PERMEATION STUDY OF VITAMIN C EMULGEL WITH VARIATIONS IN GELLING AGENT CONTENT

Dea Tri Novianti
Polytechnic Piksi Ganesha
Bandung, Indonesia

Meiti Rosmiati
Polytechnic Piksi Ganesha
Bandung, Indonesia

Abstract. A permeation study, also known as a penetration test or drug absorption test, is used to evaluate the absorption of a topical preparation into the skin. This is typically conducted using a Franz diffusion cell, a simulation model that mimics how a topical preparation penetrates or is absorbed by the skin. The device is designed to replicate actual skin conditions, including pH, physiological fluids, temperature, and pore size. The aim of this research is to demonstrate how permeation studies are conducted on topical preparations, specifically emulgel formulations containing vitamin C, which serves as an antioxidant. In this study, the key values measured include the cumulative penetration of vitamin C into the emulgel and the flux value of vitamin C from each sample. The results from three samples indicate that Sample 1 exhibited the highest cumulative amount of vitamin C penetration, with a value of 12,789.08 ± 83.44 µg/cm², and the highest flux value, measured at 60.34 ± 0.23 µg/cm².min⁻¹.

Keywords: *Permeation Study, Emulgels,Vitamin C*

Introduction

To achieve local effects, various formulations of therapeutics and cosmeceuticals are applied to the skin surface. However, the main obstacle is the permeability of chemical substances in the formulation to pass through the permeable skin membrane (Shashi, 2012). The outermost layer of the skin, namely the multilayered stratum corneum, represents a strong barrier to the penetration of chemical substances into the skin, especially since most medicinal chemicals are unable to penetrate the stratum corneum (Raut SV, 2014). The development of drug delivery systems has led to new

formula modifications that can increase the bioavailability of drugs in the skin. New drug delivery systems resulting from the combination of two pharmaceutical dosage forms, such as oral suspensions and emulsifiers, are emerging. Emulgel, which combines two dosage forms, namely an emulsion preparation and a gel preparation, has been shown to increase the percutaneous absorption of drugs, especially for fat-soluble types of drug molecules (Shashi, 2012). Also, the use of penetration enhancers is being considered to increase the penetration of the drug into the skin (Raut SV, 2014). This proves that aspects of the formulation and properties of the active ingredient are very important factors that determine the penetration of drugs into the skin, as the properties of the active ingredients and excipients each have different influences on the penetration and absorption profile of drugs through the skin membrane (Shashi, 2012). In addition, anatomical and physiological factors of the patient also affect the permeation and absorption of drugs through the skin, such as the condition of the injury, pH of the skin, thickness of the skin and age of the patient.

In this case, the preparation chosen for research was vitamin C, which, among other things, acts as an antioxidant and is widely used in the field of cosmetics. Vitamin C or ascorbic acid/ascorbic acid is a general term that refers to all the biological activities of natural vitamin E, namely d-alpha-tocopherol. In nature, there are 8 substances with vitamin E activity, namely the tocopherol group (D-alpha, D-beta, D-gamma and D-delta-tocopherol) and the tocotrienol group (D-alpha, D-beta, D-gamma and D-delta-tocotrienol). These two groups differ in terms of methylation and chain. Of all of them, d-alpha-tocopherol has the highest biological activity and is therefore used as a standard for others. (Wanasundara and F. Shahidi. 2005).There should be no numbering in the sections. Except if the alignment is explicitly stated, all text must be justified. The default line spacing is 11 units.

"*Quoted texts must be used the same indentation as the abstract, which is 0.5 inches from both sides. Quotes must be placed within double quotes at both ends and must be in italics. Follow the APA 7th edition Publication Manual guidelines for the use of quotes.*"

Cosmetic preparations containing vitamin C are easier and more stable to manufacture when they are made into emulgel preparations, as this system is a form of emulsion in gel, which provides an elegant appearance and high stability. And with high effectiveness. Emulgel is a form of stable emulsion and gel preparation with the addition of a gelling agent, where the gel capacity of the emulgel preparation makes the emulsion formulation more stable.

