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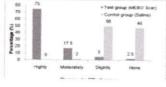
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Original Article

The Influence of Sesame Oil Addition on the Arbutin Release and Penetration in Carbomer Gel Base

Tristiana Erawati *, Widji Soeratri, Noorma Rosita, Wida Rukmanajati, Hanifa Rahma

Department of Pharmaceutics, Faculty of Pharmacy Airlangga University, Surabaya-Indonesia.

ARTICLE INFO

ABSTRACT

Received: 15 Jun 2014 Accepted: 29 Jun 2014 Hydrophilic arbutin as lightening agent with log P value -1.35, make it difficult to permeate through the skin and reach its site of action. Sesame oil addition (3, 5, and 7% w/w) was expected to increase the arbutin release and penetrations. The aim of this study was to investigate the influence of sesame oil addition on the arbutin release and penetrations in the Carbomer-940's gel base. The release (flux) of arbutin, as initial process before penetration, formulation were studied using cellophane membrane and buffer phosphate pH 7.0 as media at 37±0.5°C for six hours long. The penetration of arbutin was observation on inhibition of enzyme tyrosinase activity. Inhibition percent of tyrosinase by arbutin was determined in vitro by observing the absorbance value of dopachrome (an intermediate product of melanin formation) as a reaction product between enzyme tyrosinase and L-tyrosine as a substrate using spectrophotometer. Conclusion of this study was sesame oil addition 3 and 5% w/w decreased arbutin release, sesame oil addition 3, 5 and 7% w/w increased arbutin penetrations. Increasing of arbutin effectiveness more is caused by enhancer effect of sesame oil.

Key words: Arbutin, Carbomer-940, Penetration, Release, Sesame oil, Tyrosinase-inhibition.

1. INTRODUCTION

Arbutin widely used in cosmetic as lightening agent to inhibited enzyme tyrosinase activity in basal membrane of the skin. Tyrosinase was known as enzyme that involved in melanin formation.^{1, 2} Because of the hydrophilic of arbutin with log P value 1.35 make it difficult to penetrate through the skin. To increase the

Corresponding author *

Tristiana Erawati, Department of Pharmaceutics, Faculty of Pharmacy Airlangga University, Surabaya-Indonesia. E Mail: tristiana-e-m@ff.unair.ac.id was impregnated with isopropyl-myristate as modified lipid membrane. Then the preparation of arbutin gel in diffusion cell was put into the penetration chamber contain 500 ml of phosphate buffer pH 6.5 ± 0.05 at 37 ± 0.5 °C as diffusion medium, and then the paddle was stirred 100 rpm. The sample solution around 5 ml was collected at 360 minutes after it penetrated.

2.5 Determination of enzyme tyrosinase activity

L-tyrosine solution 0.5 ml added with 3.0 ml sample solution that collected from compartment receptor after 360 minutes penetrated through Millipore membrane which was impregnated with isopropyl-myristate. The mixture was oxygenized 5 minutes then added with 1.0 ml tyrosinase solution. After incubated for 10 minutes at 25°C the mixture was inactivated with 0.5 ml TCA solution and then the absorption value measured at maximum wavelength of dophacrome.⁵

2.6 The evaluation of inhibition of enzyme tyrosinase activity

The inhibition of enzyme tyrosinase activity was performed as inhibition percent, which found from calculation of absorption value per second enzymatic reaction with inhibitor, compared with absorption value per second enzymatic reaction without inhibitor, using the following equation. ⁶

inhibition (%) =
$$100 - \frac{(Ax100)}{B}$$

Whereas:

A = absorption value (A/second) at dophacrome λ maximum with inhibitor

 $B = absorption \ value \ (A/second) \ at \ dophacrome \ \lambda$ maximum without inhibitor

The data (inhibition %) were analyzed with ANOVA one way method (p<0.05).

3. RESULTS AND DISCUSSION

The result of this study, in table 2 shows that the pH of all formulas around 6 it mean appropriate with skin pH. The spreading profile of arbutin gel preparation shows in Figure 1 and spreading-capasity of arbutin gels at 20

gram ballast shows in table 3. Spreading-capacity was formulas spreading-diameter at same ballast weight. The result of ANOVA one way test of spreading-capacity found the value of F_{calculation} (13.741) > F_{table} (4.07). Its can conclude there were significant deference minimal one pair of spreading-capacity formulas data. To know which spreading-capacity formulas was significant deference it's tested by *Honestly Significant Deference* (HSD) tests. The result of HSD test in table 4, that can concluded the spreading-capacity of formula 1 did not deference with control but higher than formula 2 and 3.

