelength 290 nm

Determination of tyrosinase activity inhibition:

A.   Preparation of a solution of the reaction components. The solution should be prepped for the implementation of this study was 0.1 M phosphate buffer pH 6.5, solution of tyrosinase (5370 units / mg solid in 0.1 M phosphate buffer pH 6.5 to 100.0 ml volume), a solution of 5.52 mM L-tyrosine, and 30% TCA.

B.   Preparation of test sample solution. The cream (around 3 grams) was put in the diffusion cell then covers with the Millipore membrane which was impregnated with isopropyl-myristate as modified lipid membrane. Then the preparation of cream in diffusion cell was put into the penetration chamber contain 500 ml of phosphate buffer pH 6.5 ± 0.05 at 37 ± 0.52C as diffusion medium, and then the paddle was stirred 100 rpm. The sample solution around 3 ml was collected at 6 hours after it penetrated.

Determination of the dopakrom maximum wavelength. L-tyrosine solution of 5.52 mM has taken a number of prepared and then added 0.5 ml 3 ml of buffer solution pH 6.5 ± 0.05. Then the mixture add with 1.0 ml of tyrosinase and oxygenated for 5 minutes. The mixture was incubated for 15 minutes at 26 ±

0.5 2C then added 0.5 ml of 30% TCA. The solution is then inserted into cuvet and placed on the sample position in the electrophotometer. Used as a blank solution 0.1 M phosphate buffer pH 6.5. Then do the reading of absorbance values from a wavelength of 400 nm to 500 nm, and the selected wavelength that gives the greatest absorption.

Inhibition Tyrosinase Activity Determination L-tyrosine solution 5.52 mM added with 0.5 ml 3 ml of sample solution. Then the mixture added with 1.0 ml of tyrosinase and oxygenated for 5 minutes. The mixture was incubated for 15 minutes at 26 ± 0.5 ℃. Then add 0.5 ml of TCA 30% and observed the absorbance at dopacrom maximum wavelength. Inhibition tyrosinase activity is the percent inhibition values obtained from the later absorbance value calculated by the equation:

$$\% \text{ Inhibition} = 100 - \frac{A \times 100}{B}$$

A = Absorbance at the maximum with the skin lightening

B = Absorbance at maximum A without the skin lightening

RESULT AND DISCUSSION

All the formulas had either pH in skins pH range or similar spreadability.

The SPF value increased in formula 3. The complex formation of the sunscreen agent with BCD was suspected to be responsible for this phenomenon. It might because of complex formation of the sunscreen agent with β- cyclodextrin increased sunscreen agent solubity.

Based on one way ANOVA test, known that the addition of BCD decreased inhibition percent compared to the control constraints. Nevertheless the addition of sunscreen does not affect the inhibition percent of tyrosinase activity. Addition of BCD on combination kojic acid-sunscreen preparations showed the

same phenomena such as decreased its inhibition percent of tyrosinase activity. Decreasing of inhibition percent of tyrosinase activity after addition of BCD or combination BCD-sunscreen was might be caused by decreasing of kojic acid penetration. It was probably caused by some of the things that the release process of kojic acid from base and in the process of penetration through the membrane. Before reach basal membrane where the kojic acid site of action, kojic acid molecule should released from base further more penetrate through the skin. Increasing viscosity will decrease the molecules mobility of active ingredient that caused barriers against the release of kojic acid.

Table 2. Average of pH

FORMULA	pH±SD
1	4.51 ± 0.08
2	4.29 ± 0.03
3	4.04 ± 0.03
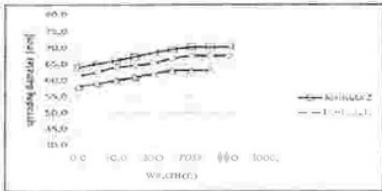
Figure 3. Profile of Spreading ibitity
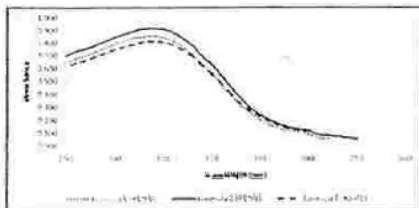
Figure 4. Profile of sunscreens absorbance measurement

Table 3. Average of SPF

Formula	SPF (average)
1	10,607 ± 0,432
2	11,741 ± 0,479
3	19,113 ± 0,293

Table 5. Average percent of tyrosinase inhibition

Formula	% tyrosinase activity Wtihilion
1	28,39 ± 0,51
2	60,20 ± 0,98
3	23,69 ± 0,61

CONCLUSION

1. The complex formation of the sunscreen agent with BCD decreased the SPF value of sunscreen product.

2. BCD decrease penetration kojic acid based on inhibition of tyrosinase enzyme activity in invitro.

3. The existence of BCD and combination sunscreen oxybenzone and octyl dimethyl PABA (3:7)% w/w decrease penetration kojic acid based on inhibition of tyrosinase enzyme activity in invitro.

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FINAL GRADE

/0

GENERAL COMMENTS

Instructor