When assessing the penetration, permeation or absorption of a topical preparation, the evaluation of the permeation study into the skin is done using an in vitro evaluation using the Franz diffusion cell device, a model of how a topical preparation can penetrate or penetrate the skin, to calculate the cumulative amount of penetration as well as the percentage of active ingredients penetrating the skin.

This research aims to determine the absorption of the active ingredients contained in vitamin C emulgel, as it is believed to be widely used by manufacturers. Apart from that, vitamin C is quite effective as an antioxidant based on various studies (Gilbro, JM, 2011).

Basic Theory

Methodology

A. Tools and materials

The tools used are glassware, analytical balance, micropipette, magnetic stirrer, Franz diffusion cell with a receptor volume of 13 ml, PTFE membrane, water bath, pH meter, UV spectrophotometer. The material used for emulgel formulation is Vitamin C (Brataco Chemical), Span 20 (Brataco Chemical), Tween 20 (Brataco Chemical), Parrafin liquidum (Brataco Chemical), Propylene glycol (Brataco Chemical), Methyl and Propyl Paraben (Brataco Chemical), Triethanolamine (Brataco Chemical), HPMC (Brataco Chemical), Phosphate buffer pH 7.4, Aquamineralisata (Brataco Chemical).

B. Method

Emulgel base orientation

The base orientation was performed using different concentrations of HPMC as gelling agent, 0.5% (F1), 1% (F2), 1.5% (F3). The composition of various emulgel formulations is shown in Table 1

Table 1. Composition of emulgel base orientation

Komponen	Prosentase (%)		
	F1	F2	F3
Vitamin C	2	2	2
HPMC	0.5	1.00	1.50
Liquid Paraffin	7.5	7.5	7.5
Tween 20	1	1	1
Span 20	1.5	1.5	1.5
Propylene Glycol	10	10	10
Nipagin	0.03	0.03	0.03
Nipasol	0.01	0.01	0.01
TEA	2	2	2
Aqua ad.	100	100	100

Preparation of liquid paraffin vitamin emulgel while the water phase is prepared by dissolving Tween 20 in distilled water. 0.03 g of methylparaben and 0.01 g of propylparaben dissolved in 10 g of propylene glycol and vitamin C mixed with the water phase. The oil and water phases are heated separately to 70–80 °C. Then the oil phase was added to the water phase with continuous stirring until it cooled to room temperature. The emulsion is poured into the gel with gentle stirring until a homogeneous emulsion is formed (V. Naga Sravan et al., 2014). Methylparaben and other preservatives are greatly reduced by the presence of non-ionic surfactants as a

result of micellization. However, propylene glycol (10%) has been shown to enhance the antimicrobial activity in the presence of non-ionic surfactants (Rowe, 2006).

Evaluation of emulgel physical parameters of the prepared formulations. All prepared formulations were visually inspected for color, appearance, homogeneity and phase separation and then subjected to freeze-thaw test.

Determination of pH pH measurements were performed using a digital pH meter (Mettler Toledo). The gel (1 g) was dissolved in 25 ml of distilled water and the electrode was then immersed in the gel formulation until a constant reading was observed. Determination of pH values for each formulation was done in three replicates (V. Naga Sravan et al., 2014).

Determination of Viscosity

The viscosity of each formulation was determined at ambient temperature using a Brookfield digital viscometer with spindle no. 5 at 50 rpm (V. Naga Sravan et al, 2014).

Spreadability test

Weigh (350 mg) of Emulgel on a glass plate (10 x 5 cm). Another glass plate (10 x 5 cm and 5.8 ± 1 g) was dropped from a distance of 5 cm. The diameter of the spreading circle was measured after 1 minute (V. Naga Sravan et al, 2014). The gel types based on spreading are given in Table 2.

Table 2. Gel types based on spreading (Dignesh, 2012)

Jenis gel	Pengukuran (cm)
Gel cair	Lebih dari 2,4
Gel semi-cair	1.9-2.4
Gel semi kaku	1.9-1.6
Gel kaku	1.6-1.4
Gel sangat kaku	Kurang dari 1,4

In vitro penetration test using Franz diffusion cells

The penetration test of the facial whitening serum preparation Vitamin C was performed using an FTFE membrane with a Franz diffusion cell (diffusion area 1.77 cm2, chamber volume 13 ml, receptor chamber filled with phosphate buffer pH 7.4 with a temperature of 37 ± 0.5˚C), in Figure 1 you can see a cross section of the Franz diffusion cell.

