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Study Of The Effects Of Tween 80 And Palm Kernel Oil On in vitro Ascorbic Acid Penetration Through Rabbit Skin

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Abstract :

Background: It is well known that drugs can be applied to the skin to get advantages like easy of access and the avoidance of first pass metabolism. However, the slow transport of many drugs across the skin makes a limitation. To overcome this problem, enhancers can be used.
Objective: To investigate the penetration enhancing effect of Tween 80 and palm kernel oil on the *in vitro* skin penetration of ascorbic acid.
Methods: In this study, varies ointment formula were made: F1 (without Tween 80, 35% palm kernel oil), F2 (2.5% Tween 80, 35% palm kernel oil), F3 (5% Tween 80, 35% palm kernel oil), F4 (10% Tween 80, 35% palm kernel oil), F5 (without palm kernel oil, 5% Tween 80), F6 (25% palm kernel oil, 5% Tween 80), and F7 (ascorbic acid solution, 50% glycerin as solvent), and each formula contained 10% ascorbic acid. The ascorbic acid ointment was applied to the skin and then the skin was attached to diffusion cell. At a certain interval of time, ascorbic acid content in the receptor chamber was assayed using UV spectrophotometer at wavelength 266.8 nm.
Results: This study showed that Tween 80 and palm kernel oil could enhance ascorbic acid skin penetration, but Tween 80 must be used in low concentration (2.5% and 5%). Tween 80 in high concentration (10%) decreased the penetration. For palm kernel oil, the concentration of ascorbic acid penetrated increased with the increased of the concentration of palm kernel oil used.
Conclusion: This study suggests that Tween 80 and palm kernel oil can be used to enhance ascorbic acid penetration through rabbit skin.
Keywords: *Ascorbic acid, penetration, Tween 80, palm kernel oil.*

Introduction

Drug can be delivered across the skin to have an effect on the site of application (topical delivery), the tissues adjacent to site of application, or systemic effect. Although there are many advantages to delivering drugs through the skin, like easy of access and avoidance of first pass metabolism, the barrier properties of the skin limit this method of drugs delivery[1]. The stratum corneum is the main barrier for drug absorption across the skin. It is the primary protective layer and consists of fully keratinized dead cells[2].
Ascorbic acid is a typical pharmaceutical agent that has been used for a long time in whitening cosmetics to control production of melanin. It reduces the melanin intermediate compound, dopaquinone, which produces melanin from tyrosine, and it also reduces the dark color melanin to lighter color form[3].
Tween 80 is a nonionic surfactant that usually used in pharmaceutical products. The effect of Tween 80 on the penetration of ascorbic acid through rabbit skin has been investigated and reported that the higher

concentration of Tween 80, the higher is the permeability of ascorbic acid[4]. At the study, the highest concentration of Tween 80 used was 5%. Other study reported that drug released is not proportional linearly with the concentration of penetration enhancer[5]. This author was interested in using higher concentration of Tween 80 to compare the results.
Palm oil has been reported to enhance the penetration of aspirin through skin of rabbit[6]. But as far as the author's concern, palm kernel oil has never been investigated. The author interested in the investigation of the penetration enhancing effect of palm kernel oil on the penetration of ascorbic acid through rabbit skin.

Experimental Methods

Materials

Ascorbic acid was purchased from PT. Mutifa (Medan, Indonesia), palm kernel oil was obtained from PT. Multimas Nabati (Medan, Indonesia). Glycerin, Tween 80, ethanol 96%, and sodium metabisulfite were products of Merck, Germany. Vaseline album was purchased from PT. Brataco (Medan, Indonesia),

Preparation of absorption curve for ascorbic acid

An accurately weighed 50 mg of ascorbic acid and 100 mg of sodium metabisulfite were dissolved in 50% glycerin solution and the volume was made up to 100 ml to obtain 500 µg/ml of stock solution. Then, as much as 0,1 ml of this stock solution was withdrawn and made up to 10 ml with 50% glycerin. The ascorbic acid solution was assayed using UV spectrophotometer at wavelength 200-400 nm. Maximum absorption of ascorbic acid was at λ 266.8 nm.

