PERMEATION STUDY OF CARROT EXTRACT EMULGEL (DAUCUS CAROTA L.)

Nurul Komariah
Piksi Ganesha Polytechnic
Bandung, Indonesia

Meiti Rosmiati
Piksi Ganesha Polytechnic
Bandung, Indonesia

Abstract.

A permeation study, also known as a penetration or drug absorption test, is a key method for assessing how a topical preparation is absorbed into the skin. Using a Franz diffusion cell, this study models how a topical formulation penetrates and is absorbed by the skin, simulating actual skin conditions, including pH, physiological fluids, temperature, and pore size. This research focused on conducting a permeation study for emulgel preparations containing vitamin C, valued for its antioxidant properties. The study measured two main values: the cumulative penetration amount of carrot root extract (Daucus carota L.) in the emulgel, and the flux of carrot extract in each sample. Among the three samples tested, Sample 1 demonstrated the highest cumulative penetration of active ingredients, reaching 12,789.08 ± 83.44 µg/cm², and achieved the highest flux value at 60.34 ± 0.23 µg/cm²/min. These findings highlight Sample 1's superior effectiveness in delivering the carrot root extract’s antioxidant benefits through the emulgel formulation.

Keywords: Permeation Study, Emulgels, carrot extract emulgels

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 a carrot extract, which, among other things, acts as an antioxidant and is widely used in the field of cosmetics. Carrot extract or Dautus carrota is a general term to designate all biological activities, 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).

Cosmetic preparations containing carrot extract 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 that too with high effectiveness. Emulgel is a form of stable emulsion and gel preparation with the addition of a gelling agent, the gel capacity of the emulgel preparation makes the emulsion formulation more stable

To assess the penetration, permeation or absorption of a topical preparation, the permeation study into the skin must be evaluated using an in vitro evaluation using the Franz diffusion cell device which is a model of how a topical preparation can penetrate or penetrate the skin, the cumulative penetration amount and also the percentage of active ingredients that penetrate the skin.

This research aims to determine the absorption of the active ingredients contained in carrot extract emulgel, since it is considered to be widely used by manufacturers,

apart from various studies based on the fact that carrot extract is quite effective as an antioxidant (Gilbro, JM , 2011).

Basic Theory

Methodology

Tools and materials

The tools used were 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 was carrot extract (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).

Procedure Emulgel base alignment

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

Table 1 Composition of Emulgel Base Orientation.

Component	Percentage (%)		
	F1	F2	F3
Ekstrak wortel	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 carrot extract emulgel

Preparation of emulgel by dispersing HPMC in distilled water (75 °C) with constant stirring at medium speed with a mechanical stirrer and adjusting the pH to 5.5-6.5 with triethanolamine (TEA). The oil phase is prepared by dissolving Span 20 in liquid

paraffin. The water phase was prepared by dissolving Tween 20 in distilled water. 0.03 g of methylparaben and 0.01 g of propylparaben were dissolved in 10 g of propylene glycol and carrot extract was 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 obtained (V. Naga Sravan et al, 2014)

Methylparaben and other preservatives are greatly reduced in the presence of non-ionic surfactants due to micellization. However, propylene glycol (10%) has been shown to enhance antimicrobial activity in the presence of non-ionic surfactants (Rowe, 2006).

Emulgel Evaluation

Physical parameters of prepared formulation

All prepared formulations were visually examined for color, appearance, homogeneity, phase separation and 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. Types of gel based on distribution (Dignesh, 2012)

Types of gels	Measurement (cm)
Gel cair	Over 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	Less than 1.4

In vitro penetration test using Franz diffusion cells

The penetration test of the face whitening serum Emulgel Carrot Extract was performed using a 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 at a temperature of 37 ± 0.5˚C) , in Figure 1 you can see a cross section of the Franz diffusion cell.