Figure 1. Franz diffusion cell device

The composition of the diffusion equipment used consists of a water bath, a magnetic stirrer, a beaker, a peristaltic pump to control the flow rate, a Franz diffusion cell and a 5 mm diameter tube. 1 g sample of each facial whitening serum sample (samples 1, 2 and 3) was fixed on the upper surface of the membrane in the diffusion cell and placed on the water bath. The receptor section consists of a beaker filled with Daparphosphate pH 7.4, which is placed on a magnetic stirrer at a speed of 300 rpm. During the process, the temperature is maintained at 37 ± 0.5 °C, which is the same as the temperature of the human body, using a water jacket. Then, a tube is connected between the receptor section and the Franz diffusion cell. This diffusion test was carried out for 3 hours and samples were taken 5 times at 30, 60, 90, 120 and 180 minutes. At the time of sampling, 1 ml of the sample was taken and then the receptor was filled with Daparphosphate pH 7.4 to the same volume. The same. The absorbance of all samples was measured with a UV-Vis spectrophotometer.

The phases carried out include:

1. Preparation of a standard solution of vitamin C 500 ppm

Vitamin C was weighed at 25 mg and dissolved in phosphate buffer to a volume of 50 ml in a volumetric flask.

2. Determination of the maximum wavelength (λ) of vitamin C

1) Take 5 ml of the standard vitamin C solution with a pipette and then place it in a cuvette

2) Then, as a blank, add 5 ml of phosphate buffer pH 7.4 to the cuvette.

3) Then the two cuvettes are placed in a UV-Vis spectrophotometer and look for the highest λ.

3. Preparation of vitamin C standard curve

1) Vitamin C standard solutions are prepared with different concentration series, namely 100, 200, 300, 400 and 500 ppm

2) Fill 0.5 ml into 5 10 ml glass vials; 1 ml; 1.5 ml; 2 ml; and 2.5 ml of vitamin C standard solution.

3) Then each vial is diluted with buffer phosphate solution pH 7.4 to a volume of 5 ml.

4) The absorbance is measured at the maximum wavelength (λ) and a relationship curve between vitamin C concentration and absorbance is prepared to obtain the absorbance value (y). Thus, a linear equation and correlation coefficient (r) can be obtained from the calibration curve.

4. Calculate the cumulative penetration amount of drug and the drug flux of vitamin C

5.

Table 3. Physical Evaluation Results of Emulgel Base

Formula	Warna	Bau	Konsistensi	Phase Separation
F1	Putih	Tidak Berbau	Kental, mudah disebar	Tidak terjadi pemisahan fasa
F2	Putih	Tidak Berbau	Kental, mudah disebar	Tidak terjadi pemisahan fasa
F3	Putih	Tidak Berbau	Kental, mudah disebar	Tidak terjadi pemisahan fasa

The cumulative amount of active substance (vitamin C) penetrating per diffusion area (µg/cm2) is calculated using the following formula:

Q = (Cn.V+ ∑_(i=1)^(n-1)▒〖Ci.S〗)/A

Where :

Q = Cumulative amount of penetrated vitamin C per diffusion area (µg/ml)

Cn = Vitamin C concentration (µg/ml) in the nth minute of sampling

∑_(i=1)^(n-1)▒〖Ci〗 = = Total concentration of vitamin C (µg/ml) at sampling (minute (n-1) until before the nth minute

V = Franz diffusion cell volume

S = Sampling volume

A = Membrane area (cm2)

(Thakker and Chern, 2003)

Calculation of drug flux based on Fick-I law:

J===M/(S x t)

Where:

J = flux (µg.cm-2.hour-1)

M = cumulative amount of vitamin C penetrating the membrane (µg)

S = diffusion area (cm-2)

T = time (hours)

From these data, a graph of cumulative amount of vitamin C penetrated (µg) per diffusion area (cm-2) versus time (hours) and a graph of flux (µg. cm-2 hour-1) versus time (hours) were made.