Preparation of calibration curve of ascorbic acid

Aliquot of 0.02, 0.03, and 0.04 ml of stock solution were diluted to 50 ml with 50% glycerin solution to produce 0.2, 0.3, and 0.4 µg/ml of ascorbic acid solution, respectively. Aliquot of 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.16, 0.18, 0.2, 0.28, 0.32, 0.36, and 0.4 ml of stock solution were diluted to 10 ml with 50% glycerin solution to produce 0.5, 1, 2, 3, 4, 5, 8, 9, 10, 14, 16, 18, and 20 µg/ml of ascorbic acid solution, respectively. The absorbance of these solutions were determined using UV spectrophotometer at 266.8 nm.

Preparation of rabbit skin

A male rabbit weighing 1.5-2 kg was used in this study. The dorsal area hairs of the rabbit were removed carefully using scissors and razor and cleaned. This procedure was conducted one day prior to excising the skin to allow the skin to condition itself to the environment[4].

The rabbit was sacrificed using diethyl ether. The skin was excised using surgical scissors. Any attaching fat was removed and washed with distilled water.

The skin was wrapped with aluminum foil and stored at -20ºC until the experiments were carried out[7].

Preparation of formulations

The components of each formulation are showed in Table 1.

Table 1. Components of each formulations

Components	F1	F2	F3	F4	F5	F6	F7
Ascorbic acid (%)	10	10	10	10	10	10	10
Ethanol 96% (%)	20	20	20	20	20	20	-
Tween 80 (%)	-	2.5	5	10	5	5	-
Palm kernel oil (%)	35	35	35	35	-	25	-
Vaseline album (%)	34.9	32.4	29.9	24.9	64.9	39.9	-
Na.metabisulfite (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sol. of 50% Glycerin (%)	-	-	-	-	-	-	89.9

Ascorbic acid, sodium metabisulfite, and ethanol were put into the mortar and triturated until dissolved and homogenized. Tween 80 was added to this mixture if the formula consist it. Then, palm kernel oil and vaseline album were also put in the mortar and mixed until a homogeneous ointment was obtained.

For ascorbic acid solution, the weighed ascorbic acid was mixed directly with 50% glycerin solution until completely dissolved.

Penetration studies of ascorbic acid through rabbit skin *in vitro*

The apparatus used was diffusion cell, consisted of a donor and a receptor chamber. The volume was 10.8 ml each. Rabbit skin (1,28 cm^2) was applied with 0.15 g of ointment, and put between the donor and receptor chambers. The receptor chamber was filled with 50% glycerin solution and during the experiment, it was stirred using a magnetic bar. The temperature of the diffusion cell was maintained at 37ºC by a thermostat.

At certain interval of time, 1 ml of sample was withdrawn from the receptor chamber, diluted to 25 ml with 50% glycerin solution and the ascorbic acid content was determined using UV spectrophotometer at 266.8 nm. On each sampling occasion, 1 ml of fresh receptor medium at 37ºC was added to the receptor chamber to replenish the receptor medium so that the volume of medium in the receptor chamber was kept constant. The experiment was conducted up to nine hours and in triplicate.

Statistical analysis

Statistical analysis of the experiment data was carried out by one way analysis of variance (ANOVA) at significant level $P < 0.05$, follow by Post Hoc test analyzed by Tukey HSD using SPSS 17.0 software.

Results And Discussion

Effect of Tween 80 concentration on the *in vitro* penetration of ascorbic acid through rabbit skin

Figure 1 and Table 2 show the effect of Tween 80 on the penetration of ascorbic acid.