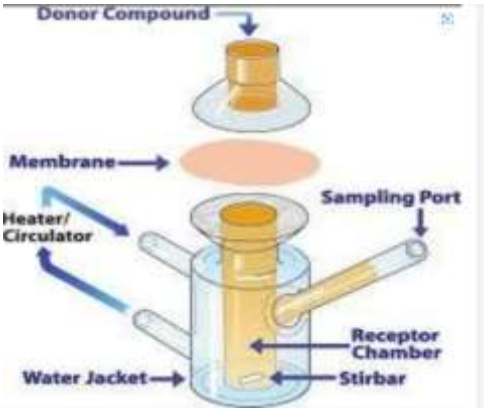

Figure 1. Franz Diffusion Cell Apparatus

The arrangement of the diffusion apparatus used consists of a water bath, magnetic stirrer, beaker, peristaltic pump to regulate the flow rate, Franz diffusion cell, then a 5 mm diameter hose. 1 g sample of each sample of facial lightening serum (samples 1.2 and 3) is attached to 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 phosphate buffer pH 7.4 and placed on a magnetic stirrer at a speed of 300 rpm. During the process, the temperature is maintained using a water jacket at 37 ± 0.5oC where the temperature describes the state of human body temperature. Then the hose is connected between the receptor section and the French diffusion cell. then the receptor is refilled using phosphate buffer pH 7.4 with the same volume. All samples were measured for absorbance using a UV-Vis spectrophotometer.

The stages carried out include:

1. Preparation of standard solution of 500 ppm carrot extract

25 mg of carrot extract was weighed and dissolved in phosphate buffer in a volumetric flask to a volume of 50 ml.

2. Determination of maximum wavelength (λ) of carrot extract emulgel

    1) Take 5 ml of the standard solution of carrot extract emulgel with a pipette and then place it in a cuvette

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

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

3. Preparation of standard curve for carrot extract emulgel

    1) Carrot extract emulgel standard solutions are prepared with different concentration series namely 100, 200, 300, 400 and 500 ppm

    2) Fill 0.5 ml in 5 10 ml glass vials; 1 ml; 1.5 ml; 2 ml; and 2.5 ml of Emulgel standard solution of carrot extract.

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

    4) The absorbance is read at the maximum wavelength (λ) and a relationship curve is constructed between the Emulgel concentration of carrot extract and the absorbance to obtain the absorbance value (y). Thus, a linear equation and a correlation coefficient (r) can be obtained from the calibration curve.

4. Calculation of the cumulative amount of drug penetrated and the drug flux Emulgel carrot extract

The cumulative amount of drug (Carrot extract Emulgel) penetrated per diffusion area (µg/cm2) is calculated using the following formula:

$$Q = \frac{Cn.V + \sum_{i=1}^{n-1} Ci.S}{A}$$

Q	=	Cumulative amount of carrot extract Emulgel penetrated per diffusion area (µg/ml)
Cn	=	Carrot extract emulgel concentration (µg/ml) at the nth minute of sampling
$\sum_{i=1}^{n-1} Ci$	=	The concentration of carrot extract Emulgel (µg/ml) at sampling (minute (n-1) to before minute n).
V	=	Franz diffusion cell volume
S	=	Sampling volume
A	=	Membrane area (cm2)

(Thakker and Chern, 2003) Calculation of drug flux based on Fick's I law:

Results and Discussion

(Thakker and Chern, 2003) Calculation of drug flux based on Fick's I law:***Equations***

To aid legibility, equations should be typed in the same word processor as the rest of the text, ideally without handwritten symbols. Equations must be numbered sequentially, with the numbers in parenthesis and justified to the right.

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

Table 4 Freeze Thaw Test Results

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

Desc : (-) No phase separation

(+) Phase separation occurs

Carrot Extract Emulgel Formulation (Daucus carota L.)

Freeze thaw test showed that F1, F2 and F3 have good stability, so all three can be combined with carrot extract. All formulations can be seen in Table 5.

Table 5. Carrot Extract Emulgel Formulation

Komponen	Prosentase (%)		
	F1	F2	F3
Ekstrak wortel	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 results showed that F1 has a better formula based on parameters such as pH measurement and spreading test F1 continued the stability test and Figure 4 shows the stability study data of the F1 formula.

