EFFECT OF SKIN FAT ON CAPSAICIN TRANSFORMSOME GEL: IN VITRO PENETRATION STUDIES USING FRANZ DIFFUSION CELLS

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INTRODUCTION

The lipophilicity and hydrophilicity of a substance have a significant impact on penetration, especially in percutaneous absorption [1]. The skin is an organ used in the dermal or transdermal route and has several core layers, namely the epidermis, dermis, and hypodermis [2]. The outermost layer of the epidermis is the stratum corneum. In the stratum corneum, there is a combination of lipids in a multilayer that has lipophilic and hydrophilic fields parallel to the cell surface [3, 4]. Under the dermis layer is the hypodermis or subcutaneous fat layer, which is 85% of the total body fat and the essential component of the skin [5]. For a drug to penetrate through the skin, the drug should have a balance of hydrophobicity and hydrophilicity [6]. In skin penetration tests to see drug release in semisolid products, the Franz diffusion cell is generally used as an in vitro test. It has the advantage of resembling penetration in human skin (containing hydrophobic and hydrophilic parts with biological membranes) with synthetic or biological membranes [7]. In its development, various kinds of diffusion mediums are used in testing the Franz diffusion cell, such as water or buffer solutions with the addition of organic cosolvents [8]. This organic cosolvent is used to increase the solubility of hydrophilic drugs in the media, fluidize skin fat, and the possibility to extract compounds trapped under fat from the basolateral [9].

Capsaicin is a highly lipophilic drug with limited ability to penetrate the deeper layer of the skin because it is chemically bound to protein or trapped in fat [10, 11]. Having a nonpolar phenolic structure and highly lipophilic, capsaicin poses a unique difficulty for transdermal drug delivery. There have been studies using the addition of organic cosolvents in a diffusion medium for capsaicin. The use of the cosolvent serves to increase the solubility of capsaicin and its partition in the stratum corneum, thereby increasing the penetration of capsaicin by fluidizing fat [12, 13]. Research on the effect of subcutaneous fat on the penetration of lipophilic substances is important due to the possibility of fluidization of fat by the diffusion medium used. This study aimed to investigate the penetration of hydrophobic substances through skin membranes with and without subcutaneous fat to determine the effect of fat on penetration.

MATERIALS AND METHODS

Instruments

The instruments used in this study: analytical balance (Sartorius, Germany), rotary vacuum evaporator (Buchi R-100, Switzerland), particle size analyzer (Malvern, UK; Beckman Coulter, USA), vortex mixer (VM–300 Germany Industrial Corporation, USA), sonicator (Qsonica, USA), pH meter (Eutech Instruments pH 510, Singapore), Brookfield viscometer (Brookfield, USA), transmission electron microscope (TEM Tecnai G2 20 S-Twin, America), UV-Vis spectrophotometry (Shimadzu UV-1800, Japan), cuvette (Hellma), USP standard Franz diffusion cell (Mitra, Indonesia), Mini extruder set (Avanti Polar Lipids), polycarbonate membrane (Whatman), hot plate (IKA, Germany), centrifuge (Spectraflue 16M), centrifuge tube (Kubota, Japan), thermometer, magnetic stirrer (Boeco MSH-300, Germany), oven (Mennert, Germany), micro-dropper (Socorex), degassing (Elmasonic S40H), desicator, refrigerator (LG, Korea), IKA-Ultra-Turax T25 homogenizer (Staufen, Germany), and tools glassware.

Materials

The materials used in this study were Capsaicin (Formosa, Taiwan), Vitamin-C (DSM, UK), Phospholipone 90G (Lipoid, Germany), Carbomer (SIL, Indonesia), Tween 80 (KAO, Indonesia), Triethanolamine (Petronas, Indonesia), Propylene glycol (Caelo), Sodium Hydroxide (Merck, Indonesia), Propylene glycol (Caelo), Sodium Hydride (Merck, Indonesia), Propylene glycol (Caelo), Potassium dihydrogen phosphate (Merck), aquadest, Ethanol (Merck), Dichloromethane (Merck), Methanol (Merck).