Results and Discussion

Emulgel-Base Orientation Results

Based on the results in Table 3 and Table 4, formulas F1, F2 and F3 show good results in consistency, phase separation and freeze-thaw tests.

Table 4. Freeze-thaw test results

Formulas	Phase separation at the cycling-					
	1	2	3	4	5	6
F1	(-)	(-)	(-)	(-)	(-)	(-)
F2	(-)	(-)	(-)	(-)	(-)	(-)
F3	(-)	(-)	(-)	(-)	(-)	(-)

Note: (-) No phase separation

(+) Phase separation occurs

Vitamin E emulgel formulation

Freeze-thaw test shows that F1, F2 and F3 have good stability so all three can be combined with vitamin C. All formulations are shown in Table 5.

Table 5. Vitamin C Emulgel Formulation

Komponen	Prosentase (%)		
	F1	F2	F3
Vitamin C	2	2	2
HPMC	0.5	1.00	1.50
Liquid Paraffin	7.5	7.5	7.5
Tween 20	1	1	1
Span 20	1.5	1.5	1.5
Propylene Glycol	10	10	10
Nipagin	0.03	0.03	0.03
Nipasol	0.01	0.01	0.01
TEA	2	2	2
Aqua ad.	100	100	100

Physical stability study of vitamin E emulgel

Table 6 and Figure 1 show the physical properties of emulgel in F1, F2 and F3. The research results show that F1 has a better formula based on parameters such as pH measurement and dispersion test. F1 continues the stability test and Figure 4 shows the stability study data of F1 formula.

Table 6. Physical properties results of vitamin C emulgel

Formula	Characteristic Organoleptik	Storage Time (Days)				
		0	7	14	21	28
F1	Phase separation	No White	No White	No White	No White	No White
	Color	Odorless	Odorless	Odorless	Odorless	Odorless
	Odor	Slippery	Slippery	Slippery	Slippery	Slippery
	Texture	Thick	Thick	Thick	Thick	Thick
	Consistency Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F2	Phase separation	No White	No White	No White	No White	No White
	Color	Odorless	Odorless	Odorless	Odorless	Odorless
	Odor	Slippery	Slippery	Slippery	Slippery	Slippery
	Texture	Thick	Thick	Thick	Thick	Thick
	Consistency Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F3	Phase separation	No White	No White	No White	No White	No White
	Color	Odorless	Odorless	Odorless	Odorless	Odorless
	Odor	Slippery	Slippery	Slippery	Slippery	Slippery
	Texture	Thick	Thick	Thick	Thick	Thick
	Consistency	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

Homogeneity

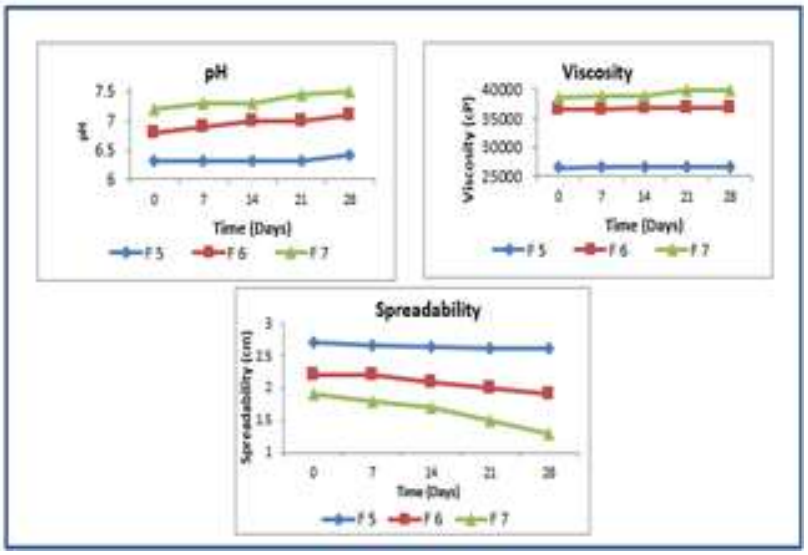

Figure 2. Results of pH, Viscosity and Spreadability Evaluation

Vitamin C Emulgel Permeation Study

Results of Preparation of Standard Curve for Vitamin C in Phosphate Buffer pH 7.4