Table 6. Results of Physical Characteristics of Carrot Extract Emulgel

Formula	Karakteristik Organoleptik	Waktu Penyimpanan (Hari)				
		0	7	14	21	28
F1	Pemisahan fasa	Tidak	Tidak	Tidak	Tidak	Tidak
	Warna	Putih	Putih	Putih	Putih	Putih
	Bau	Tidak Berbau	Tidak Berbau	Tidak Berbau	Tidak Berbau	Tidak Berbau
	Tekstur	Licin	Licin	Licin	Licin	Licin
	Konsistensi	Kental	Kental	Kental	Kental	Kental
	Homogenitas	Homogen	Homogen	Homogen	Homogen	Homogen
F2	Pemisahan fasa	Tidak	Tidak	Tidak	Tidak	Tidak
	Warna	Putih	Putih	Putih	Putih	Putih
	Bau	Tidak Berbau	Tidak Berbau	Tidak Berbau	Tidak Berbau	Tidak Berbau
	Tekstur	Licin	Licin	Licin	Licin	Licin
	Konsistensi	Kental	Kental	Kental	Kental	Kental
	Homogenitas	Homogen	Homogen	Homogen	Homogen	Homogen
F3	Pemisahan fasa	Tidak	Tidak	Tidak	Tidak	Tidak
	Warna	Putih	Putih	Putih	Putih	Putih
	Bau	Tidak Berbau	Tidak Berbau	Tidak Berbau	Tidak Berbau	Tidak Berbau
	Tekstur	Licin	Licin	Licin	Licin	Licin
	Konsistensi	Kental	Kental	Kental	Kental	Kental
	Homogenitas	Homogen	Homogen	Homogen	Homogen	Homogen

Emulgel Permeation Study Carrot Extract Results of Emulgel Standard Curve Preparation for Carrot Extract in Phosphate Buffer pH 7.4

The Emulgel absorption standard curve of 500 ppm carrot extract 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 $r2 = 0.9994$

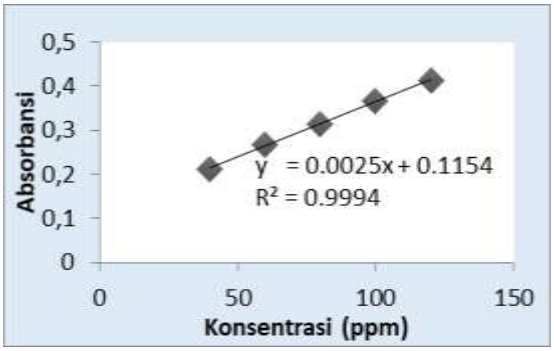

Figure 2 Linear Regression of Carrot Extract Emulgel in Phosphate Buffer

Permeation Test Results

The permeation test in this study was performed in vitro using a Franz diffusion cell. The test was performed to determine the amount of carrot extract that was able to permeate through the skin from the carrot extract emulgel preparation during a specific time interval.

The membrane used is the PTFE (polytetrafluoroethylene) membrane, a synthetic membrane commonly used for in vitro testing. The membrane selection 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 point of view is that this membrane is more practical because it does not need to undergo any special treatment, as is the case when using animal skin. Human skin, for example when using leather, must first be shaved and the 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 solution 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 dissolving 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 during the process is maintained at 37 ± 0.5 °C using a water jacket, as a simulation of the human body, which uses water flowing from the thermostat. The test time was 3 hours, with samples taken after 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 carried out 5 times. The receptor compartment fluid was immediately replaced with the same volume of phosphate buffer solution at pH 7.4 at each sampling, with the aim of keeping the receptor fluid volume constant

during the test. Next, the sample absorbance was measured with a UV-Vis spectrophotometer at the emulgel wavelength of carrot extract in phosphate buffer at a wavelength of 510 nm. For each formula, the permeation test was performed three times (triplo).