Animals

Abdomen skin of female rat, Sprague Dawley strain, weighing 180-200 grams at 2-3 mo old. The Ethical Committee of Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia,

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approved the study under registration number KET-755/UN2.1/ETIK/PPM.00.02/2022.

**Transfersome preparation**

The transfersome was made using the thin layer method (table 1). The phospholipid and the surfactant (ratio 85:15) were dissolved in dichloromethane [7]. The mixture then evaporated at a speed of 50-150 rpm and a temperature of 40 °C. After forming the thin layer, it was streamed with N2 gas and stored at a low temperature for 2 h.

**Table 1: Formulation of transfersome**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Formulation (85:15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>50 mg</td>
</tr>
<tr>
<td>Fosfolipon 90G</td>
<td>850 mg</td>
</tr>
<tr>
<td>Tween 80</td>
<td>150 mg</td>
</tr>
<tr>
<td>Phosphate buffer (pH 7.4)</td>
<td>Ad 20 ml</td>
</tr>
</tbody>
</table>

**Transfersome characterizations**

**Particle size, PDI (polydispersity index), and zeta potential**

The particle size, particle distribution, and zeta potential were determined using a particle size analyzer according to the DLS method by Malvern Zetasizer. Before measurements, one drop of the transfersome suspension was dissolved in 20 ml of distilled water.

**Morphological characterization**

The morphology characterization used Transmission Electron Microscope (TEM Tecnai G2 20 S-Twin (America)) to determine. A drop of diluted dispersion (1:50) was applied to a film-coated copper grid and observed after drying.

**Deformability index**

The extrusion method is a way to measure the elasticity and flexibility of vesicles which describe the deformability index. Using a 0.1 μm polycarbonate membrane by passing the transfersome suspension through a mini extruder set over a hot plate at 40 °C. The deformability index can be expressed as the following equation

\[ D = J \left( \frac{\sigma V}{E} \right) \]  (1)

**Entrapment efficiency**

Entrapment efficiency was determined with the "indirect method," which used free concentration and total concentration via centrifugation with a filtered centrifugation tube at 7500 rpm for 90 min, broken down by methanol. The amounts of drugs in the vesicle were measured using Spectro UV-Vis at 281 nm. Trapping efficiency was measured using Formula 2:

\[ EE = \frac{C_{total} - C_{free}}{C_{total}} \times 100\% \]  (2)

**Gel preparation**

The three gel formulations were prepared, capsaicin transfersome, capsaicin non-transfer some, and vitamin C, by dispersion of carbomer in distilled water, then left for a day until the carbomer swelled. Propylene glycol and triethanolamine were added, homogenized, and stirred at 1000 rpm until a thick gel base formed. Finally, the capsaicin/vitamin C was added to the gel and homogenized at 250 rpm for 10 min.

**Table 2: Gels formulation**

<table>
<thead>
<tr>
<th>Formula</th>
<th>F1 (%)</th>
<th>F2 (%)</th>
<th>F3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>0,15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Transfersome Capsaicin</td>
<td>0</td>
<td>0,15</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0</td>
<td>0</td>
<td>0,15</td>
</tr>
<tr>
<td>Carbomer</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol 96%</td>
<td>0,5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Triethanolamine (TEA)</td>
<td>Ad pH5</td>
<td>Ad pH5</td>
<td>Ad pH5</td>
</tr>
<tr>
<td>Aquadest</td>
<td>Ad 100</td>
<td>Ad 100</td>
<td>Ad 100</td>
</tr>
</tbody>
</table>

**Gel evaluation**

The gel was evaluated by observation organoleptic, pH measurement, homogeneity, viscosity, and physical stability at low temperatures (4±2 °C), room temperature (20±2 °C), and high temperatures (40±2 °C); tests were conducted for eight weeks with a 2-week interval between each observation and cycling test.

**In vitro penetration test**

**In vitro** penetration test was carried out using Franz diffusion cells with membranes from the skin of Sprague-Dawley female rats aged 2-3 mo and weighed 180–200 grams. Rats were sacrificed by ether anesthesia. To remove the skin of the abdomen, carefully shave the hair, then cut the skin (thickness: 0.7±0.05 mm). The skin was immersed in phosphate buffer pH 7.4 for 30 min at 5 °C. The skin layer was then hydrated at 40 °C for 60 min, and sonication was carried out for 1 min to reduce the particle size.