The standard curve for the absorbance of 500 ppm of vitamin C in a phosphate buffer solution of pH 7.4 shows a maximum wavelength at 510 nm. The standard solution was prepared at a concentration of 500 ppm, then diluted to several concentrations and the absorbance was measured at a wavelength of 510 nm and a standard curve was prepared. The vacuum curve equation obtained is:

$y = 0.0025x + 0.1154$

with $r2 = 0.9994$

The linear regression plot can be seen in Figure 3 below:

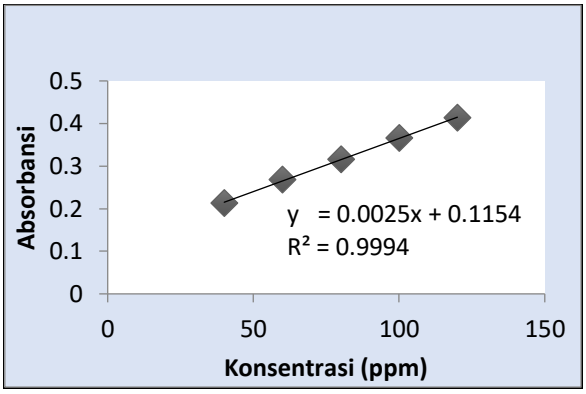

Figure 3 Linear Regression of Vitamin C in Phosphate Buffer

The permeation test in this study was performed in vitro using a Franz diffusion cell. The test was performed to determine the amount of vitamin C that was able to penetrate through the skin during a specific time interval after vitamin C emulgel preparation.

The membrane used is the PTFE (polytetrafluoroethylene) membrane, a synthetic membrane commonly used for in vitro testing. The selection of the membrane is based on considering the least resistance rather than speed control. Apart from that, the membrane is selected to minimize errors. This PTFE membrane is a lipid-free synthetic membrane that resembles human skin and offers many advantages in terms of economy and stability during storage. Before use, the membrane must be hydrated, first soaked in 7,4 phosphate buffer (simulating the pH values of human biological fluids) and set at a constant temperature of 37 °C for 3 minutes. Then the PTFE membrane layer can be used. The pore size of PTFE, 0.45 microns, is the same as the human pore size, namely 0.2 microns to 50 microns. Another aspect is that this membrane is more practical because it does not require special treatment, as is the case when using animal skin or human skin, for example, when using leather, rats must first be shaved and their subcutaneous fat layer removed, and especially when human skin is used, it is definitely more difficult to obtain.

Before use, the PTFE membrane is first placed in the receptor dissolution medium, namely phosphate buffer pH 7.4 for the hydration process. For the receptor fluid, phosphate buffer was chosen as a simulation of the pH conditions of human biological fluids with a pH of 7.4. The membrane is then placed between the receptor and donor compartments. In this case, the membrane must be in contact with the receptor fluid so that the preparation applied to the membrane can penetrate through the skin into the receptor fluid. Stirring in the receptor chamber serves to homogenize the fluid so that the process of dissolution of penetrated substances can be accelerated and the concentration of substances can be evenly distributed in the receptor fluid. Stirring with a magnetic stirrer at a speed of 300 rpm to avoid the formation of air bubbles due to excessive rotation. The temperature is maintained at 37 ± 0.5 °C during the process

using a water jacket, as a simulation of the human body using water flowing from the thermostat. The test time was 3 hours, with samples taken at 30, 60, 90, 120 and 180 minutes, i.e. 5 points. Each time, a sample amount of up to 1 ml was taken and diluted to 5 ml, which means that the dilution was performed five times. The receptor compartment fluid was immediately replaced with the same volume of phosphate buffer solution with pH 7.4 at each sample collection, with the aim of keeping the receptor fluid volume constant during the test. Next, the sample absorbance was measured using a UV-Vis spectrophotometer at the vitamin C wavelength in phosphate buffer at a wavelength of 510 nm. For each formula, the permeation test was performed three times (Triplo).