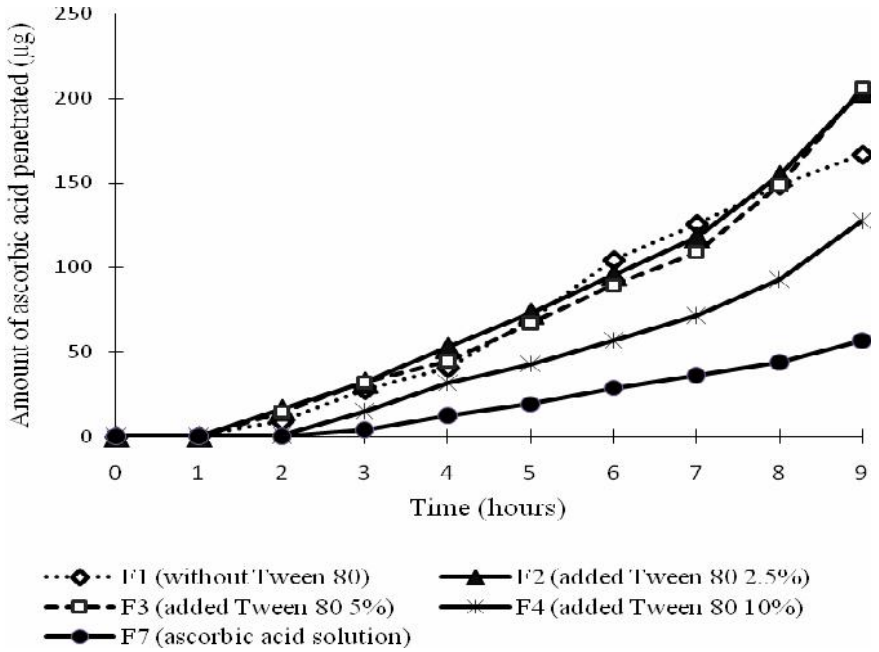

Figure 1. Effect of Tween 80 concentration on the *in vitro* penetration of ascorbic acid in 50% glycerin solution at 37ºC.

Table 2. Amount of ascorbic acid penetrated from various formulations in different Tween 80 concentrations.

Time (hours)	Amount of ascorbic acid penetrated (µg)				
	F1	F2	F3	F4	F7
0	0.0	0.0	0.0	0.0	0.0
1	0.5	0.0	0.0	0.0	0.0
2	8.9	15.8	14.5	0.7	0.0
3	27.7	32.0	31.9	14.9	4.0
4	40.7	52.6	44.6	31.6	12.3
5	69.1	72.8	67.0	42.7	19.3
6	104.3	95.4	89.2	56.8	28.8
7	125.6	117.6	109.0	71.5	36.1
8	148.4	153.9	148.7	92.56	43.8
9	166.5	203.7	205.9	127.3	56.9

The results showed that at concentrations of 2.5% and 5%, Tween 80 increased ascorbic acid penetration, but the used of 10% Tween 80, it decreased ascorbic acid penetration.

The concentration of ascorbic acid penetrated through rabbit skin to the medium in receptor chamber was higher in the formula contained Tween 80 in low concentrations (2.5% and 5%) compared to formula without Tween 80.

This was due to the effect of Tween 80 as a non-ionic surfactant. Tween 80 has the potential to solubilize the lipid of stratum corneum thus enhances the penetration of ascorbic acid[8]. Beside that, Tween 80 can interact onto the stratum corneum, thereby disorganizing its structure, increased its permeability, thus enhances the penetration[9]. But, from the statistical analysis, the enhancement was not significant (P>0.05). Instead of enhancing the penetration, the used of 10% Tween 80 decreased the penetration of ascorbic acid. This may be due to micelle formation of Tween 80 with ascorbic acid trapped in the micelle. Tween 80 can form micelles with long polyoxyethylene chains that can trap the ascorbic acid. The ascorbic acid trapped in the micelle could not pass through the stratum corneum because of the large size of the micelles. If the concentrations of the used Tween 80 were lower, the enhancement effect may be become significant. Higher polymer concentration has been reported to decrease the drug released from topical formulation[5].

Furthermore, the effect of Tween 80 on the penetration rate of ascorbic acid through rabbit skin was shown in Figure 2.