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<td>0</td>
<td>0,15</td>
<td>0</td>
</tr>
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<td>0</td>
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<td>Aquadest</td>
<td>Ad 100</td>
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</tbody>
</table>
of the diameter distribution. Transfersome obtained a particle size of 113.03±1.88 nm. Vesicles smaller than 300 nm have been reported to be able to penetrate deeper layers of the skin, while those larger than 600 nm are unable to do so [15]. The size of the resulting vesicle indicates the possibility of vesicles being able to penetrate deeper layers of the skin, thereby increasing the amount of drugs penetrated.

Another data obtained from the Zeta-sizer is the polydispersity index (PDI). PDI is a value that shows the level of homogeneity of a vesicle size. A smaller value, which is close to 0, indicates the narrow distribution of particle size (homogenous), and a value close to 1 indicates heterogeneous size distribution. PDI value of the resulting capsaicin transfersome is 0.213±0.03. From the results, a PDI value of transfersome indicates a homogenous dispersion system [16]. The zeta potential is a crucial aspect of nanovesicles as well. The zeta potential is an essential parameter in predicting the physical stability of vesicles. Particles with a zeta potential of more than (+/-) 30mV are proven stable from aggregation [17, 18]. The zeta potential value was 38.36±1.7 mV, indicating the formulations’ favorable stability.

**Morphology characterization**

Morphological characterization shows a structural picture of the nanoparticles obtained from the transmission of electrons to very thin specimens [19]. A magnification of 29000 and 43000 showed that vesicles have spherical shapes with various particle sizes. However, a vesicle shape that is not perfectly round is called an irregular shape with a unilamellar structure that has one layer inside.

**Deformability index**

The ability to deform due to surfactants, which tend to disrupt the bilayer of lipids and increase the fluidity and elasticity of the vesicle membrane, is one of the advantages of the transfersome vesicle. Vesicles with great deformability can squeeze through the intercellular pores and pass through lipid membranes without losing their integrity [20]. The deformability index is calculated by multiplying the volume of transfersomes that pass through the membrane by the square of the ratio of the size of the particles that pass through the membrane to the size of the membrane; then, the result is 3.83±0.12. The greater deformability index value makes more flexible vesicles [16].

**Entrapment efficiency**

The entrapment efficiency results obtained in this formulation were 75.91%. Transfersome composition and its concentration can affect the entrapment efficiency, as the use of lecithin from soybeans has a smaller entrapment efficiency and the HLB value of surfactants. Lipid formulation and surfactant with a ratio (of 85:15), which are used by high lipids, will increase entrapment efficiency [16]. Previous studies had shown similar outcomes when hydrophilic surfactants with high HLB values like Tween 80 were used, showing a high entrapment efficiency of the lipophilic drugs. In contrast, lipophilic surfactants show lower entrapment efficiency because lipophilic surfactants would compete with lipophilic molecules in the lipid bilayers. This may lead to larger vesicle size and cause less space for lipophilic molecule entrapment, explaining the low entrapment [21].

**Gel evaluation**

An organoleptic observation showed that non-transfersome gels are more transparent white than transfersome gels, and vitamin C gel has a slightly yellowish-clear color. For each preparation, the non-transfersome capsaicin gel with transfersome capsaicin gel had a pH of 5.65 and 5.95, and for vitamin C gel, 5.36. The pH decreased after eight weeks, although it remained at a balanced level for the skin (4.5-6.5). Viscosity measurements showed that all gel preparations had thixotropic flow rheology characteristics. In addition, homogeneity tests showed that both gel compounds were homogeneous. Finally, a stability test was performed in six cycles; results showed no syneresis in either gel.