Spreading-ability was the slope of linier-regression between spreading-diameter (cm) and ballast weight (gram), its shows in table 5. The slope value from its formulas was tested by ANOVA one way method, it's found that the value of $F_{\text{calculation}}$ (0.274) $< F_{\text{table}}$ (4.07). So that can conclude it's was no significant deference between spreading-ability of all formulas.

Arbutin release (flux) was calculated from the linier regression of the correlation curve between square root of time versus arbutin release accumulation. Slope (flux) of linier regression showed in table 6. To make sure if there is any difference of arbutin flux between formulas was done by statistical testing using ANOVA one way. It is showed that $F_{calculation}$ (50,918) > F_{table} (4.07). From HSD result know that flux formula 1 and 2 not statistically different, but if compare with control and formula 3 were decrease. This might be caused by interaction between arbutin, sesame oil, and Tween. Tween is anionic surfactant which is amphiphil, it have affinity towards polar or non polar substance, such as arbutin and sesame oil. This interaction makes arbutin more difficult to release from bases. Another factor that may influence was viscosity from formula 1 and 2 which is more viscous than control, it cause arbutin molecules difficult release from bases also. The

penetration enhancer can be add in the formula. Sesame oil as an oily enhancer has total protein (25%) and globulin (67.3%) its can increased penetration trough polar pathway by enlarge aqueous channel. Sesame oil also can use as healing effect from sunburn.3 It was known Sesame oil effective concentration as enhancer up to 10%.4 The aim of this study was to investigate the influence of sesame oil (3, 5, and 7% w/w) addition on the arbutin (3% w/w) penetrations in the Carbomer-940 gel base through the modified lipid membrane. It was observation on inhibition of enzyme tyrosinase activity. However sesame oil is a viscous fluid can increase the viscosity of base so that it feared inhibits the release of arbutin and decrease penetration. In this study determined arbutin release from the base using cellophane membrane and buffer phosphate pH 7.0 as media at 37±0.5°C for six hours long.

2. MATERIALS AND METHODS

2.1 Preparation of the arbutin gel as Lightening product

The arbutin in Carbomer-940 gel base formulas as lightening product was shown in table 1. In this research Carbomer-940 gel base contained tri ethanol ammine (TEA) as alkalizing agent, propylene-glycol as humectants, methyl-parabene and propyl-paraben as preservative, Na-EDTA as chelating agent, butylated hydroxyl toluene (BHT) as anti-oxidant and Tween-80 as surfactant. Arbutin 3% w/w in Carbomer-940 gel base was used as control. Arbutin 3% w/w with sesame oil 3% w/w in Carbomer-940 gel base named as F1, Arbutin 3% w/w with sesame oil 5% w/w in Carbomer-940 gel base named as F2 and Arbutin 3% w/w with sesame oil 7% w/w in Carbomer-940 gel base named as F3.

2.2 The Characteristics determination of the arbutin gel

The Characteristics determination of arbutin gel included:

- 1. Determination of gel pH
- 2. Determination of the spreading-ability

Determination of gel spreading-ability was performed using a pair of glass plate (20 X 20 cm). The gel preparation (1 gram) was put in the middle of the first glass plate that given the scale. Then put the second glass plate on the first glass plate and measured the diameter of gel spreading. After that put ballast on the second glass plate then measured the diameter spreading-ability of the gel. The weight of ballast that put on the second plate was increased until spreading-ability of the gel was constant.

2.3 Determination of arbutin release

Determination of arbutin release from the bases was done by the dissolution tester *Hanson Research SR-6* with paddle stirrer. Each cell diffusion fill with arbutin gel (± 2 grams), in 500mL buffer phosphate pH 7.0, temperature 37°C, agitation 100 rpm. Samples (5mL) were taken at 5, 10, 15, 30, 45, 60, 90, 120, 180, 210, 240, 270, 300, 330, and 360 minutes, replace with 5mL buffer phosphate pH 7.0 to keep volume constant. The absorbance of arbutin in the sample measured by spectrophotometer. The arbutin release (flux) from the base obtained from the slope of the linear regression of the correlation curve between arbutin releases accumulations versus square root of time.

2.4 The penetration evaluation arbutin gel (United Stated Pharmacopoeia, 2002)

In vitro study for the penetration of the arbutin in Carbomer gel base was measured by the modification method of the penetration test USP XXV and British Pharmacopoeia, 2002 with diffusion apparatus ERWEKA DT 700.

The in vitro study was evaluated as follows: The arbutin gel (around 3 grams) was put in the diffusion cell then covers with the Millipore membrane which

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increase of viscosity may inhibit the movement of molecules to release from bases.⁷

Flux value of formula 3 not statistically different with control but higher than formula 1 and 2. It might be caused by addition of sesame oil reduce amount of water from formula. Decrease amount of water caused increase of arbutin concentration on water phase. Substance release from bases is known as diffusion. Based on Fick's law, diffusion is the process by which molecules moved from compartment with high concentration to low concentration.