The results of the three-hour penetration of vitamin C into the PTFE membrane from the emulgel preparations Vitamin C Formula 1,2 and 3 were 12,789.08 ± 83.44 µg/cm2, 7,025.39 ± 85.21 and 4,325.78 ± 40.37 µg/cm2. What can be seen in Figure 4 below:

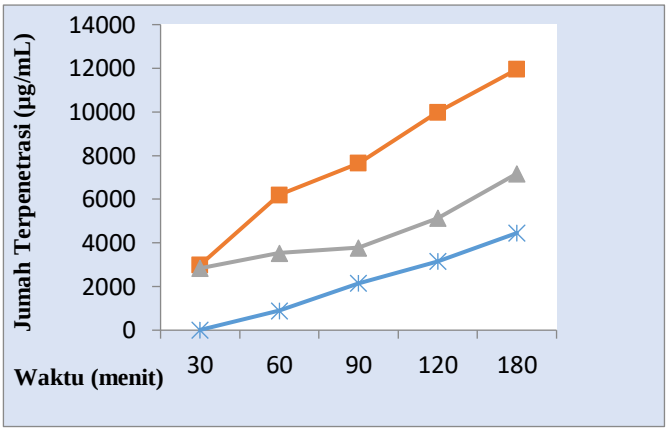

Figure 4. Cumulative amount of penetrated vitamin C per unit membrane area from emulgel preparations (Formula 1, Formula 2 and Formula 3).

Based on these results, the highest amount of vitamin C was penetrated in formula 1 emulgel preparations. Although the active ingredient used in the three samples was the same, namely the use of the facial whitening agent vitamin C, the formulas of the three emulgels were identical. All of these formulas met the physicochemical and stable physical criteria, so this permeation test can be used to determine which formula is the most suitable in terms of permeation or penetration into the skin, which can roughly simulate the penetration of the active ingredient into the skin to its site of action where it can exert the desired effect.

Then the flow is obtained in a steady state following the rules of Fick's law. Fick's first law gives the flow (diffusion rate through a unit area) in a flow in a steady state (Martin and Cammarata, 1983). The cumulative amount of vitamin C penetrated through the PTFE membrane is plotted against time and a linear regression equation is constructed so that the value of vitamin C flux can be determined from the slope of the steady state graph. The steady state is shown as a horizontal line on the flux curve plotted against

units of time. The value of vitamin C flux for formulas 1,2 and 3 are 60.34 ± 0.23; 36.18 ± 0.24; and 22.34 ± 0.33 µg.cm-2.minute-1, respectively. The formula with the highest flux value during the three hours of testing was formula 1, with this formula having the highest penetration rate of the active ingredient. The flux of each sample can be seen in Figure 5 below:

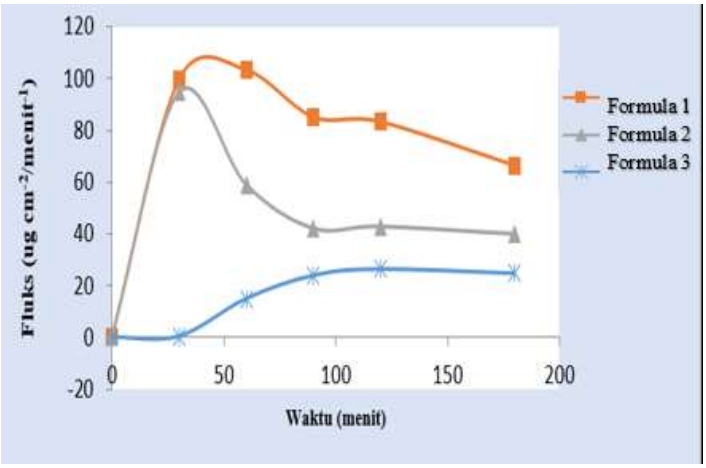

Figure 5. Vitamin C flux at each sampling time from Samples 1, 2 and 3

In Figure 5, it can be seen that Sample 1 and Sample 2 have high flux values in the first few minutes, which shows that these samples allow for rapid drug release, while for Sample 1, the graph tends to drop and then increase, so it does not mean that absorption tends to take longer.

The difference in the concentration of the gelling agent shows the influence of the penetration power of these formulas, as can be seen from the cumulative penetration amount and flux value of each sample.

Conclusion

Based on the permeation study results of the three vitamin C emulgel formulas, the results obtained in formula 1 had better cumulative penetration amount and flux value than the other two formulas, where the results respectively for the cumulative penetration amount obtained from samples 1, 2 and 3 were 12,789.08 ± 83.44 µg/cm2; 7,025.39 ± 85.21 and 4,325.78 ± 40.37, while the flux value is 60.34 ± 0.23; 36.18 ± 0.24; and 22.34 ± 0.33 µg.cm-2.minute-1.

References

Dignesh, M., Ashish, D., Dinesh, R., 2012. Formulation design & development of piroxicam emulgel. Int. J. Pharm. Tec. Res. 4 (3), 1332-1344.

Draelos, Z.D., & Thaman, L.A. Ed. 2006. *Cosmetic Formulation of Skin Care Products*. Vol. 30. New York: Taylor and Francis Group, LLC.

Gillbro JM, Olsson MJ. The melanogenesis and mechanisms of skin-lightening agents – existing and new approaches. International Journal of Cosmetic Science. 2011;33(3):210-21

Kolarsick PAJ, Kolarsick MA, Goodwin C. 2009. *Chapter 1 :Anatomy and Physiology of the Skin*, dalam Skin Cancer. Journal of the dermatology nurses' association. Hal 1-11.

Martin A., Swarbick, J., & cammarata, A. 1983. *Farmasi Fisik. Edisi ke-3*. Penerjemah: Joshita Djajadisastra. Jakarta: Penerbit Airlangga UI Press.

Rowe, RC., Sheskey, PJ.,Owen, SC. 2006. Handbook of Pharmaceiticals Excipient. 5th ed. Washington Pharmaceutical Press and American Pharmacist Association. P.441-444.

Saeedi, M., Eslamifar, M., & Khezri, K. (2019). Kojic acid applications in cosmetic and pharmaceutical preparations. Biomedicine and Pharmacotherapy, 110(November 2018), 582–593.

Shashi P, Anroop N, Vipin S, Neelam S. 2012. *Skin Kinetict and Dermal Clearance,* Review article. Int. Res. J. Pharm, 3 (8): 14 - 21.

Shimizu, Hirosi. 2007. *Chapter 14 Keratinization.* Hokaido University press, Dalam http://www.derm-hokudai.jp/shimizu-dermatology/pdf/01-03.pdf diakses pada 19 Juni 2016.

Singla, Anil K. Alka Garg. Deepika Aggarwal. Sanjay Garg. 2002. Spreading of Semisolid Formulations An Update. *Pharmaceut. Technol.*

Swarbrick, J. dan Boylan, J. 1995. *Percutaneous Absorption*, in Encyclopedia of Pharmaceutical Technology, Volume 11. New York : Marcel Dekker Inc. Hal. 413-445.

Thakker, K.D. & Chem, W.H. 2003. Development and Validation of in Vitro Release Tests for Semisolid Dosage Forms-Case Study. Dissolution Technology. 10-15.

V. Naga Sravan Kumar Varma et al. Calcipotriol delivery into the skin as emulgel for effective permeation. Saudi Pharm.J. 2014; 22: 591-599.

Rizka Amalia, A., & Usviany , V. (2023). Evaluasi Efek Samping Obat Antihipertensi pada Pasien di RSAU Dr. M Salamun Periode Juni 2023. Health Information : Jurnal Penelitian, 15(2), e1117.

visilia, novia, & Usviany, V. U. (2023). EVALUASI WAKTU TUNGGU

PELAYANAN RESEP INSTALASI FARMASI RAWAT JALAN RSUD SAYANG CIANJUR PERIODE APRIL SAMPAI MEI 2023. FASKES : Jurnal Farmasi, Kesehatan, Dan Sains, 1(3), 78–84.