**In vitro penetration test**

In vitro penetration tests were performed using a Franz diffusion cell to determine drug penetration through the skin layers. Penetration test results in fig. 1 showed that the cumulative amount of drug penetrated the skin membrane of rats with subcutaneous fat within 8 h for transfersome capsaicin gel, non-transfersome capsaicin gel, and vitamin C gel was 762.22±1.73 µg/cm, 595.60±0.32 µg/cm, and 654.69±3.36 µg/cm, respectively. Compared to the non-fatty subcutaneous membrane, the cumulative amount of penetrated for transfersome capsaicin gel was 920, 28±3, 42 µg/cm, non-transfersome capsaicin was 833, 33±0.84 µg/cm, and vitamin C gel was 776,45±1.19 µg/cm². These findings show that the cumulative amount of drug penetrated for both hydrophilic and lipophilic drugs significantly (p<0.05) decreased in the presence of subcutaneous fat. This is due to the fact that lipophilic substances have a high affinity for the subcutaneous fat layer and cause it to be deposited in the subcutaneous tissue. In contrast, hydrophilic substances with a different polarity from the subcutaneous fat layer would also find it challenging to penetrate this fat layer [22].

These results also indicate that the transfersome succeeded in increasing the cumulative amount of capsaicin penetrated compared to non-transfersome capsaicin gel, and these findings show that the cumulative amount penetrated for lipophilic drugs was higher than for hydrophilic drugs. A drug has to partition into the membrane to pass through the stratum corneum, making partitioning a crucial step in the penetration of the membrane. For the transdermal route to be successful, either lipophilic or hydrophilic drugs must penetrate the hydrophilic and lipophilic layers, respectively. The partitioning of a drug between the lipophilic and hydrophilic
The epidermis is well represented by the n-octanol-water partition coefficient (log P). The chemical with a lower log P value is considered hydrophilic. As a result, compounds with log P lower than -1, like vitamin C (log P -1.85), would find it challenging to enter the stratum corneum, and the molecules with a log value between 1-3 had both lipophilic and hydrophilic properties, adequate to obtain proper transdermal penetration like capsaicin (log P 3.04) [23].

Fig. 1: The cumulative amount of capsaicin and vitamin C penetrated from the gel preparation on the (A) skin with subcutaneous fat and (B) without subcutaneous fat. Each value shown is the mean (n = 3)

Fick's first law states that flux can be calculated from the slope of the regression equation on the graph of the cumulative amount of drug that penetrates in a steady state condition [24]. There was a significant increase (p<0.05) in the flux of the drug on the non-fatty membrane (TL) compared to the value of the flux on the membrane with subcutaneous fat (L), namely in the capsaicin transfersome gel of 116.84±0.47 µg/cm²/h, and capsaicin gel non-transfersome was 104.96±0.28 µg/cm²/h and the vitamin C gel was 93.36±0.28 µg/cm²/h as shown in fig. 2. The graph of the relationship between the cumulative amount penetrated and the time at a steady state can determine the lag time. This time value crosses the x-axis when y = 0. The data shows that the non-fat membrane (TL) produced a significantly lower (p<0.05) lag time compared to the membrane with fat (L) for capsaicin transfersome gel 0.19±0.01 h and 0.25±0.01 h and for non-transfersome capsaicin gel 0.17±0.02 h and 0.24±0.02 h, while the lag time is non-significantly lower (p>0.05) on vitamin C gel 0.14±0.01 h and 0.16±0.01 h.

The significant difference (p<0.05) in flux and lag time between membranes with subcutaneous fat (L) and without subcutaneous fat (TL) is due to the nature of the capsaicin substance, which is bound and soluble in fat (lipophilic), while the vitamin C is hydrophilic so penetration is limited by the presence of fat in the membrane. Another reason for these findings is the thickness of the membrane. The thickness of the membrane will vary depending on whether subcutaneous layers are present or not. The membrane’s thickness impacts the drugs’ flux and lag time. Since the membrane layer containing subcutaneous fat is thicker, the flux would be lower, and the lag time would be longer [25]. These results are parallel with Wilkinson’s studies on the penetration of testosterone and caffeine using different thickness membranes [26].

CONCLUSION

According to the results of this study, it can be confirmed that the presence of subcutaneous fat on the membrane inhibits drug penetration as it decreases the cumulative amount of drugs that penetrated, the flux of penetration, and the lag time were all lower on the membrane without subcutaneous fat.

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AUTHORS CONTRIBUTIONS

All authors were responsible for every part of this work and contributed to data analysis, drafting, and manuscript revision.
CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES