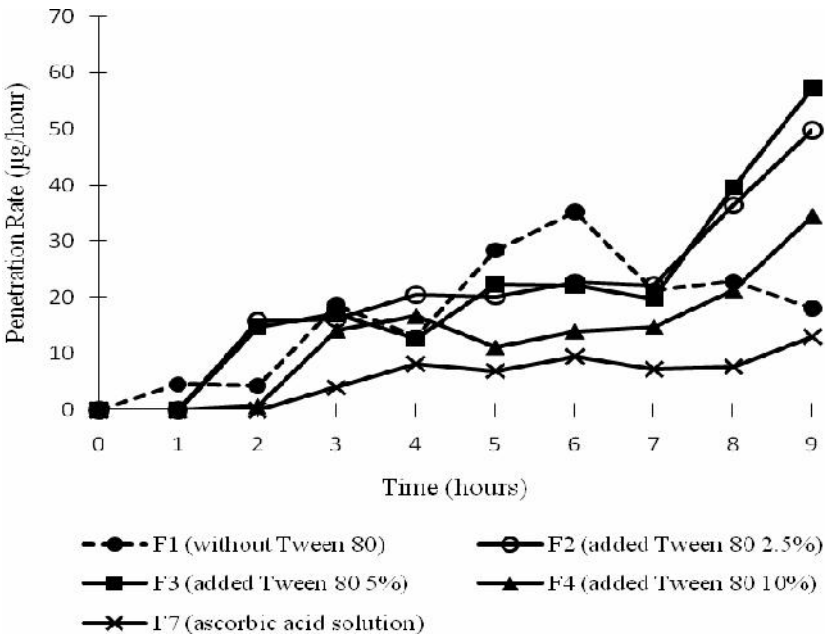

Figure 2. Effect of Tween 80 on the penetration rate of ascorbic acid through rabbit skin *in vitro* in 50% glycerin solution at 37ºC.

The penetration rate of ascorbic acid was increased with the used of Tween 80 at concentration 2.5% and 5%, but decreased with the use of 10% Tween 80. This may due to the same reasons described previously.

Effect of palm kernel oil concentration on the *in vitro* penetration of ascorbic acid through rabbit skin

Figure 3 and Table 3 show the effect of palm kernel oil on the penetration of ascorbic acid *in vitro*.

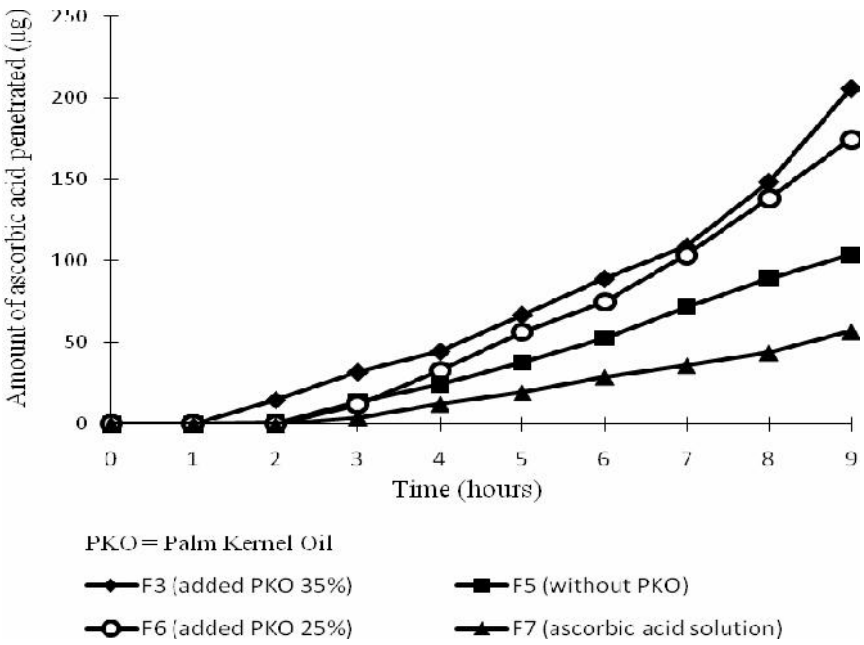

Figure 3. Effect of palm kernel oil concentration on the *in vitro* penetration of ascorbic acid in 50% glycerin solution at 37ºC.

Table 3. Amount of ascorbic acid penetrated from various formulations in different palm kernel oil concentrations.