From the results of three-hour penetration of carrot extract emulgel into the PTFE membrane from carrot extract emulgel formulas 1, 2 and 3, the values obtained 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

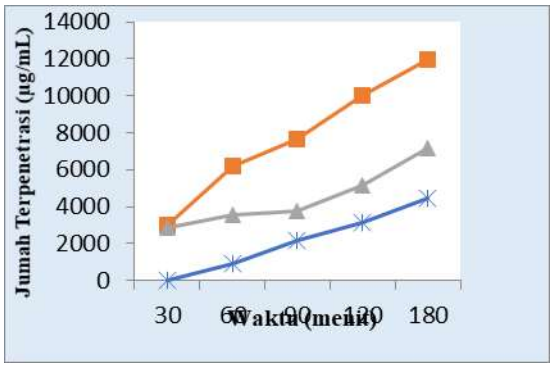

Figure 3. Cumulative amount of carrot extract penetrated per unit area of membrane from emulgel preparations (Formula 1, Formula 2, and Formula 3).

Based on these results, the highest amount of carrot extract emulgel penetrated into the formula 1 emulgel preparation. Although the active ingredient used in the three samples was the same, namely the use of facial whitening agent emulgel carrot extract, the formula of the three emulgels was identical because all of these formulas met the physicochemical properties and stability that were tested, so this permeation test can be used to determine which formula is the best in terms of permeation or penetration into the skin, which can roughly simulate the entry 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 carrot extract emulgel that penetrated through the PTFE membrane was plotted against time and a linear regression equation was constructed so that the value of carrot extract emulgel flux could be determined. The flux is determined from the slope of the graph at steady state. The steady state is shown as a horizontal line on the flux curve plotted against units of time. The values of emulgel flux of carrot extract formulas 1,2 and 3 were 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

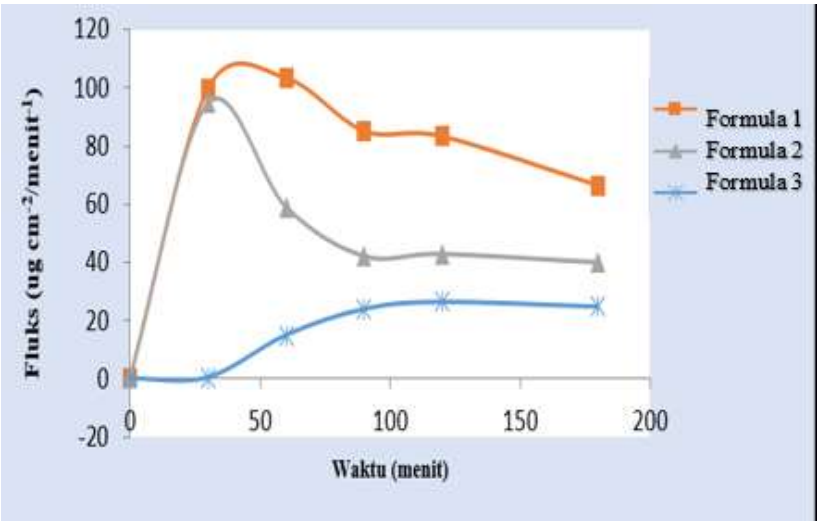

Figure 4. Emulgel flux of carrot extract at each sampling time from Samples 1, 2 and 3.

In Figure 4, 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 curve tends to decrease 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 results of the permeation study of the three carrot extract emulgel formulas, the results obtained in Formula 1 had better cumulative number of penetrations and flux values than the other two formulas, where the results obtained for the cumulative number of penetrations from samples 1, 2 and 3 respectively were

13,179.08 ± 55.44 µg/cm2; 6,905.79 ± 28.32 and 4,046.65 ± 39.67, while the flux value is 59.34 ± 0.89; 35.98 ± 0.54; and 22.64 ± 0.63 µg.cm-2.minute-1.

Suggesttion

For further investigation, it is recommended to add categories and the number of samples examined and to further develop the existing formula to improve it.

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