The arbutin effectiveness as lightening agent calculated as inhibition percent (%) of enzyme tyrosinase activity. The result of arbutin inhibition percent (%) with enhancer sesame oil in Carbomer gels shows in table 7. The result of ANOVA one way test of the arbutin effectiveness in carbomer gel formulas found the value of $F_{calculation}$ (23,582) > F_{table} (4.07), and from the HSD test result was found inhibition percent of control < formula 1 = formula 2 < formula 3. Increasing of arbutin effectiveness more is caused by enhancer effect of sesame oil.

Table1: Formulas of lightening product

		Concentra	tion (%	6 w/w)	
Material	Base	Control	F1	F2	F3
Arbutin	-	3	3	3	3
Sesame oil	-		3	5	7
Carbonier 940	1	1	1	1	1
TEA	1	1	1	1	1
Propylene glycol	20	20	20	20	20
Methyl-parabene	0,15	0,15	0,15	0,15	0,1
Propyl-parabene	0,05	0,05	0,05	0,05	0,0
Na EDTA	0,05	0,05	0,05	0,05	0,0
BHT	0,05	0,05	0,05	0,05	0,0
Tween 80	0,5	0,5	0,5	0,5	0,5
Water up to	100	100	100	100	100

Table 2: The Arbutin gel pH values

Formula			p	H value	
	\mathbf{R}_1	R_2	R_3	Mean	% CV

			Volume 2 (3), 2014, Page-241-24		
Control	6.24	6.21	6.04	6.16 ± 0.11	1.75
F1	6.34	6.34	6.38	6.35 ± 0.02	1.88
F2	6.16	6.36	6.37	6.30 ± 0.12	1.88
F3	6.43	6.38	6.38	6.40 ± 0.03	0.45

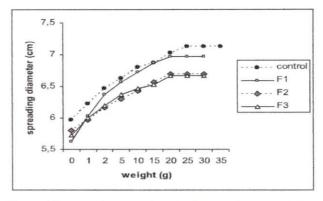


Fig 1: The spreading profile of arbutin gel with various concentration of sesame oil. Each value represents the mean of 3 determinations

Tabel 3: Spreading-capasity of arbutin gels at 20 gram ballast

Formula	Spreading Diameter (cm)* at 20 gram ballast
control	7.03 ± 0.06
F1	6.97 ± 0.15
F2	6.70 ± 0.00
F3	6.67 ± 0.06

^{*} The result were obtained from an average of 3 times replication

Table 4: HSD test result of spreading- capacity value of arbutin

Formula	N	Value groups of spreading-capacity (cm)		
	_	1	2	
F3	3	6.667		
F2	3	6.700		
F1	3		6.967	
control	3		7.033	

Tabel 5: Arbutin gels spreading-ability

Formula	Spreading-ability (cm/g)*		
control	0.0444 ± 0.0033		
F1	0.0545 ± 0.0091		
F2	0.0396 ± 0.0044		
F3	0.0310 ± 0.0034		

^{*} The result were obtained from an average of 3 times replication

Tabel 6: Flux of arbutin release from gel bases

Formula	Replication	Flux (µg/cm²/menit ^{1/2})	Mean ±SD
	1	438.4026	
Control	2	422.1708	125 2052 . 1 1 7/
	3	445.0424	435.2053±11.76
	1	359.0422	
F1	2	388.6486	274 4402 114 84
	3	375.6301	374.4403±14.84
	1	377.4143	
F2	2	388.6486	201 2250 (05
	3	386.9422	384.3350±6.05

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	1	440.0723	
F3	2	462.3000	
	3	458.1690	453.5138±11.82

Table 7: The arbutin effectivity (inhibition %) in carbomer gel formulas

	Inhibition (%)				
Formula	R1	R2	R3	Mean	% CV
control	37.50	35.98	36.53	36.67 ± 0.77	2.10
F1	41.77	40.87	41.80	41.48 ± 0.53	1.27
F2	44.82	45.51	43.96	44.76 ± 0.78	1.73
F3	52.44	48.61	45.51	48.85 ± 3.47	7.11

4. CONCLUSION

Conclusion of this study was sesame oil addition 3 and 5% w/w decreased arbutin release, sesame oil addition 3, 5 and 7% w/w increased arbutin penetrations. Increasing of arbutin effectiveness more is caused by enhancer effect of sesame oil.

5. ACKNOWLEDGEMENT

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