Time (hours)	Amount of ascorbic acid penetrated (µg)			
	F3	F5	F6	F7
0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0
2	14.8	0.7	0.0	0.0
3	31.9	13.9	11.9	4.0
4	44.6	24.1	32.7	12.3
5	67.0	38.0	56.2	19.3
6	89.2	52.9	74.8	28.8
7	109.0	72.0	103.5	36.1
8	148.7	89.4	138.3	43.8
9	205.9	104.0	174.5	56.9

Application of oily material such as palm kernel oil will block the pores and sweat ducts so that the water cannot evaporate out of the skin. This will cause hydration of the skin. The water content in the skin will interact with the polar head group of the lipid bilayers present in the intercellular spaces through hydrogen bonding. This bonding will stretch and loosen the lipid packing so that the bilayer region becomes more fluid. This will enhance the migration of ascorbic acid through the stratum corneum[10]. Hydration increases the penetration of polar molecules[2].

Furthermore, the penetration enhancement may be due to the content of fatty acids of palm kernel oil. The fatty acids in palm kernel oil are: caprylic (4.2-4.4%), capric (3.5-6%), lauric (44.3-48.7%), miristic (14.4-15.6%), palmitic (7.5-8.2%), stearic (1.8-2.5%), oleic (14.8-16.9%), and linoleic (2.5-2.9%)[11]. The penetration enhancing effect of fatty acids have been described many times in the literature[8]. Lauric acid increases the flux of ozagrel for 24 fold[12]. Oleic acid increases the absorption of tenoxicam. This increment is due to the alteration of the structure of stratum corneum caused by this enhancer[13]. Although the fatty acids are in triglyceride form, but some present as free fatty acids. So, it was assumed that the free fatty acid is playing the role of enhancement.

The effect of palm kernel oil on the penetration rate of ascorbic acid through rabbit skin was shown in Figure 4.

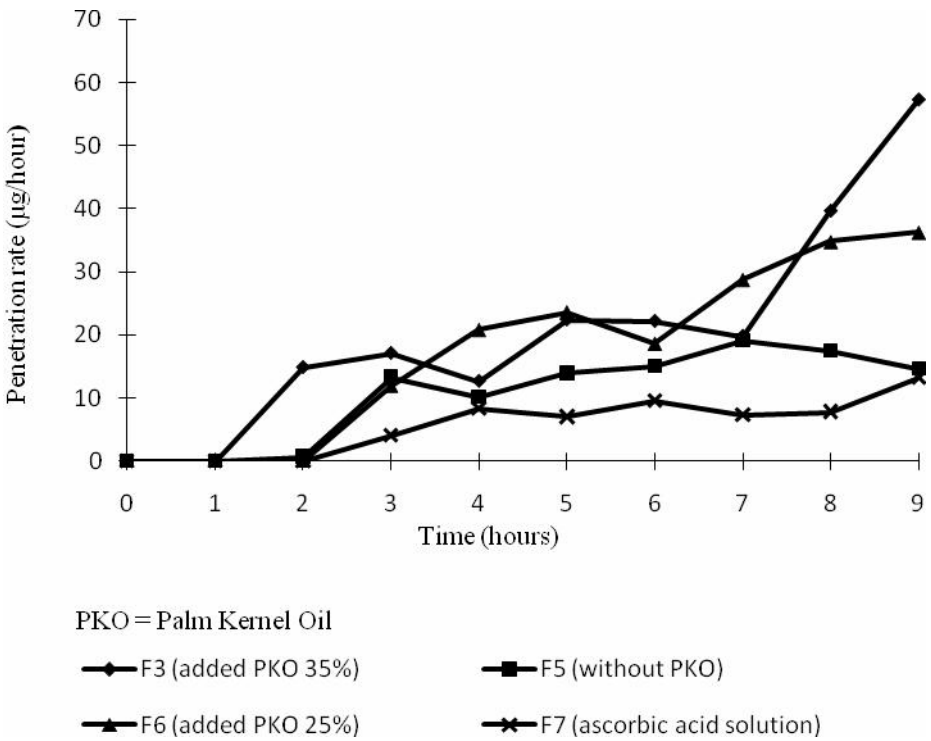

Figure 4. Effect of palm kernel oil on the penetration rate of ascorbic acid through rabbit skin *in vitro* in 50% glycerin solution at 37ºC.

As the concentration of ascorbic acid penetrated, the penetration rate of ascorbic acid was also increased with the increased of concentration of palm kernel oil used in the formulation. This may due to the same reasons described previously.

Effect of the combination of Tween 80 and palm kernel oil on the *in vitro* penetration of ascorbic acid through rabbit skin

The effect of the combination of Tween 80 and palm kernel oil on the *in vitro* penetration of ascorbic acid compare to usage of Tween 80 and palm kernel oil separately is showed in Figure 5.

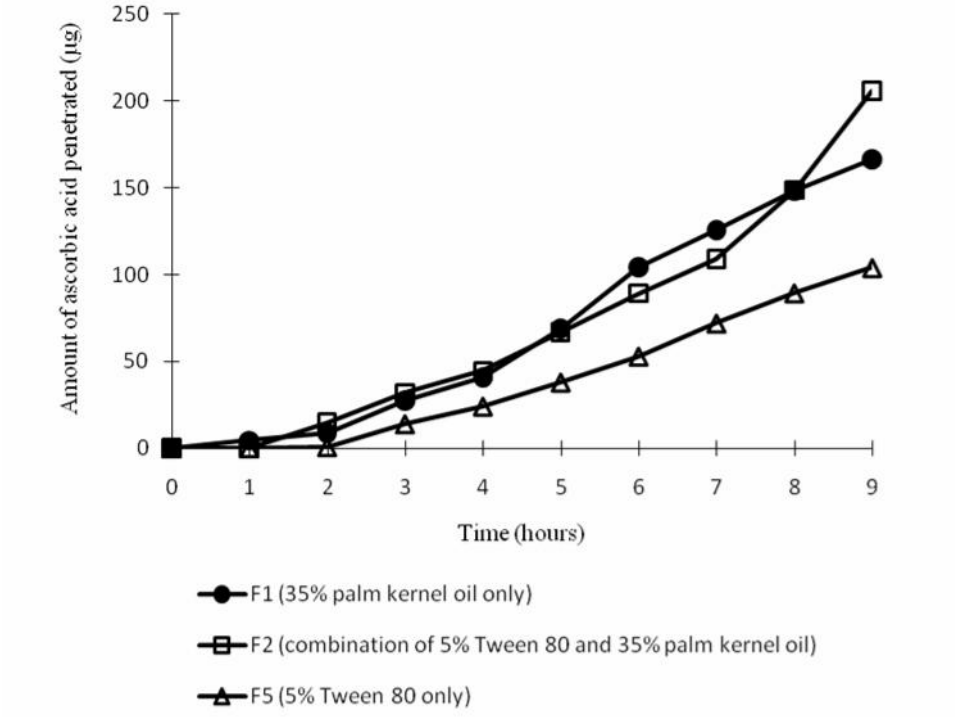

Figure 5. Effect of the combination of Tween 80 and palm kernel oil compared to Tween 80 and palm kernel oil alone.

It shows that formula with combination of Tween 80 and palm kernel oil produced higher amount of ascorbic acid penetrated compare to formula that only contained Tween 80 and only contained palm kernel oil. It may be due to combination of the enhancing effect of Tween 80 and palm kernel oil, which are solubilization of the lipid of stratum corneum by Tween 80 and hydration of stratum corneum by palm kernel oil. It is also shows that formula with palm kernel oil only produced higher ascorbic acid penetration compared to formula with Tween 80 only. It shows that palm kernel oil has a greater role of enhancing the penetration of ascorbic acid than that of Tween 80.

Conclusion

This study shows that Tween 80 can enhances ascorbic acid penetration, but it must be used at low concentration (2.5% and 5%). At high concentration (10%), it decreases ascorbic acid penetration. Palm kernel oil can enhances ascorbic acid penetration. As the concentration of palm kernel oil increases, the concentration of the penetrated ascorbic acid is also increases. Combination of Tween 80 and palm kernel oil produces higher penetration enhancing effect if the concentration of Tween 80 used is low.

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